Peptide mapping study on human erythropoietin (EPO): a complex N- and O-linked glycoprotein

Streamlined N- and O-linked glycopeptide characterization using the ZenoTOF 7600 system with the new Biologics Explorer software from SCIEX

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This technical note describes a newly integrated and easy-to-use peptide mapping workflow for the analysis of standard monoclonal antibodies (mAbs) as well as complex therapeutic proteins using the new Biologics Explorer software from SCIEX. The software provides a streamlined workflow for the analysis of both collision induced dissociation (CID) and electron activated dissociation (EAD) data, and it includes Genedata algorithms for data processing, reviewing and final reporting.

Peptide mapping is now considered a common analysis approach, and it is used throughout the biopharma industry. Sequence confirmation as well as identification and localization of post-translational modifications (PTMs) are all necessary to ensure that biotherapeutic proteins pass criteria for safety and efficacy. Although interrogation of CID data is well established, there is an ongoing drive to simplify and harmonize data analysis while providing meaningful information faster. Interpretation of alternative fragmentation data, which is needed to elucidate certain PTMs, can add complexity due to the nature of the data

and the potential unfamiliarity of data analysts with such techniques. To address these challenges, the new Biologics Explorer software was developed by SCIEX in collaboration with Genedata to streamline the task of submitting, processing and reviewing results for both CID and EAD data. Biologics Explorer software is highly optimized for SCIEX CID and EAD peptide mapping data and includes improved elucidation of PTMs using EAD.[1-4]

In this technical note, the peptide mapping analysis of erythropoietin (EPO) is shown, with a specific focus on complex N- and O-linked glycans, using the prebuilt peptide mapping workflow in Biologics Explorer software. Key features for facilitating peptide mapping with CID and EAD are highlighted.

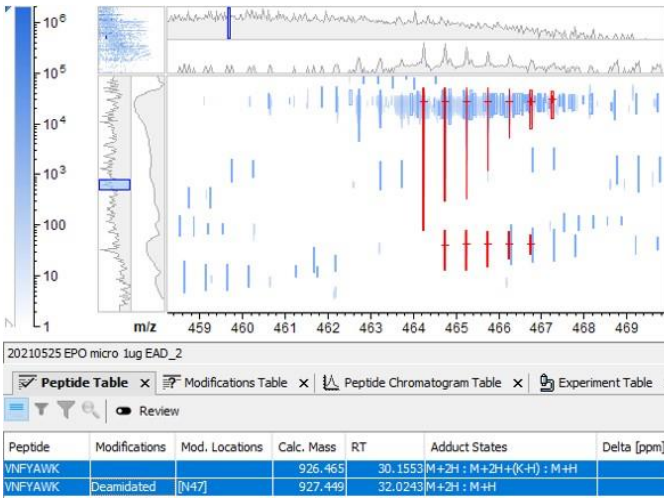

Figure 1. Heat map for a deamidated versus non-deamidated peptide of EPO digest. The heat map is zoomed in at a specific retention time (RT). Blue bars in the heat map show identified signals with darkness indicating their intensities. Red bars highlight the selected peptides from the table below. In this case, the non-deamidated and deamidated peptides of VNFYAWK were chosen. With the help of the heat map, the identification can be confirmed without missing information on other signals at the relevant RT.

Key features of Biologics Explorer software

- Provides a quick start for new and advanced users with prebuilt peptide mapping workflows that leverage highly optimized processing algorithms for SCIEX CID and EAD data and include example data
- Enables accelerated data analysis through a customizable interface for curation of results and comparison of samples, and offers the ability to save an interface setup
- Supports better decision making enabled by powerful visualization options for raw data (Figure 1)

Methods

Sample preparation: A denaturing buffer of 7 M guanidine hydrochloride (HCl) and 100 mM Tris-HCl, pH 7.4, was added to 100 µg protein to dilute the samples to 1 µg/µL. The samples were reduced by adding 2 µL of 0.5 M DL-dithiothreitol. Samples were incubated for 30 min at 50°C and allowed to cool to room temperature. Alkylation of the samples was performed by adding 2 µL 1.0 M iodoacetamide and incubating for 30 min in darkness at room temperature. Samples were buffer exchanged into 1 M guanidine HCl and 100 mM Tris-HCl, pH 7.4, using 10 kDa Amicon filters before 5 µg trypsin/Lys-C in 100 mM Tris-HCl, pH 7.4, was added to the samples. Glu-C (2 µg in 100 mM Tris-HCl, pH 7.4) was added to the samples before incubating overnight at 37°C. The digestion was quenched by adding 10 µL of 6% trifluoroacetic acid (TFA).

Chromatography: A total of 4–8 µL of enzymatically digested EPO (approximately 2 µg for CID and 4 µg for EAD, respectively) were injected onto a Waters ACQUITY CSH C18 column (2.1 ×150 mm, 1.7 µm particle size, 130 Å) using an ExionLC AD system. The aqueous mobile phase (A) consisted of water with 0.1% formic acid while the organic phase (B) consisted of acetonitrile with 0.1% formic acid. Temperature was set to 50°C on the column and a gradient profile was used (Table 1) with 0.3 µL/min.

Table 1. Chromatographic gradient.

Time [min]	%A	%B
0	*99*	*1.0*
5	*99*	*1.0*
35	*55*	*45*
36	*10*	*90*
38	*10*	*90*
38.1	*99*	*1.0*
41	*99*	*1.0*

Mass spectrometry: Data were acquired with an information-dependent acquisition (IDA) method using the ZenoTOF 7600 system from SCIEX. General method parameters were kept the same for CID and EAD and are summarized in Table 2. Parameters specific for EAD and CID can be found in Table 3.

Table 2. Mass spectrometry parameters.

