# TopDownApp: An open and modular platform for analysis and visualisation of top-down proteomics data

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## Abstract

Although Top-down (TD) proteomics techniques, aimed at the analysis of intact proteins and proteoforms, are becoming increasingly popular, efforts are needed at different levels to generalise its adoption. In this context, there are numerous improvements that are possible in the area of open science including the FAIR (Findability, Accessibility, Interoperability and Reusability) data principles. These include e.g. increased data sharing practices and availability of tailored open data standards. Additionally, the field would benefit from the development of open analysis workflows that can enable e.g. data reuse of public datasets, something that is increasingly common in other proteomics fields. We present an open and modular platform for the analysis and visualisation of TD proteomics data called TopDownApp. It can be used as a flexible analysis platform, through the use of a common workflow engine, common data formats for input/output, and software containerisation. It can also serve as a tool for visual inspection through its simple setup. As a key point, it can also be used as a development platform for new tools through the use of Python, a modular design, software containerisation and common data formats. TopDownApp is open source and freely available at: https://github.com/mwalzer/TopDownApp.

## Technical Brief

TopDownApp: An open and modular platform for analysis and visualisation of top-down proteomics data

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## Abstract

Although Top-down (TD) proteomics techniques, aimed at the analysis of intact proteins and proteoforms, are becoming increasingly popular, efforts are needed at different levels to generalise its adoption. In this context, there are numerous improvements that are possible in the area of open science including the FAIR (Findability, Accessibility, Interoperability and Reusability) data principles. These include e.g. increased data sharing practices and availability of tailored open data standards. Additionally, the field would benefit from

the development of open analysis workflows that can enable e.g. data reuse of public datasets, something that is increasingly common in other proteomics fields.

We present an open and modular platform for the analysis and visualisation of TD proteomics data called TopDownApp. It can be used as a flexible analysis platform, through the use of a common workflow engine, common data formats for input/output, and software containerisation. It can also serve as a tool for visual inspection through its simple setup. As a key point, it can also be used as a development platform for new tools through the use of Python, a modular design, software containerisation and common data formats. TopDownApp is open source and freely available at: *https: // github. com/ mwalzer/ TopDownApp* .

**Main Text**

Proteomics techniques are increasingly tackling the identification and quantification of intact proteoforms, via the so-called top-down (TD) proteomics mass spectrometry (MS)-based approaches, in addition to the more widely used 'bottom-up' proteomics analysis. Proteoforms are the distinct molecular *species* of an expressed and translated gene including the precise combination of sequence variants, alternative splicing events, post-translational proteolysis (e.g. signal peptide cleavage) and post-translational modifications (PTMs) [1].

The increasing popularity of open science practices in proteomics has resulted in the dramatic growth of publicly available MS-based proteomics datasets. The PRIDE database (*https://www.ebi.ac.uk/pride/*) [2] is the largest proteomics data repository worldwide and is one of the founding members of the ProteomeXchange consortium [3]. As of May 2023, PRIDE stores approximately 34,000 datasets. ProteomeXchange resources adopt the FAIR data principles (Findability, Accessibility, Interoperability and Reusability) [4]. One of the main benefits of making data publicly available is to enable data reuse and the reproducibility of the analysis, facilitating an independent assessment of the results described in the corresponding publications. Additionally, as is already happening in other proteomics fields, new knowledge and tools are being generated from data reuse activities, for instance by applying machine learning techniques [5]. Furthermore, in the context of data interoperability, it is important to highlight that ProteomeXchange resources implement the main open data standards developed by the Proteomics Standards Initiative [6], such as mzML (for MS data) [7] and mzTab (for peptide/protein identification and quantification) [8,9].

