MetaXtract: Extracting Metadata from Raw Files for FAIR Data Practices and Workflow Optimisation

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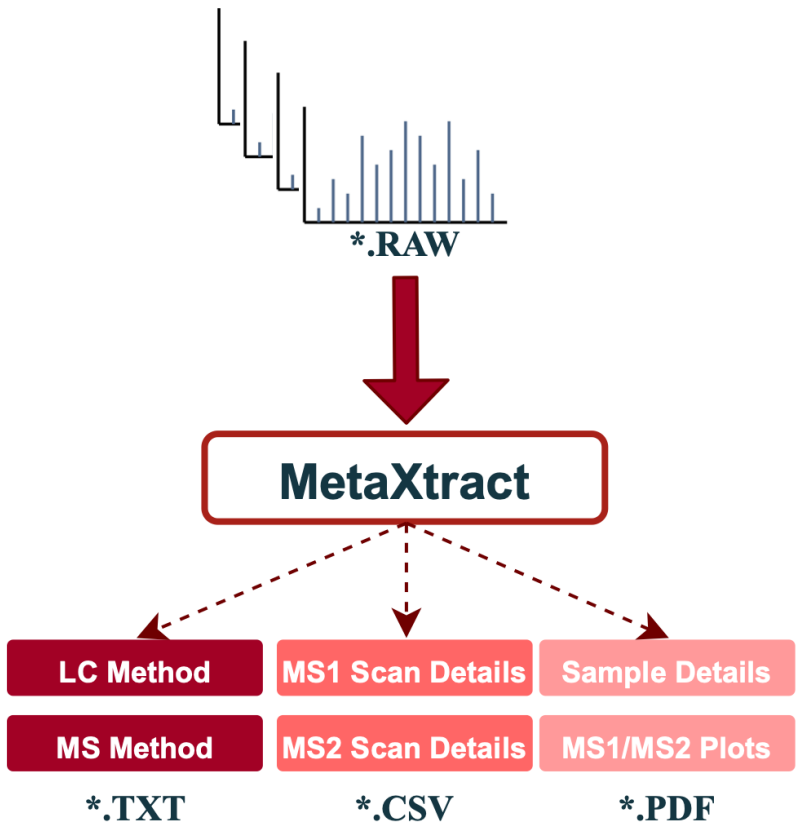
Graphical Abstract



Highlights

- Metadata extraction from Thermo Fisher *raw* files
- Enhanced findability, accessibility, and reusability of deposited data
- Integration into workflows via GUI and command-line modes
- Troubleshooting support by visualizing MS1/MS2 scan details

Abstract

Mass spectrometry (MS) experiments generate rich acquisition metadata that are essential for reproducibility, data sharing, and quality control (QC). Because these metadata are typically stored only in vendor-specific formats, they often remain difficult to access. MetaXtract is a lightweight tool that extracts detailed parameters directly from Thermo Fisher raw files and exposes them in structured, tabular formats. By capturing sample information, LC-MS method settings, and scan-level metrics such as retention time, total ion current, and ion injection time, MetaXtract increases transparency and ensures that essential acquisition details accompany published data and results in easy readable form. This supports FAIR data practices by improving the findability, accessibility, interoperability, and reusability of MS datasets, thereby increasing the value of deposition in public repositories. The importance of such metadata accessibility was recently highlighted by the crosslinking mass spectrometry community in efforts to advance FAIR data principles, and it extends to MS-based omics approaches more broadly. Beyond data sharing, the tool streamlines QC and troubleshooting through simple visualisations of MS1/MS2 scans and enables integration into automated pipelines. By embedding acquisition parameters into routine data handling, MetaXtract strengthens reproducibility, optimises method development, and supports large-scale applications, including machine learning and secondary data analysis.

Keywords
mass spectrometry, metadata extraction, quality control, proteomics, FAIR data principles, computational mass spectrometry, method optimization.

Availability
MetaXtract is available for free download as an executable file at [Rappsilber Laboratory GitHub repository](), the software is licensed under the Apache-2.0 license.

Abbreviations

CSV	Comma-Separated Values
MS	Mass Spectrometry
QC	Quality Control
LC	Liquid Chromatography
FAIR	Findable, Accessible, Interoperable, and Reusable
LIMS	Laboratory Information Management System

Introduction

Mass spectrometry (MS) generates large binary raw files containing both spectral data (MS1 and MS2) and essential acquisition metadata such as precursor charge states, retention times, ion injection times, and fragmentation energies (1). Although these parameters are critical for data interpretation, reproducibility, and quality control, they are difficult to access because vendor-specific formats typically require proprietary software. Existing vendor tools offer only limited flexibility for automated extraction, batch processing, or integration into modern bioinformatics pipelines. Consequently, researchers lack practical means to obtain comprehensive and structured metadata for QC, method optimisation, and large-scale computational workflows (2, 3), highlighting the need for more accessible and robust extraction solutions (1).

Recent community efforts to standardise crosslinking MS have highlighted the importance of complete and machine-readable metadata to achieve FAIR data practices and reproducibility. The Crosslinking MS Roadmap (4) emphasises that, while mzIdentML 1.3 provides a unified format for reporting identified crosslinks (5, 6), critical acquisition parameters such as LC-MS method settings and scan-level metrics remain largely inaccessible. Extracting these parameters directly from mass-spectrometric raw data is considered essential for automated repository submission and transparent provenance tracking. Addressing this community-defined need across proteomics demands practical tools capable of reliably capturing such metadata and providing it in structured, reusable formats.