Parameter	MS	MS/MS
Scan mode	*TOF-MS*	*IDA dependent*
Gas 1	*50 psi*	
Gas 2	*50 psi*	
Curtain gas	*35 psi*	
Temperature	*450°C*	
Ion spray voltage	*5500 V*	
Declustering potential	*80 V*	
Collision energy	*12 V*	*
CAD	*7*	
Maximum candidate ion	*15*	
Intensity threshold	*125 cps*	
Charge states	*1–10*	
Exclusion time	*6 s for 2 occurrences*	
Start mass	*200 m/z*	*100 m/z*
Stop mass	*2000 m/z*	*3000 m/z*
Accumulation time	*250 msec*	*
Time bins to sum	*8*	*10*

*specific for EAD/CID (see Table 3)

Table 3. Mass spectrometry parameters specific for CID and EAD.

Parameter	CID	EAD
Collision energy	*Rolling*	*12*
Electron KE	*NA*	*7 eV*
Electron beam current	*NA*	*5500 nA*
ETC	*NA*	*100%*
Zeno trap	*ON*	*ON*
Accumulation time	*0.05 s*	*0.09 s*

Data processing: The EPO digests were processed using both CID and EAD data in WIFF2 format in the PeptideMapping_Extended workflow (template 3 in Figure 2) in Biologics Explorer software 1.0. Data RT range was restricted from 5 min to 65 min. A FASTA file containing the sequence of EPO was input into the peptide mapping activity nodes. The Wildcard Mapping activity node was turned off. Both peptide mapping (PepMap) activity nodes contained fixed modification of carbamidomethylation, while variable modifications included

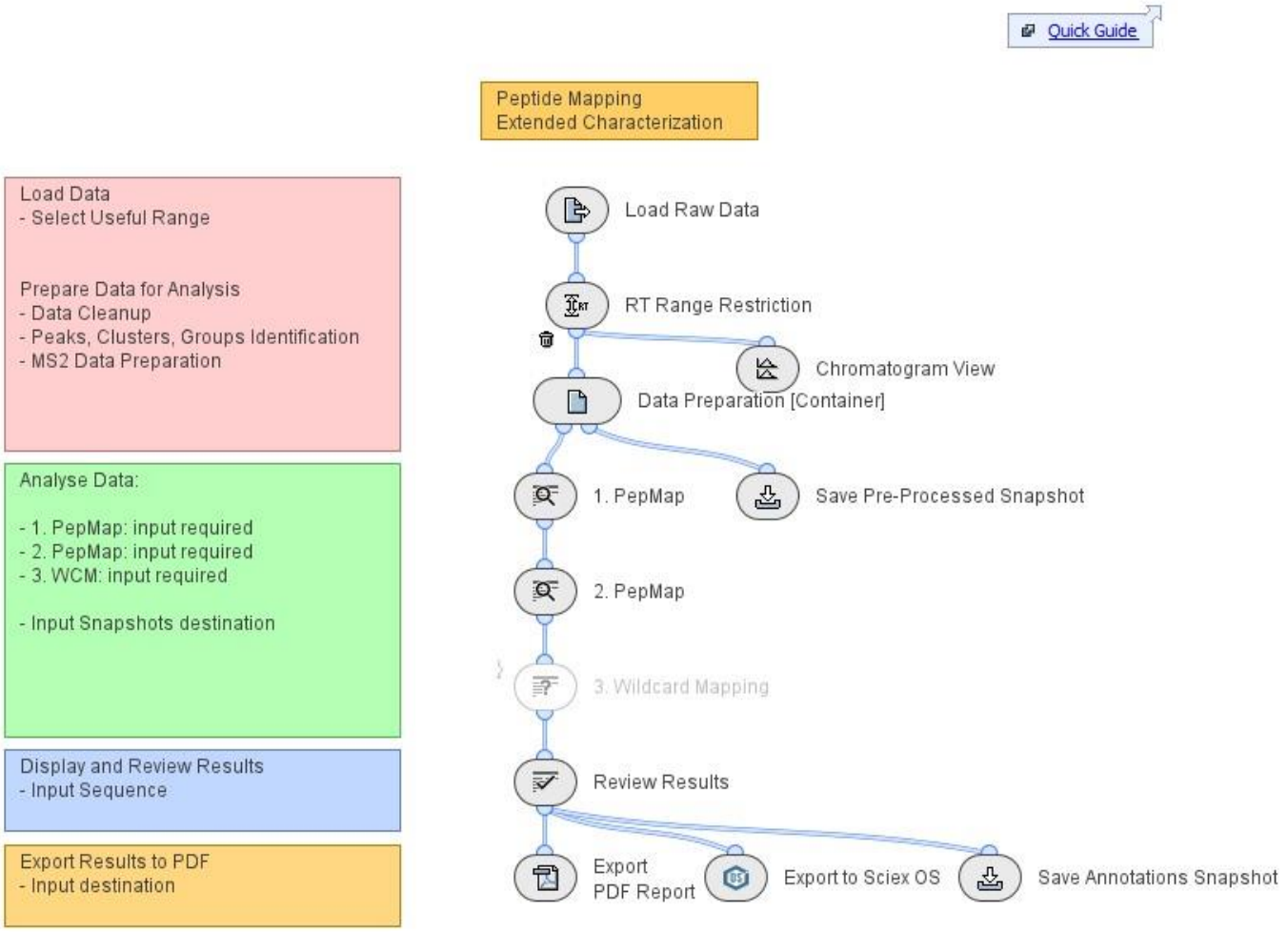

Figure 2. Overview of the extended peptide mapping workflow. Biologics Explorer software offers several workflow templates for processing SCIEX raw data (see left-hand side). The PeptideMapping_Extended template was used for the example to process peptide mapping data from EPO with a particular focus on N- and O-linked glycopeptides, using libraries for each in the first and second peptide mapping (PepMap) steps.