However, in the context of open science practices, TD proteomics lags behind others, more widely implemented, proteomics fields. For instance, the total number of submitted TD proteomics datasets to PRIDE is still small (approximately 231 datasets, of which are still 50 private, as of May 2023), although the trend is changing slowly. In our view, this is due to two reasons. On one hand, the number of TD proteomics practitioners is still relatively low when compared with bottom-up approaches. On the other hand and more importantly, TD datasets are not being made available at the same pace as in other proteomics fields. Furthermore, to increase data reuse of TD proteomics data, it is important to develop open software for enabling computational data (re-)analysis and visualisation. Indeed, advances in topics such as data integration and the reproducibility of the computational analyses, including scaling-up analysis capabilities for increasingly large experiments, are key to match the developments in other proteomics fields, and in the life sciences as a whole [10].

We here introduce an open, modular and flexible platform called TopDownApp for the analysis and visualisation of TD proteomics data, which can be applied for instance to public datasets. Since the TD data analysis methodology is still quite dynamic, a flexible and modular approach to data analysis was necessary. This is why TopDownApp was implemented using automated and modular analysis workflows – i.e. a flexible succession of tools, inputs/outputs connected through open data standard formats (as interoperability layer) and a modular choice of tools (through software containerisation) through Nextflow [11]. In the context of TD data analysis, currently, the most common and generally successive tasks are: MS raw file access, deconvolution and proteoform identification.
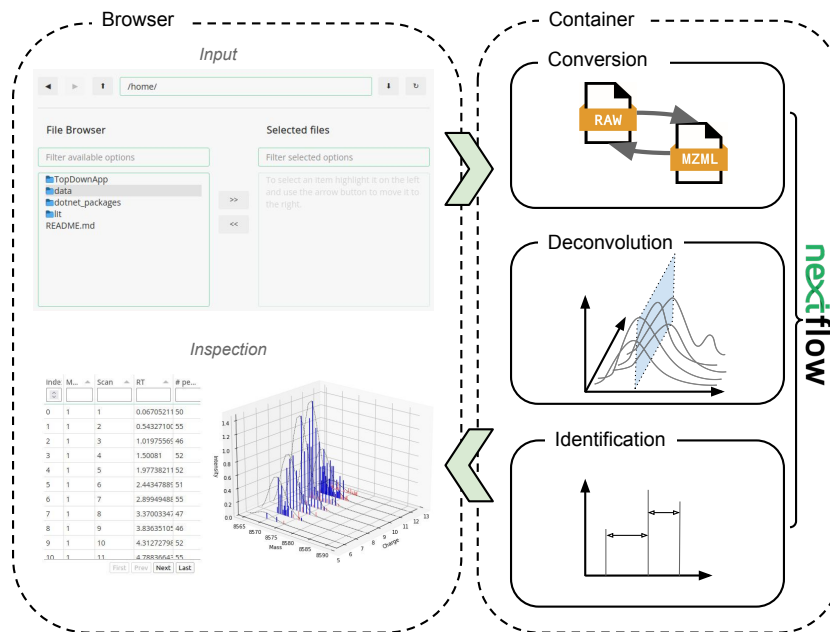
Figure 1: **Figure 1.** Overview of the use (left panel) and the processes (right panels) for TopDownApp. Local input selected through the browser (of *.RAW* files and a *.fasta* sequence database) is processed via Nextflow and dedicated tool software containers. The workflow is then started through the browser, and after processing the results can be inspected in the browser. The Nextflow workflows can also be used independently to process large batches of data.

Additionally, workflows that already support the deconvolution visualisation, can be used through a user interface (UI), as shown in Fig.1. The workflow results are reported in multiple formats, the tool native output, and of note, the mzML and mzTab format adapted for TD data. Table 1 includes the tools and the corresponding versions that are part of TopDownApp at the time of writing.

| Tool Name | Tool Use | Tool Version |
|---|---|---|
| https://sciwheel.com/work/citation?ids=8113952&pre=&suf=&sa=0 | Conversion from Thermo Raw files to mzML | v1.4 |
| https://sciwheel.com/work/citation?ids=9929122&pre=&suf=&sa=0 | Deconvolution | v3-pre.51bcc6e |
| https://sciwheel.com/work/citation?ids=12172363&pre=&suf=&sa=0 | Deconvolution & Identification | v1.5 |
| https://sciwheel.com/work/citation?ids=3552253&pre=&suf=&sa=0 | Workflow management system | v22.1 |
| https://sciwheel.com/work/citation?ids=14763476&pre=&suf=&sa=0 | Graphical User Interface | v0.14 |
| https://sciwheel.com/work/citation?ids=8519389&pre=&suf=&sa=0 | Computational access to mzML | v4.5 |

Table 1: Tools in use with the current version (May 2023) of TopDownApp. See the code repository for details about the most recent versions (if applicable).

