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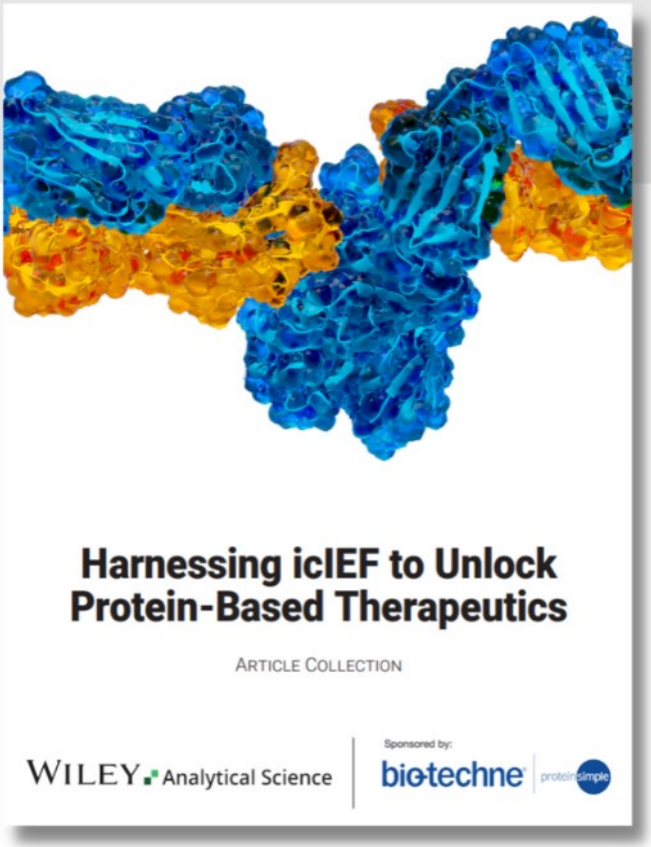


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TECHNICAL BRIEF

# Workflows for automated downstream data analysis and visualization in large-scale computational mass spectrometry

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MS-based proteomics and metabolomics are rapidly evolving research fields driven by the development of novel instruments, experimental approaches, and analysis methods. Monolithic analysis tools perform well on single tasks but lack the flexibility to cope with the constantly changing requirements and experimental setups. Workflow systems, which combine small processing tools into complex analysis pipelines, allow custom-tailored and flexible data-processing workflows that can be published or shared with collaborators. In this article, we present the integration of established tools for computational MS from the open-source software framework OpenMS into the workflow engine Konstanz Information Miner (KNIME) for the analysis of large datasets and production of high-quality visualizations. We provide example workflows to demonstrate combined data processing and visualization for three diverse tasks in computational MS: isobaric mass tag based quantitation in complex experimental setups, label-free quantitation and identification of metabolites, and quality control for proteomics experiments.

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Research in proteomics and metabolomics is mainly driven by MS coupled to LC (LC–MS). Running long gradients and acquisition of high-resolution mass spectra yield an enormous amount of data that needs to be processed efficiently. Various instruments and experimental techniques are employed in current research in order to answer complex biological questions. This variety in experimental setups raises the need for highly flexible and efficient computational approaches to analyze experimental data. Workflow systems that combine small, reusable building blocks into larger, experiment-specific analysis workflows provide this flexibility.

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**Abbreviations: KNIME**, Konstanz Information Miner; **TOPP**, The OpenMS Proteomics Pipeline

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We demonstrate how the integration of OpenMS [1, 2] into the workflow system KNIME (Konstanz Information Miner) [3] significantly extends data processing capabilities allowing for sophisticated downstream analysis and visualization. OpenMS is an open-source software framework for computational MS. It provides more than 100 tools for signal processing, identification, and quantification including adapters to established search engines such as MASCOT [4], OMSSA [5], or X!Tandem [6]. OpenMS workflows can be constructed based on The OpenMS Proteomics Pipeline (TOPP) [1] in the graphical user interface TOPPAS (TOPP Assistant) [7]. Designed for fully automated processing, TOPPAS does not provide functionality for downstream analysis and only limited visualization capabilities. KNIME is an open-source integration platform providing a powerful and flexible workflow system combined with advanced data analytics, visualization, and reporting capabilities. KNIME integrates nodes for machine learning, statistical data analysis, and interfaces to various scripting languages, for example, the statistical programming language R. KNIME's functionality can be easily extended with nodes provided via an online plugin repository (the so-called KNIME extensions). As the execution of a KNIME workflow usually runs locally on a compute machine, it does not require extra IT security provisions beyond the usual steps on the operating system or file system level. For larger data, the execution can also be run on (often commercially) available cloud/cluster or server solutions, which meet today's security standards.