pyroglutamine, oxidation, deamidation, ammonia loss and lysine loss, with a maximum of 3 modifications per peptide. Enzymes used were trypsin and V8-E (endoproteinase Glu-C from *Staphylococcus aureus*) with a maximum of 4 missed cleavages and a minimum length of 3 amino acids. The mass tolerance was set to 20 ppm for MS and MS/MS data and the minimum score was set to 50. The first peptide mapping activity node contained an EPO N-linked glycan library that was created specifically for this sample with the inclusion of up to 4 substituents consisting of either acetate or methyl modifications. The second peptide mapping activity node contained an EPO O-linked glycan library that was created specifically for this sample and was subsequently searched against all non-annotated data with the inclusion of the same substituents for glycans that were used in the first peptide mapping activity node. Data were reviewed manually for sequence coverage and modifications such as glycans before report creation.

EPO: a complex glycoprotein

Erythropoietin, or EPO, has a 166-amino-acid-long, 18-kDa protein backbone (Figure 3), and it is well known for its use in the treatment of anemia by increasing blood cell counts. It has also been used in sports doping and athletes are routinely tested for its presence. This small protein was reported to have multiple N- and O-linked glycans with 3 confirmed N-linked glycan sites and 1 confirmed O-linked glycan site, although other sites have been implicated with biosimilars.[5] Previous characterization studies of EPO suggest that N-linked glycans are very heterogenous, formed of bi-, tri and tetra-antennary glycans containing LacNAc units, α-2,3-linked sialic acids and single fucosylations.[6-7] Furthermore, it was found that these glycans may also have O-acetylation, making this an ideal surrogate to evaluate complex sample processing in Biologics Explorer software.[8]

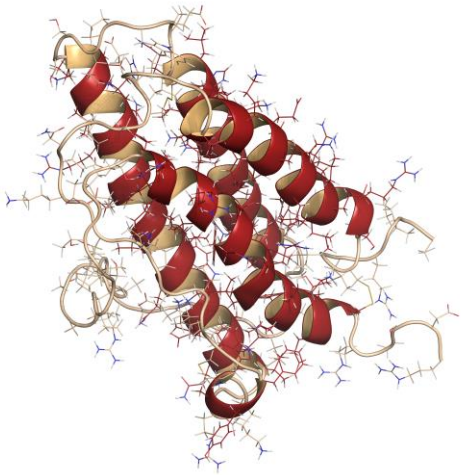

Figure 3. 3D schematic of EPO. The protein consists of several alpha helices as secondary structure. Glycosylations are not depicted here.

Overview of peptide mapping workflow

Biologics Explorer software is template-driven, offering 5 different workflow templates for processing intact proteins and subunits as well as protein digests (see the example in Figure 2). The 5 workflow templates are:

- Intact protein
- Peptide mapping simple
- Peptide mapping extended
- Peptide mapping comparative
- Peptide mapping review snapshots

Intact protein analysis allows for the processing of intact protein data, as discussed earlier,[9] while the other 4 workflow templates focus on peptide mapping and reviewing snapshots. Snapshots of processed data are saved so they can be reviewed later along with other data files acquired or processed at different points in time. The workflow templates allow the user to select the type of data processing required, from simple to complex, and they include the ability to look for more adducts and to do wildcard mapping to comparative processing methods. The comparative analysis template allows for the comparison of control data versus new batch data, stability studies or any other comparison by looking for present/absent and changing *m/z*.

The workflow templates connect different processing steps (activity nodes) in a logical manner and serve as a fast way to get from raw data to results. After each processing step, the workflow can be paused while data is reviewed. While templates cannot be modified, certain activity nodes can be turned off if they are unnecessary. In Figure 2, the CID and EAD data of the EPO digests were both processed as WIFF2 format using the PeptideMapping_Extended workflow. The workflow template offers transparency into the predefined and sequential activity nodes and their chronological connection for a user. It is easy to understand which processing is being applied to the raw data at which step in the workflow.

Data is imported using the Load Raw Data activity node, where visualization tools such as heat maps and 3D viewing (Figure 4) are available to provide a quick view of the data for setting retention times (RTs) for the next activity node, which is RT Range Restriction. The software then allows for viewing the chromatogram of the restricting RT ranges or continuing through for processing. The following container, Data Preparation, is where noise subtraction, optional RT alignment (if processing multiple samples), peak detection, clustering of charges and adducts and processing of MS/MS data activity nodes are housed to simplify the workflow view. This container can be opened to view all the activity nodes within it. The processing can be modified, although the settings have been optimized for SCIEX CID and EAD data, so that background is reduced while maintaining the integrity of low-abundant analytes.

The subsequent steps (1. PepMap, 2. PepMap, 3. Wildcard Mapping) involve analyzing the data and associating it with the theoretical masses and modifications. In the case of the PeptideMapping_Extended workflow, this is separated into multiple tasks to increase the amount of information that can be pulled from the data. The data is initially processed in the first PepMap activity node. Any MS/MS information that is not associated with a peptide or modifications during the first PepMapactivity nodeis then processed with the parameters in the second PepMap activity node. Using 2 steps like this allows searching for additional modifications or unexpected enzymatic cleavages. In the Wildcard Mapping step, the software associates masses instead of modifications to the data, taking into account the sequence of the protein selected. This allows for identification of peptides with unexpected modifications. For the data derived from the EPO digest, the first PepMap activity node used trypsin/Glu-C as digestion agent, with an N-linked library for searching, while the second PepMap contained an O-linked library for searching. Separating N- and O-linked searches allows for using specific N- and O-linked libraries. Biologics Explorer software comes with prebuilt glycan libraries, which can be appended, or new libraries tailored specifically to a protein can easily be created. Both peptide mapping activity nodes also include oxidation, deamidation, ammonia loss, Glu→pyro-Glu transformation and lysine loss as modifications. The Wildcard Mapping step was not used for the example discussed here.