Tool Name Tool Use Tool Version ThermoRawFileParser [12] Conversion from Thermo Raw files to mzML v1.4.2 FLASHDeconv [13] Deconvolution v3-pre.51bcc6e TopPIC-suite [14] Deconvolution & Identification v1.5.3 Nextflow [11] Workflow management system v22.10.4 Panel (Python) [15] Graphical User Interface

v0.14.4 Pyteomics [16] Computational access to mzML v4.5.6 Table 1: Tools in use with the current version (May 2023) of TopDownApp. See the code repository for details about the most recent versions (if applicable). Table 1: Tools in use with the current version (May 2023) of TopDownApp. See the code repository for details about the most recent versions (if applicable). Table 1: Tools in use with the current version (May 2023) of TopDownApp. See the code repository for details about the most recent versions (if applicable).

TopDownApp is open source and freely available under an Apache 2.0 licence to allow for free use and software development. The source code can be found at *https://github.com/mwalzer/TopDownApp*. The application is designed to work on local machines as well as in remote settings. Yet, the framework can be used for development of scaled-up and/or automated use cases, since the different parts are functional on their own or in combination. For example, the workflow can be used to automate the analysis, in particular on research compute infrastructures, which frequently support the use of Singularity containers [17]. All tools used in the workflow have to be containerised and can be specified via a customisable configuration file. Note however that the workflow needs to reflect potential tool command and parameter changes first, and that data hand-over compatibility needs to be ensured (preferably via the use of mzML and mzTab).

In addition to the containerisation of the tools, the processes of running the workflow and the data visualisation are containerised as well. As a result, the minimal setup for a user is the container system (Singularity/Docker) and a combined container (see Supplementary Material). The application can be started via a single command and then used through a web browser (see Supplementary Material and the code repository at *https://github.com/mwalzer/TopDownApp* for more details). Here, a local MS raw file and a protein sequence database (in fasta format) can be selected as input, and the protein modification parameters can also be set. Currently, the user interface allows to select a number of standard protein modifications and combinations thereof. From there, the analysis workflow can be started, and the results can be inspected once the data analysis process is finished. The successfully deconvolved spectra can be selected for visualisation against their original form from a table (see Supplementary Figure S2). Within each spectrum, each peak can be selected for deconvolution visualisation. Identified spectra are listed in a table, from which one can also select the corresponding spectra to be visualised. New visualisations can be developed using a Python notebook and added to the user interface (see Supplementary Material). The identification results are also available to download in the form of a development version of the mzTab standard format for TD proteomics data (see Supplementary Material).

The mzTab format was chosen because it has proven to be effective for representing identification data in other quickly developing specialisations of MS technologies, including metabolomics and lipidomics [8]. Furthermore, the format adaptations necessary for TD data are minor, and we expect a straightforward process to establish a community agreed extension of mzTab. Likewise, the representation of TD peak data in mzML is already supported by the data standard. However for complete compatibility, the deconvolution data needs to be represented and formalised in a format extension, for which we developed a functional proposal here. We used a TD spectrum reporting convention that attaches deconvolution information such as charge and isotope target as the userParam section of the spectrum schema representation and an implicit $m/z$ array value reinterpretation as the mass. This is compatible with the current release of the widely used Python library for mzML consumption (and other PSI data standards), Pyteomics [16] and many other mzML capable software libraries. For now, only FLASHDeconv supports the deconvolution specific userParam section in mzML output, and thus visualisation of deconvolution is exclusive to FLASHDeconv. For both mzML and mzTab, input from the community will be needed for both to become accepted data standards for representing TD data. For details on the format specification extensions, see the Supplementary Material.