The integration of OpenMS into KNIME is based on the GenericWorkflowNodes project (https://github.com/genericworkflownodes) that generates KNIME nodes for any command line tool that provides an XML-based description of the tool interface. We extended OpenMS to automatically generate those XML files for each TOPP tool. In contrast to regular KNIME nodes, the OpenMS nodes expect files and not tables as input. To allow an interaction between the file-based OpenMS nodes and the regular KNIME nodes, we implemented a set of nodes to load the content of proteomics data files into KNIME tables. These nodes either use the OpenMS specific TextExporter format or the recently published mzTab [8] format as input.

Integrating OpenMS tools into KNIME enables the user to combine automated data processing of the raw MS data (signal processing, quantification, identification) with KNIME's data-mining and visualization capabilities in a single workflow, for example, by directly integrating the initial raw data analysis with well-known R packages for proteomics data analysis (e.g., isobar [9], see Example 1) or by utilizing the existing KNIME packages for cheminformatics (e.g., to visualize chemical structures of metabolites, see Example 2).

A KNIME/OpenMS workflow is composed of multiple nodes that are connected by ports. Ports represent single or multiple files that are passed from one tool to another. The number of incoming and outgoing ports depends on the individual tool, for example, a database search engine such as OMSSA will have two incoming ports, one for the file containing the spectra to be analyzed and one for the protein database to be searched. Nodes are added by drag and drop to the workbench and connected by drawing a line from the outgoing to the desired incoming port. For each generated connection between OpenMS nodes, the workflow engine will check if the file types are compatible, i.e., that only files of supported formats are given to a node. The parameters of nodes and their documentation are available via a configuration dialog. On execution, each node checks if the incoming data meets all requirements, for example, the required amount of input files, and the workflow engine will abort the execution with a meaningful error message if requirements are not met.

Workflows are very rarely so simple that they contain only a linear sequence of nodes to process a single file. Therefore, additional nodes are provided to construct more complex workflows including loops and merge nodes. Loops allow applying the same series of nodes to multiple input files one at a time. Merge nodes allow combining two files into a list that can be given to nodes that require more than one input file.

To further structure a workflow, KNIME provides so-called Meta-nodes to group a collection of nodes. Grouping into Meta-nodes can be used to hide a very complex series of nodes and instead provide a high-level view on the data flow.

When the initial analysis steps are finished, one often wants to load the generated results into the KNIME environment, that is, represent them in the KNIME table structure. Using the OpenMS conversion nodes allows the user to load the file-based results of an OpenMS pipeline into KNIME tables. These tables can afterwards easily be processed using the hundreds of KNIME nodes or custom R code implemented in the KNIME R nodes.

KNIME can export complete, preconfigured workflows into self-contained ZIP files. Thus, workflows can easily be shared with collaborators, uploaded to web archives, or otherwise be made accessible to the scientific community.

Once configured, workflows can also be run from command line for fully automated batch processing on a large number of files. With minor extensions, using KNIME's flow-variable concept, one can also configure KNIME such that the input files or other variable parameters of the workflow can be set from the command line when executed in batch mode. Consequently, workflows can be configured and tested in a desktop environment, using small subsets of the original dataset, and subsequently be deployed to large computational infrastructures.

In the remainder of this article we will present a series of example workflows adapted from existing projects. Quantitative experiments are one of the most basic approaches in proteomics. Here we present a workflow for the analysis of a complex sample labeled with tandem mass tags [10].