Alongside the processing, snapshots can be saved. Users can return to snapshots of processed or partially processed files to review and/or process them in another batch using the review template in Biologics Explorer software. This allows for quick retrieval of information from the processed files. In addition, data from different batches can be compared for instance supporting the development of biotherapeutic drugs.

The final 2 activity nodes in the workflow are Review Results and Export. The Review Results activity node allows viewing of the processed data and assignment of peptides. A user is free to accept or reject them, which in turn allows for filtering and tailoring reports to a user's needs.

Sequence coverage of proteins

Confirming the sequence is a critical step for all therapeutic proteins in development, and peptide mapping approaches are frequently employed for this purpose. However, data matching results first must be verified and curated as needed. This step can be time consuming as many software tools lack visibility options. Biologics Explorer software allows raw data to be visualized in multiple ways, including via a 3D display and an ion map view. The ion map view of the raw EAD data plots the RT versus the *m/z* (Figure 4, left) while the 3D plot adds an intensity dimension (Figure 4, right). Both the 3D and ion map views allow for a deep dive into the data to quickly understand if any interferences are present or if overlaying spectra are suggesting another underlying molecule that may be important.

Selecting a peptide and using the zoom function shows the intensity and the data for the sample. Selecting 2 samples—for instance, non-deamidated and deamidated versions of the peptide (Figure 1)—enables the RT difference as well as the change in isotope pattern between the 2 to be used to validate the identification of a deamidation event. The heat map allows for this validation without missing relevant information since all analytes and their isotope series are displayed as *m/z* for the RT range of interest. The MS/MS spectra for the identified peptide would then confirm the peptide identification and the localization of the modification.

The ion map is also directly linked to the TOF-MS and MS/MS spectra. When a peptide is selected in the table of matched peptides, any and all charge states and adducts associated with it are highlighted in red in the ion map (Figure 4, left). A list of all MS/MS data from all data files (if multiple files have been searched) are listed in the fragment matching pane, and individual data specific for a particular data file can be selected. The data interactivity is multi-directional, allowing for the selection of a certain RT cut across the ion map (Figure 4), which provides all the TOF-MS data associated with that RT across the *m/z* range and highlights user-selected peptides in red in the ion map. The connectivity of data can greatly enhance the review speed of assignments.

After verifying matching results, sequence coverage results can be reviewed. Biologics Explorer software allows for a simple or more complex viewing of sequence coverage using the sequence viewing pane (Figure 5). In addition to the coverage of the sequence, identified PTMs are visualized. The color coding in the sequence pane reflects whether the peptides were identified with or without MS/MS data and if particular modifications were found within a sample. In this case, the

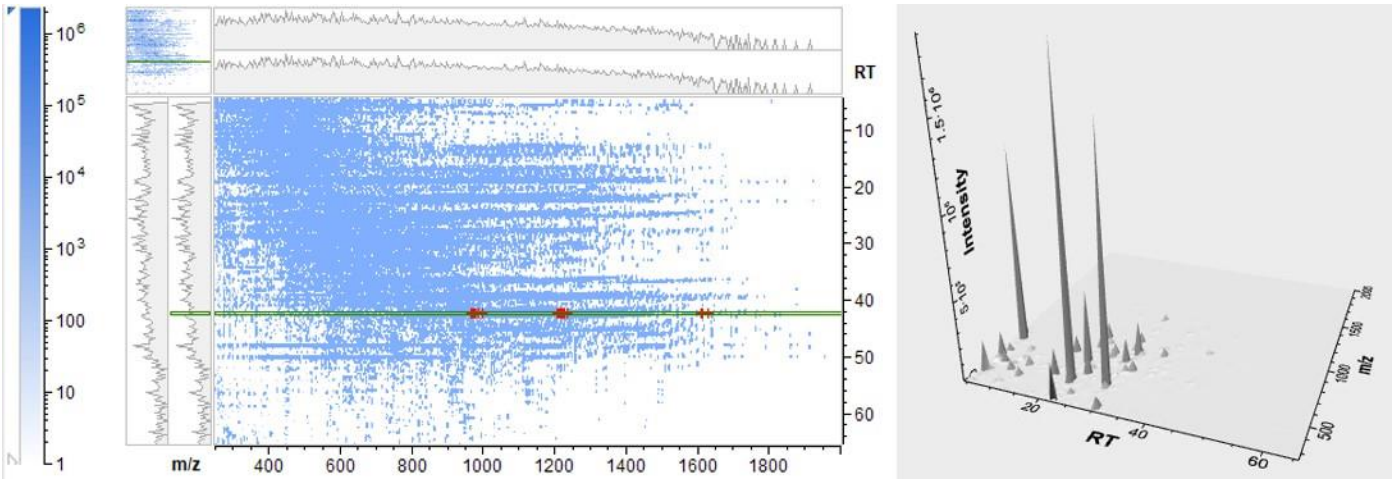

Figure 4. Ion map view and 3D view from Biologics Explorer software. Peptide GQALLVNSSQPWEPLQLHVDK with glycosylation FA13G3S3_OAc4 was highlighted in the peptide mapping table (not shown). The subsequent charge states and adducts are then automatically highlighted in the ion map in red (left-hand side). A 3D image can be created to view the data, taking into account the intensities of the analytes. The selection of a specific peptide allows for any information from that peptide and identified related species to be pulled out from all samples. All associated MS/MS data can be found in another table allowing for review by a user (not shown).