We demonstrate the utility of TopDownApp with two datasets, the first to showcase examples of visualisation, and the second to provide a re-assessment of a previously published human dataset. The first is an *E. coli* lysate, measured using a Thermo Scientific Orbitrap Eclipse (MassIVE dataset accession MSV000087484) [18]. There, we used an *E. coli* (strain: K12 MG1655 i) proteome database from UniProtKB/SwissProt (canonical; release 2023_01), and selected one modification (Oxidation [unimod:35]). The workflow configuration was FLASHDeconv for deconvolution and TopPIC for identification. We used the UI to visualise

the precursor deconvolutions. Figure 2 shows how TopDownApp can be used to examine the signal quality of the precursors corresponding to the identified proteoforms, which is crucial information for the quality control of identification [19] [20]. Figure 2 (left and right panels) show visualisation examples of high and low quality precursor signals, respectively. For each precursor mass, TopDownApp shows all its differently charged isotope packets in the input raw spectrum (blue colour coded peaks) as well as noisy peaks around them (red peaks). Users can easily distinguish signal and noise components by colour and do not need to search for separate $m/z$ value regions to observe peaks with different charges. In this way, TopDownApp organises the precursor information from different places in the spectrum for easy appraisal.
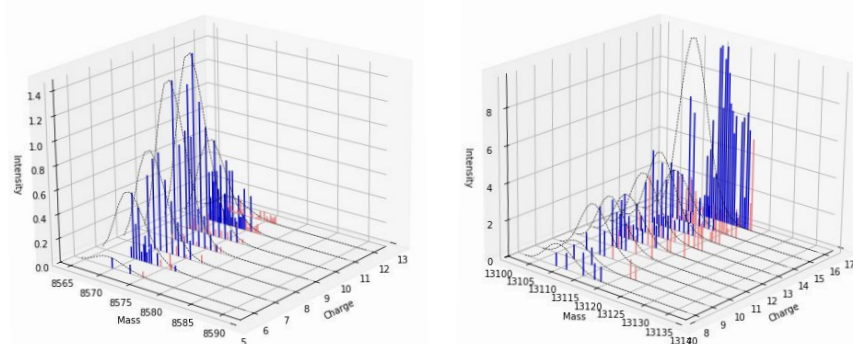


Figure 2: **Figure 2.** Example of a successful and unsatisfactory deconvolution process from the analysis of the E.coli dataset. **Left Panel:** Deconvolution of scan 8557, monoisotopic peak mass 8564.733, exemplifying a successful deconvolution with blue peaks for target peaks, pink peaks declared noise, and dashed curve showing a good approximation of the isotope traces. **Right Panel:** deconvolution of a poor quality signal in scan 8722, monoisotopic peak mass 13101.864.

A second benchmarking dataset included erythrocyte data from the human Blood Proteoform Atlas: concretely, datasets *PXD026123* (DS-01: 35 LC-MS/MS runs) and *PXD026159* (DS-53: 6 LC-MS/MS runs). We applied a minimal modification setting (Oxidation [unimod:35], Methylation [unimod:34]) and used a human sequence database from UniProtKB/SwissProt (canonical; release 2023_01), applying a spectrum-level and proteoform-level FDR (False Discovery Rate) cutoff of 1%.

Table 2 shows the analysis results using different tools for identification, split by identified proteins (accessions), number of truncated sequences for PrSM with or without PTMs, number of proteoforms, and PrSMs. The samples of PXD026123 were subject to an enrichment strategy, explaining the overall greater number of identifications as opposed to PXD026159. Switching from TopMG identification software to TopPIC also noticeably affects the number of observed identifications. This can be explained considering the different goals of the identification analysis for the two. TopMG is designed "for identifying highly modified proteoforms", whereas TopPIC is built for the characterisation of proteoforms at the proteome level [21]. This is further corroborated by comparing the overlap of accessions (75 of 209(TopPIC)/101(TopMG) in PXD026123 and 7 of 55/24 in PXD026159) and overlap of proteoform sequences (126 of max. 4826 unique PrSM sequences and 50 of max. 3085 unique PrSM sequences) for both methods (for details see Supplementary Figure S1).