Human lung adenocarcinoma epithelial cells (A549) were treated with nano-copper oxide (CuO) or left untreated (three

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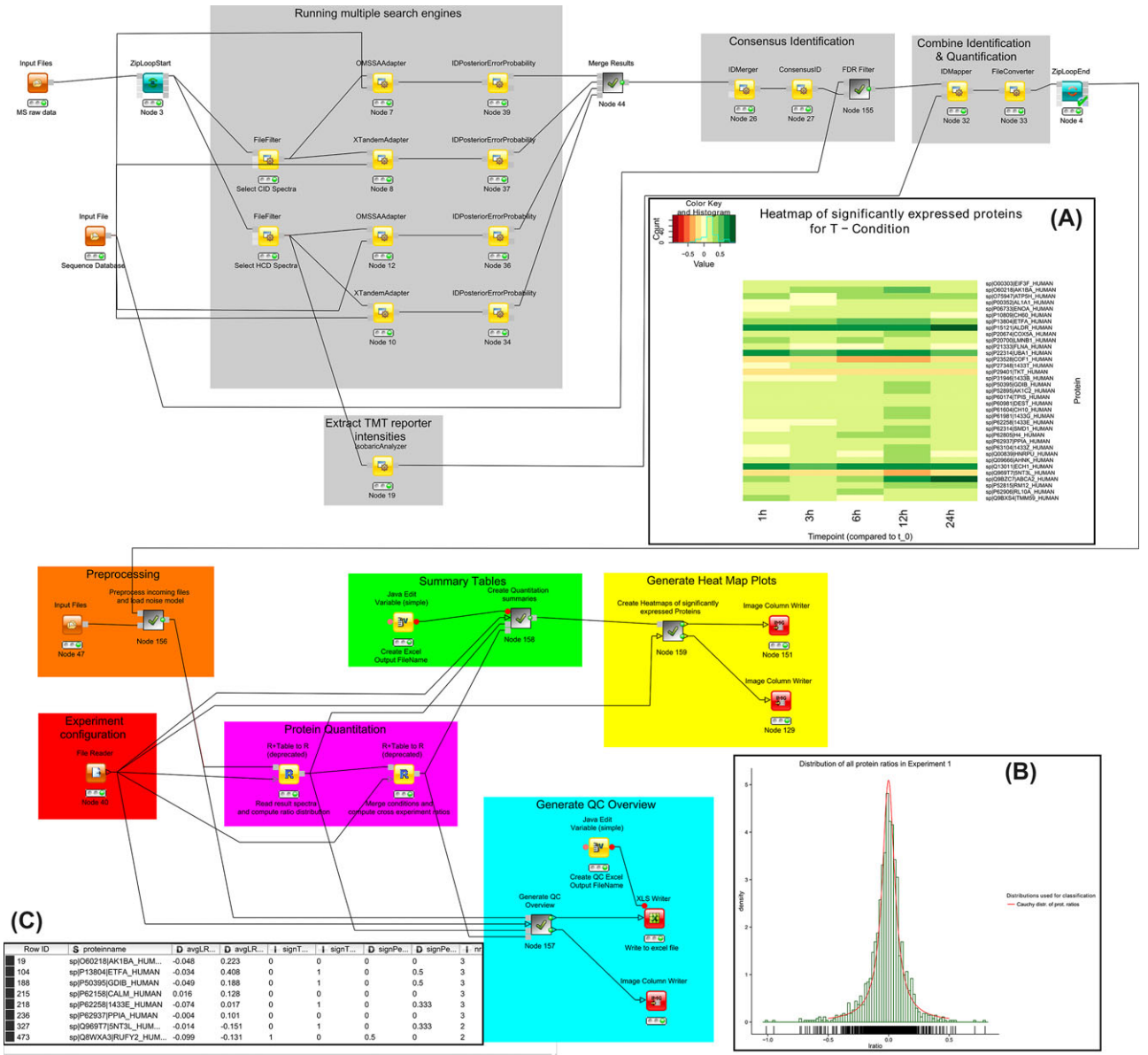


**Figure 1.** Tandem mass tags quantitation workflow performing reporter extraction, identification, and differential protein quantitation. Results are generated in form of images and Excel spreadsheets. (A) One of the heat maps generated by the workflow showing significantly expressed proteins in the treated condition, (B) the distribution of protein ratios for one of the biological replicates that can be used for quality control, and (C) small part of the final report table as it will be written to the resulting Excel file.

biological replicates). Cells were harvested at six different time points (0, 1, 3, 6, 12, and 24 h after exposure to CuO or medium only controls) and were subsequently measured and analyzed. For details on the experimental procedure, see Supporting Information.