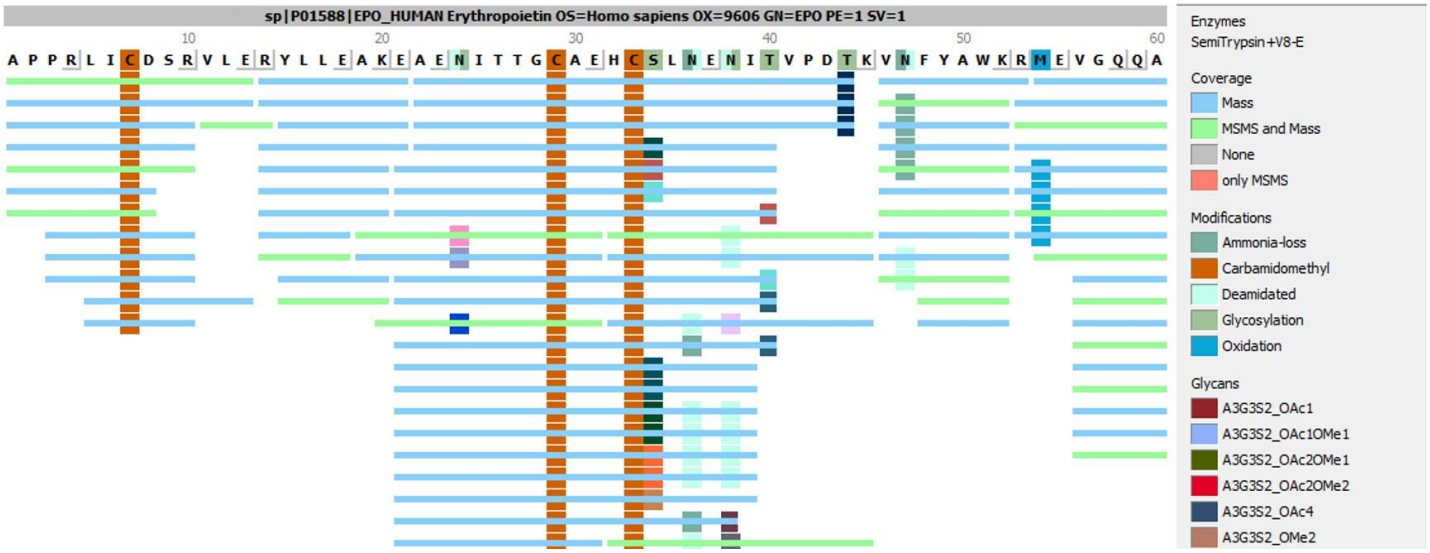

Figure 5. Example part for the sequence coverage of EPO. Expanded view to show which peptides were identified and whether MS/MS data were associated with the sample. The information also shows where modifications were identified, and a larger index refers to the type of modification based on the color used. An example of the colors listed for glycans is seen in the truncated legend on the right side.

coverage of EPO was 97.6% for both CID- and EAD-derived data. While both gave the same coverage, differences were seen in the identification of glycopeptides with EAD, showing more identifications all around for glycopeptides than its CID counterpart, which is in alignment with previous results on other types of proteins.[4]

N-linked glycosylations

EPO is known to have 3 major N-linked glycan sites: N24, N38 and N83. These sites host multiple different glycan structures, and it has been suggested in literature that some of these glycans are phosphorylated/sulphated. Additionally, O-acetylation is making identification even more complex. A comparison of the EAD and CID data in Biologics Explorer software showed significant differences between the identification of these glycopeptides and their potential for assignment of the glycans and their exact location. An example of the EAD data from N24 is given in Figure 6. Despite the low abundance of this glycopeptide, the *z*7 and *c*4 ions clearly confirm the localization of the glycosylation, with *c*4 carrying the intact modification on the fragment while *z*7 contains no modification. While the gentle fragmentation with EAD leaves most of the precursor intact (green peak in Figure 6), it can also keep dissociation-fragile modifications intact on the fragments, providing localization information. CID, on the other hand, usually cannot provide this level of information.

Selecting an *m*/*z* of interest in the MS/MS spectra pulls up information on the peak, including the ppm error, volume, type of ion, the sequence it conforms to and the modification being associated with it. This allows for the quick confirmation and review of the fragments of interest, especially around modifications, and increases confidence in the correct assignment.

For positions N38 and N83, data were scrutinized more closely due to the proximity of other asparagines to the expected location of the modification. Peptide assignments were reviewed and only accepted if there was clear evidence that the glycan was identified at the specific site. If there were no fragments supporting the localization, data were rejected, resulting in a robust list of identified glycans. While the software was able to confirm some of the glycans on these 2 sites based on the CID data, the identification was limited due to limited fragmentation of the peptide backbone. This impacts confidence in the correct assignment and especially in the verification of the localization. Since CID usually results in the dissociation of glycans from the peptide backbone, data are rarely comprehensive enough to provide information on the exact location of the modification. In this case, mostly oxonium ions are seen. In addition, the fragment coverage of such peptides is impacted with CID since the peptide backbone does not get effectively dissociated.

As mentioned, some of the glycans on EPO contain O-methylation or acetylation, which helps to improve the stability of the biotherapeutic in plasma.[3] However, this creates greater complexity for the analysis by increasing the search space.