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| Data Set | Deconvolution | Search Engine | Protein Accessions | Trunca-tions | Proteo-forms | PrSMs |
|---|---|---|---|---|---|---|
| PXD026123 | FLASHDe-conv | TopMG | 102 | 228 | 303 | 1477 |
| | FLASHDe-conv | TopPIC | 209 | 731 | 1799 | 7476 |
| PXD026159 | FLASHDe-conv | TopMG | 24 | 94 | 108 | 472 |
| | FLASHDe-conv | TopPIC | 55 | 303 | 801 | 4408 |

Table 2: Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259.

Data Set Deconvolution Search Engine Protein Accessions Truncations Proteoforms PrSMs PXD026123 FLASHDeconv TopMG 102 228 303 1477 FLASHDeconv TopPIC 209 731 1799 7476 PXD026159 FLASHDe-conv TopMG 24 94 108 472 FLASHDeconv TopPIC 55 303 801 4408 Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259. Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259. Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259. Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259. Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259. Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259. Table 2. Results from the batch application of the default workflow setting (FLASHDeconv + TopPIC) in TopdownApp plus an additional workflow setting using (FLASHDeconv + TopMG) for datasets PXD026123 and PXD0261259.

All reanalysed datasets have been made available via PRIDE datasets including input, output, and intermediate output of the Nextflow workflow, as datasets PXD042651 and PXD042650, respectively.

Our TopDownApp is free and open to use, and can be used on a local computer or institutional server-setup, installation as simple as downloading and running the containerised app. However, unlike other computing environments for TD data processing like TDPortal or TopPIC Gateway [22,23], the workflows themselves are not reconfigurable via GUI. Instead, high-throughput analysis can be conducted on a huge variety of compute infrastructures through application of the underlying nextflows. Changing the sequence or the type of tools in the workflow needs to be coded in nextflow. As a beneficial side effect of this, adding new tools into the workflow can be simply achieved by the user with a few lines of configuration change for any software if it can be called from the command line and has compatible in-/output. Another distinction is the direct integration of results visualisation as main component of the UI of TopDownApp.

In the future, the TopDownApp could be enhanced with additional deconvolution and identification software modules, such as MSPathFinderT [24], to further increase the software options for data analysis. Moreover, the inclusion of a dedicated label-free quantification module, such as FLASHQuant, would be ideal to enable quantitative analysis. To ensure robust and reproducible results, adopting additional control strategies at different levels including proteins, protein isoforms, and proteoforms, would be highly beneficial. Currently,

parameter configuration through the browser is not fully implemented due to the wide variety of supported parameters. Adoption of other PSI standard data formats, such as the ProForma 2.0 notation [25] for proteoform presentation, would improve data interoperability. Additionally, we expect that the availability of an open data analysis workflow will enable the reuse and reanalysis of TD proteomics datasets in the public domain. This would open many possibilities such as the integration of the results in popular bioinformatics resources such as PRIDE, UniProtKB [26] and the Human Proteoform Atlas [27], making TD and proteoform data more FAIR. Furthermore, in our view, the availability of open source analysis platforms will be essential to the success of the envisioned Human Proteoform Project [28].

We expect that the availability of TopDownApp as an open and shared development platform will aid the reproducibility of TD data analysis, providing a basis for new, automated, and easy to share TD data analysis workflows. Further, we envision an improved accessibility to deconvolution and identification data. As we demonstrated one method of visualisation of TD identification results, we hope this to serve as a basis for more analysis results to be made (visually) accessible to current practitioners. In the medium-long term we expect that this and analogous efforts can improve the integration of proteoform data in bioinformatics resources so that all biomedical researchers can benefit from the adoption of open science practices in the TD proteomics field.

## Acknowledgements

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