The analysis workflow (see Fig. 1) performs peptide identification using multiple search engines in parallel and then combines those results first into a single identification, using the ConsensusID approach [11], and afterwards combines the identifications with the quantitative information extracted from the tandem mass tags reporter ions. The protein inference and protein quantitation are based on the R package isobar [9], which is integrated via the R KNIME nodes. The workflow generates multiple tables containing the identified proteins with their respective log ratios and *p*-values as well as informative plots that can be used directly in reports or manuscripts. Additionally, a separate file containing basic quality control information is generated.

Identification and quantitation of small molecules are basic approaches in metabolomics studies. In the second example, we present an ultraperformance LC–MS based, label-free quantitation workflow to exemplify the differential analysis

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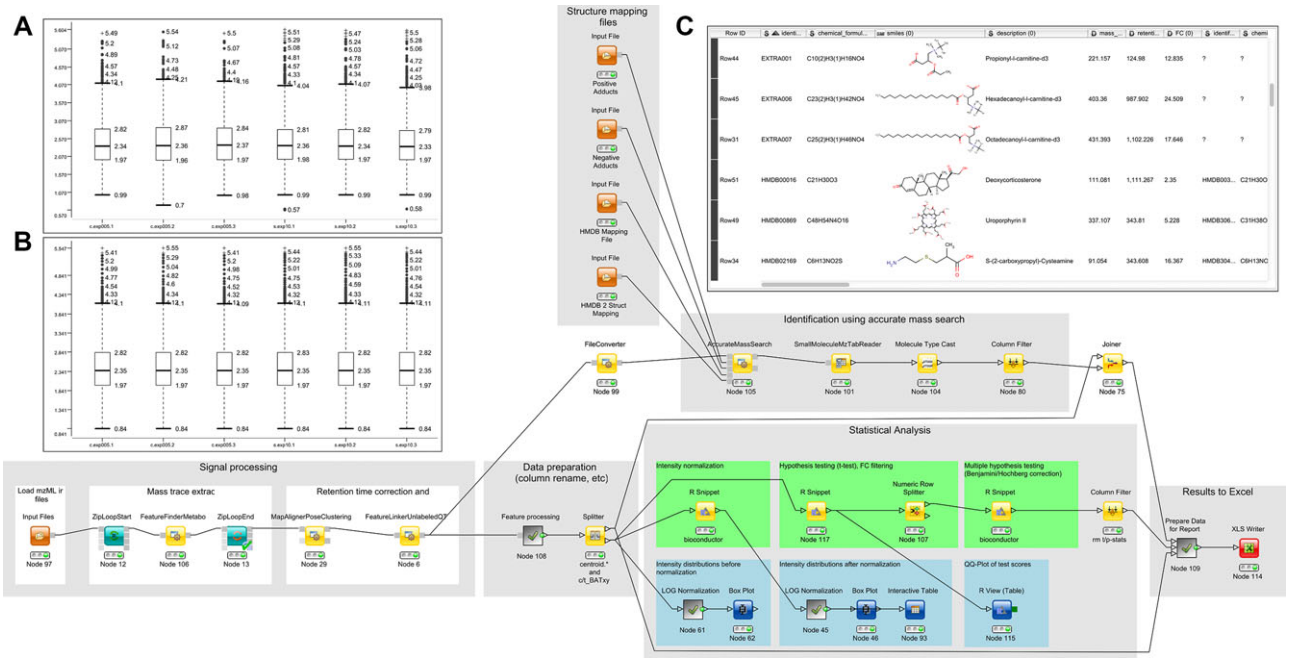


**Figure 2.** Workflow for label-free quantitation of metabolites. The insets show plots and tables generated by the workflow. The intensity distributions of the individual samples (A) before and (B) after normalization. (C) Summary table including the rendered chemical formulas for the identified and quantified metabolites.

of small molecules as typically used for biomarker discovery. Two spike-in conditions of a dilution series (male blood sample measured in triplicates, for details refer to [12]) have been processed by the analysis workflow (see Fig. 2). Eluting small molecules were detected by our recently published feature-detection approach [12]. Afterwards, interexperiment retention time shifts were corrected by map alignment [13] and subsequently features occurring at the same $m/z$ and retention time were linked between the different MS runs [14]. Identifications were assigned by accurate mass search against the HMDB database [15], which has been extended with the isotopically labeled spike-in compounds. Feature intensities