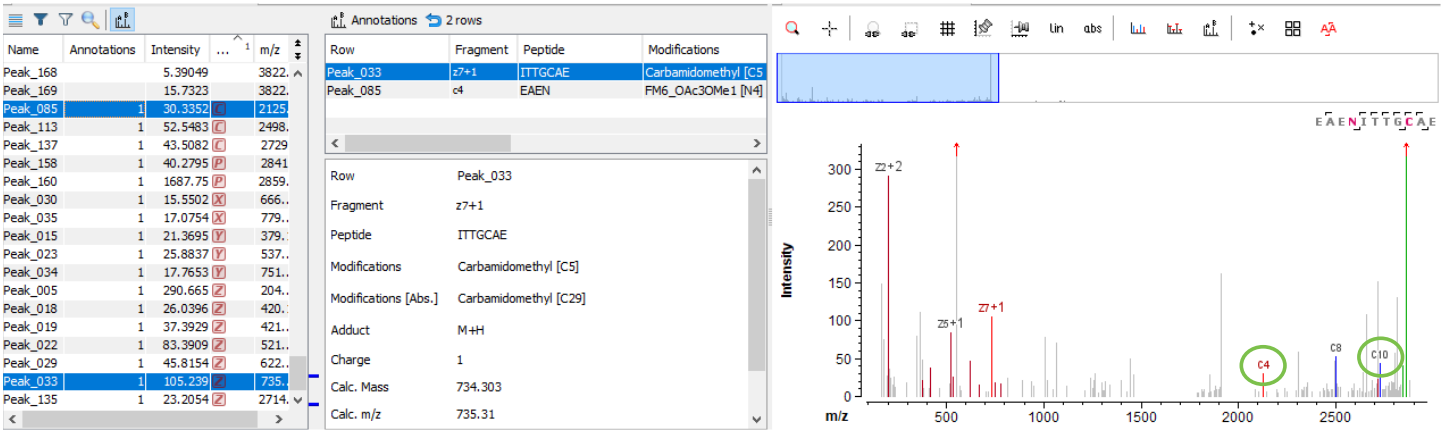

Figure 6. EAD results for N24-linked glycan FM6_OAc3OMe1 on peptide EAENITTGCAE. Left: Fragment table with identified signals. Right: MS/MS spectra of peptide with N24-linked glycosylation. Identified peaks are highlighted in red/blue and shown as an overview for the sequence (upper-right corner). The soft fragmentation with EAD mainly results in the intact precursor (green peak), but also leaves dissociation-fragile modifications, such as glycosylations, intact on fragments, allowing for exact localization (encircled ions). This mechanism is especially suited for identifying the location of glycosylations on peptides of all charge states.

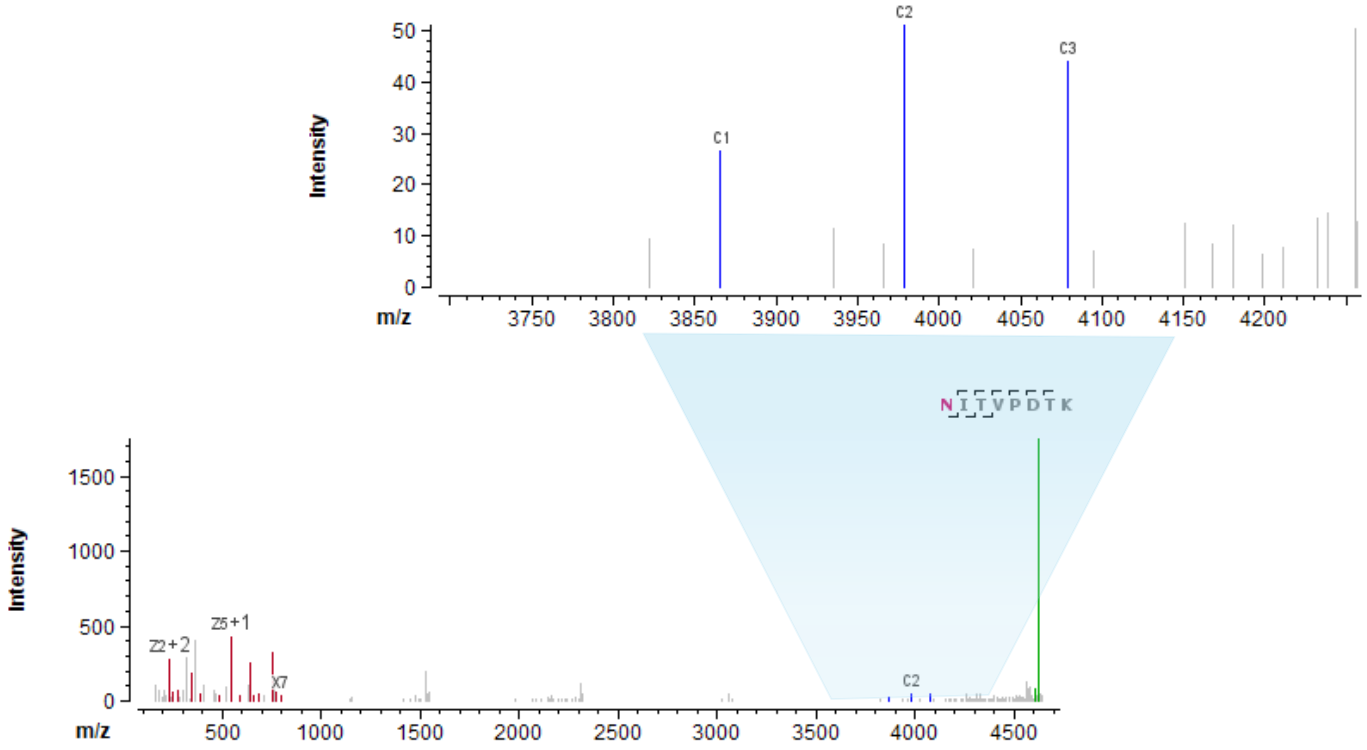

Figure 7. Assignment of F1A4G4S4_OAc1OMe2 on position N38. Top spectrum shows zoomed-in *m/z* region with *c*-ions containing the intact glycosylation, providing evidence of the presence on N with *c*1.

However, the software supports the input of the glycans for a user-defined library creation, or the use of a premade library with the selection of additional modifications that might occur on the glycans, such as methylation and acetylation. This offers significant flexibility while minimizing the manual workload of building a library of modified glycans from scratch. A library of EPO glycans was created as the base, but methyl and acetate were added as potential modifications into the search (Figure 2). As a result, around 70% more glycopeptides could be identified (see the example in Figure 7).

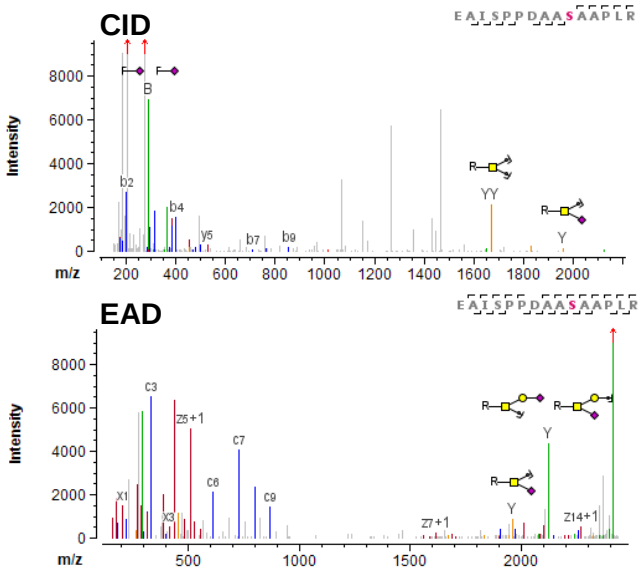

Figure 8. S126 Core1_S1 O-linked glycan on peptide EAISPPDAASAAPLR. Top: CID data lacking fragment information on the serine S126. Bottom: EAD data providing fragment information on *c*8, *c*9 and *c*10 encompassing the glycan on both sides of the amino acid sequence.

O-linked glycosylations

While EPO has 3 sites for N-linked glycans, it only has 1 site for O-linked glycans and minimal glycans associated with the site. However, the peptide containing the expected site of O-linked modifications contains 2 serines as potential sites of attachment. Both EAD and CID data provided MS/MS data for the specific peptide, but the data from CID show very little information around the glycan itself (Figure 8, top). The CID data did not unambiguously identify the site of attachment. EAD showed richer information with more fragments across the entire peptide with better signal-to-noise (S/N) than CID. Most importantly, EAD provided MS/MS confirmation for the presence of the glycan at position S126, while position S120 could be excluded (Figure 8, bottom). Facilitating the review of complex glycopeptides, Biologics Explorer software labels all *b*-/*y*-ions and *c*-/*z*-ions (red and blue), as well as peptides with fragments of glycans (yellow) and oxonium ions (yellow), to give a clear representation of the information. The data can be viewed in the spectra or listed in a fragment ion table that shows the list of identified versus unidentified fragments.

In addition, the percentage of a modified peptide is calculated automatically, including the creation of a list of all identified modifications. This allows for quick and easy sorting and filtering, facilitating data review. Despite gaps in the identification of modifications from CID data, both data sets (CID and EAD) showed consistency in terms of modification percentages (Figure 9). EAD is capable of not only providing additional information, but also providing it fast enough to ensure enough data points across peaks, allowing for relative quantification. The gaps in CID identification can be balanced out based on the EAD data as well since Biologics Explorer software will look at both data sets to assign peaks. If a sample/data file contains MS/MS information that helps with confirmation, it will use this to assign the *m*/*z* to the same peak in another sample/data file, ensuring that all available MS/MS data from all samples/data files are processed together for assigning peaks and modifications. This can significantly reduce the need for rerunning any samples.

Location	Modification	20210525 EPO micro 0.5 ug CID_3 [%]	20210525 EPO micro 1ug EAD_2 [%]
S126	Core1	0.44512	0.389338
S126	Core1_S1	0.214441	0.222724
S126	Core1_S2	52.6365	53.377
S126	S1_Core1	15.7895	17.4875

Figure 9. Percentages of O-glycans at position S126. Values show consistent results for EAD- and CID-derived data.

Reviewing and saving snapshots

Once data are processed, the peptides can be reviewed in the Review Results activity node (Figure 2). Peptides, their modifications and their associated raw data can be investigated and either accepted or rejected by a user. The user can also add comments to entries. Users can quickly accept all peptides of interest and reject all others with the click of a button. Highlighting multiple peptides at once also allows for quick acceptance by accepting all highlighted peptides. Any calculations within the software will be done using only the accepted peptides. The final results can be filtered to show only accepted peptides to simplify the view (Figure 10). The reviewed data can then be saved as a snapshot to enable viewing or editing at a later point in time (Figure 2).