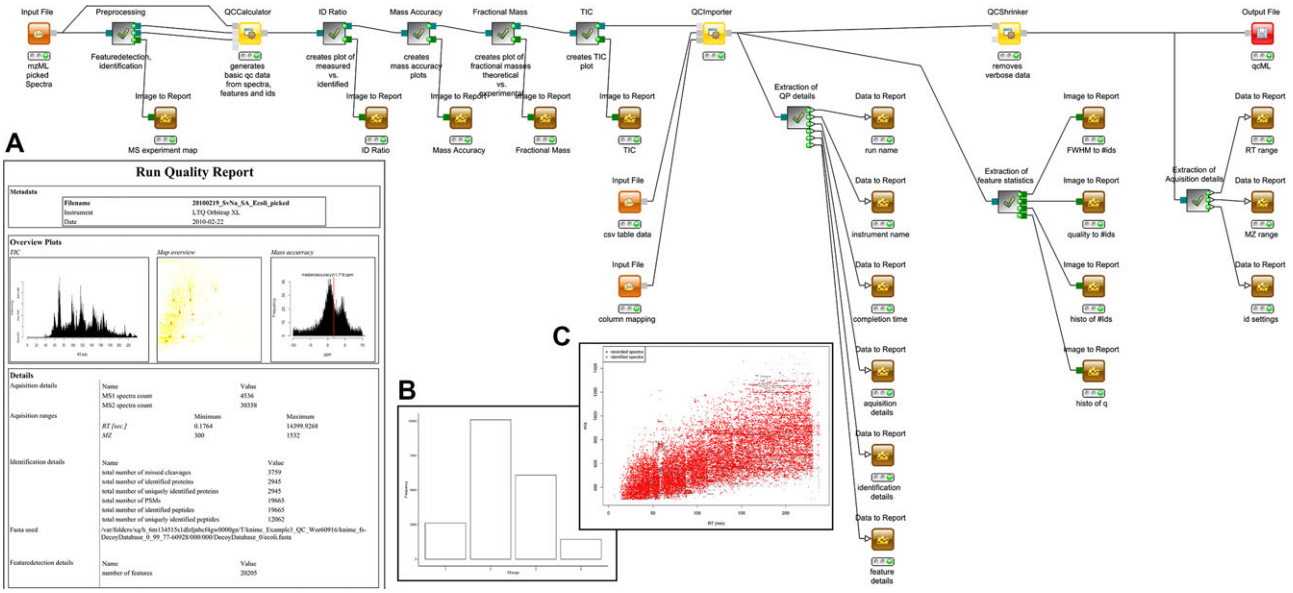


**Figure 3.** Workflow to compute and summarize a detailed quality report for an LC–MS/MS experiment. Insets (A–C) show parts of the generated quality report. (A) An overview of the analyzed file and details on the experiment, (B) charge distribution of found $MS^1$ features, and (C) a plot comparing found $MS^1$ features with peptides identified in the MS/MS spectra.

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were normalized using quantile normalization. Two-sided *t*-tests and a fold-change threshold of 2 were applied to determine differentially quantified compounds. *p*-Values have been corrected for FDR control using the Benjamini–Hochberg procedure. For reporting, identification and quantification results are joined in a table for manual inspection.

With the increasing amount of data produced in LC–MS/MS experiments, quality control has become a crucial aspect of the day-to-day usage of MS. Optimally, it should be easy to extend existing analysis workflows to also incorporate quality control. For this, qcML, a format for the representation, storage, and transfer of quality metrics has been developed [16]. The computation of the quality metrics, the generation of suitable plots, and integration in qcML can be achieved by extending existing OpenMS/KNIME workflows. Figure 3 shows the detailed QC workflow as it was presented previously [16]. It uses a combination of OpenMS nodes, R nodes, and the KNIME reporting functionality to generate a full quality control report. See reference [16] for more details on the format and the workflow.

The combination of OpenMS and KNIME allows users to utilize the numerous OpenMS tools in a user-friendly workflow environment in combination with the powerful analysis and visualization techniques provided by KNIME. With this unique combination, workflows for the analysis of MS data can easily be created, tested, and shared with collaborators and used in high-throughput scenarios. All example workflows presented in this article can be downloaded, including example data, from https://sourceforge.net/projects/open-ms/files/Papers/OpenMS_KNIME/.

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