Reporting and exporting to SCIEX OS software

Within the Review Results step, all data can be accepted, or a limited set, such as a list of critical quality attributes (CQAs) of interest, can be set for acceptance while all others are rejected. In either case, the data can then be used to create a compliant-ready method for attribute monitoring in SCIEX OS software by using the Export to SCIEX OS activity node (Figure 2). This node requires the user to set the number of charge states to be exported, as well as whether to use the monoisotope or the most intense isotope for export. Once configured, the node processes all accepted data from the Review Results step to create a table of peptides that can be directly imported into SCIEX OS

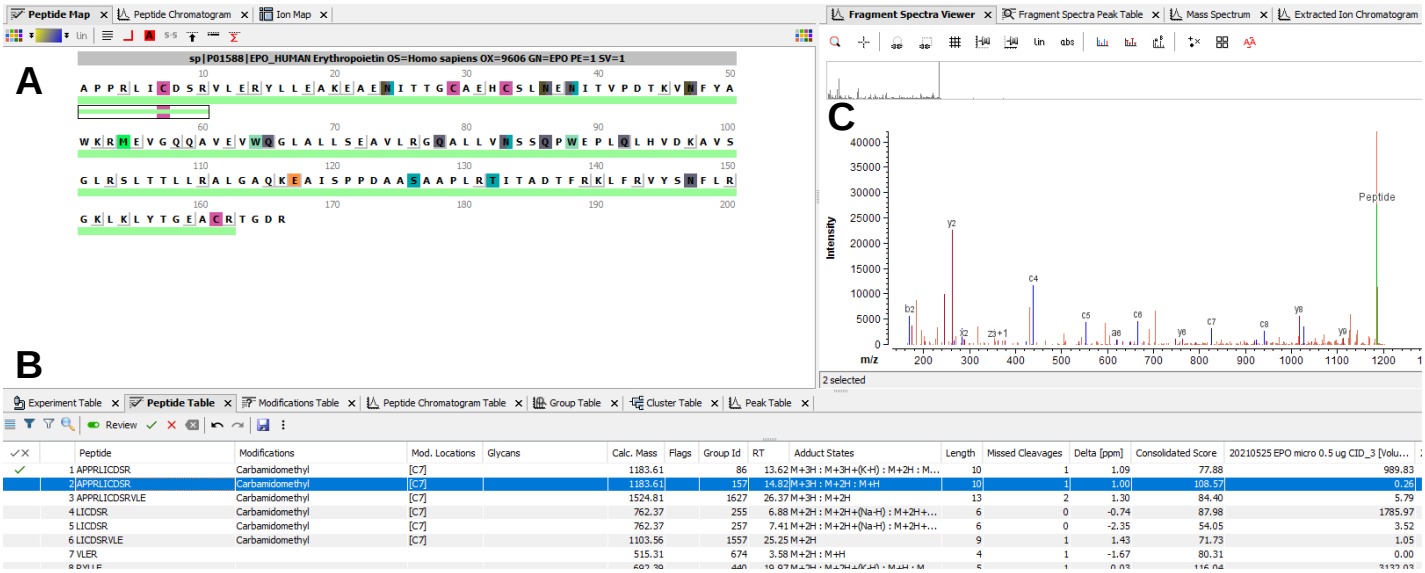

Figure 10. Reviewing pane for peptides. Sequence coverage map showing identified sequence parts and modifications highlighted with color coding that indicates the type of modification (A). Identified peptides are shown in table format (B). Selecting a particular peptide shows the MS/MS information (C). The parts are interactive to facilitate data review.

software. Any peptides or attributes exported can then be monitored and quantified in a compliant-ready environment.

After completing the sample processing, a report can be easily created with the push of a button. This opens up the Export PDF Report activity node, which allows the user to export the results into a PDF document. Complete control on the depth of information on (setting up, processing and results) that can be sent to the PDF file is offered. A template .xml file, that represents the optimized processing method, can be also attached to the PDF report, allowing for sharing of workflows among users.

Conclusions

- Faster time to answers through:
  - Intuitive workflow templates for peptide mapping tailored to various needs for simple, deep or comparative processing
  - Powerful visualization tools that facilitate workflow optimization and data reviewing
  - Proven algorithms for baseline subtraction and pre-processing to ensure that low-level analytes are not lost
- Flexibility to fit different needs with:
  - Highly customizable generation of PDF reports with embedded Excel spreadsheets of data
  - Snapshots of data at various points throughout the workflow that can be reviewed later
- Broad compatibility enabled by:
  - Compatibility with WIFF and WIFF2 data formats
  - Intuitive annotation of SCIEX CID and EAD MS/MS data for peptide mapping
  - The ability to export into the analytics module of SCIEX OS software, with attributes easily set and exported for monitoring

References

1. Evaluation of biotherapeutic sequence coverage using electron activated dissociation (EAD). SCIEX technical note RUO-MKT-02-12920-B.
2. Baba, T. et al. Electron capture dissociation in a branched radio-frequency ion trap. *Anal. Chem.* 2015, 87, 1, 785–792.
3. Baba, T. et al. Dissociation of biomolecules by an intense low-energy electron beam in a high sensitivity time-of-flight mass spectrometer. *J. Am. Soc. Mass Spectrom.* 2021, 32, 8, 1964–1975.
4. A new electron activated dissociation (EAD) approach for comprehensive glycopeptide analysis of therapeutic proteins. SCIEX technical note RUO-MKT-02-12980-A.
5. Thomson, R.I. et al. Analysis of three epoetin alpha products by LC and LC-MS indicates differences in glycosylation critical quality attributes, including sialic acid content. *Anal. Chem.* 2017, 89, 12, 6455–6462.
6. Falck, D. et al. Affinity purification of erythropoietin from cell culture supernatant combined with MALDI-TOF-MS analysis of erythropoietin *N*-glycosylation. *Sci Rep* 2017, 7, 5324.
7. Yang, Q. et al. Glycan remodeling of human erythropoietin (EPO) through combined mammalian cell engineering and chemoenzymatic transglycosylation. *ACS Chem. Biol.* 2017, 12, 6, 1665–1673.
8. Kim, U. et al. Sensitive and comprehensive analysis of *O*-glycosylation in biotherapeutics: a case study of novel erythropoiesis stimulating protein. *Bioanalysis* 2017, 9, 18, 1373–1383.
9. Intuitive software workflow for intact and subunit analysis of monoclonal antibodies (mAbs). SCIEX technical note RUO-MKT-02-13505-A.

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