Although several tools have been reported to offer access to some metadata, these existing tools present significant limitations (**Table 1**). RawMeat (7), developed by Vast Scientific, is no longer supported, making it impractical for long-term use. The MSQC software (8, 9), developed by the National Institute of Standards and Technology (NIST), facilitates QC by computing metrics from LC-MS/MS data, but it requires database search results for most metrics, limiting real-time instrument monitoring. RawBeans (10) lacks flexibility in selecting MS1/MS2 data and does not extract LC/MS method information, complicating method validation and troubleshooting. Its lack of updates also raises compatibility concerns with newer instruments. LogViewer (11) depends on log-files from RawXtract (*.ms2* and *.log* files), making it unsuitable for high-throughput workflows. It also does not extract method settings and is no longer available for download. SIMPATIQCO (12) has not been updated since 2015, cannot export selected metadata or QC metrics, and requires advanced user expertise for setup and use. Similarly, QuaMeter (13) is no longer accessible from its official source and lacks ongoing support, limiting its use in current environments. In general, these limitations point to the need for a modern and robust QC solution in proteomics: outdated software, lack of flexibility, missing method information, and download/accessibility issues.

To overcome the current limitations of QC tools, we developed MetaXtract, a lightweight Python-based solution that directly reads *raw* files using the Thermo Fisher RAWFileReader library (14). It offers comprehensive MS1 and MS2 visualizations, allowing users to explore key metrics like precursor intensity, ion injection time, charge states, and retention time distributions. MetaXtract provides customizable tabular output, enabling users to select specific parameters, making it ideal for machine learning (ML) and deep learning (DL) applications in proteomics. It also extracts sample information and LC/MS method details, ensuring quality control and reproducibility. With a user-friendly GUI, researchers can easily configure QC metrics without

advanced computational expertise. The tool can be integrated into workflow-based frameworks like Snakemake (15) and Nextflow (16) for efficient metadata extraction. MetaXtract is available for free download as an executable file at Rappsilber Laboratory GitHub repository.

Results

MetaXtract Interface and Visualization Features

MetaXtract provides a GUI and a CLI. The executable can be downloaded from GutHub and runs without installation and operating system admin rights being required, simplifying its use also in managed desktop or otherwise restricted work environments. The GUI offers an intuitive interface for browsing files, inspecting scan measurements, and generating plots interactively. The CLI, on the other hand, allows automation and scripting for large-scale batch processing. While the GUI aims at assisting individual scientists in their work, the CLI allows the construction of automated workflows in scaled projects. MetaXtract is designed primarily as a Windows-based application, but has been tested and confirmed to work on Linux systems using Wine (17), enabling cross-platform compatibility.

We manually validated that the metadata extracted by MataXtract matched the information within raw files by comparing our export with the information accessible in Freestyle version 1.7 (Thermo Fisher). **Figure 1** shows the interface of MetaXtract, which provides an interactive environment for exploring mass spectrometry data. The interface displays key scan parameters such as total ion current, retention time, base peak intensity, and injection time, allowing the user to select all or specific output values for each scan.

Workflow integration of MetaXtract to annotate PRIDE submissions

We provide a Snakemake-based workflow in the MetaXtract GitHub repository, fully open source and easily adaptable. It automatically downloads raw files from PRIDE (by year and file count) and runs our end-to-end annotation. We used this to download the last 20 raw files submitted to PRIDE in January 2025, spanning separate submissions from three different laboratories (PXD060351, PXD060271 and PXD060112), none being our own. The workflow extracted the full available set of metadata including instrument-, run-, and scan-level metadata (MS1/MS2 headers and method details) into structured TXT/CSV for straightforward reporting. MetaXtract averaged ~40 s per file on a Windows 11 laptop with 32 GB RAM and NVIDIA GeForce RTX™ 4070 with Intel® Core™ i9 i9-13900HX processor. While we here generated output files, the modular design of MetaXtract allows routing the outputs to downstream tools or LIMS with minimal changes to the workflow.

Application of MetaXtract for Systematic MS Performance Evaluation

We first demonstrated the use of MetaXtract to monitor mass spectrometer performance. We generated comprehensive visualizations of MS1 and MS2 metadata from four datasets of 50 ng HeLa Protein Digest Standard, acquired for quality control purposes. In the two reference runs, 5,055 and 5,843 peptides were identified, whereas only 31 and 5 peptides were identified in the two datasets acquired on a different day, indicating instrument underperformance. The “file-based details” showed a similar total number of MS1 and MS2 scans across both the reference runs (Ref 1, Ref 2) and the underperforming runs (UP 1, UP 2), though the UP runs had a slightly lower proportion of MS2 scans (56% and 59% versus 60% and 62% in the reference

runs) (**Figure S1**). Comparable total ion chromatograms (TICs) of MS1 signals suggest that chromatographic separation was not significantly impaired in the UP runs. TIC values for all MS1 scans showed on average 8.4% lower median MS1 signal intensity in the UP runs (**Figure 2a**). While this reduction may have contributed to fewer MS2 triggers, it alone is unlikely to account for the dramatic loss in peptide identifications. However, plotting the TIC values for all MS2 scans revealed a 9.2-fold reduction in signal intensity in the UP runs (**Figure 2b**). This significantly lower MS2 signal is very likely the primary reason for the loss of peptide identifications. Given the normal performance of MS1 scans, the issue is most likely due to a malfunction during precursor ion isolation or fragment ion transmission.
Beyond metadata, MetaXtract exposes additional run-level parameters useful for routine MS performance tracking and acquisition tuning, illustrated in Supplementary **Figures S2-S5**. These metadata can be used in conjunction with downstream identification data, but are also informative on their own. For example, precursor charge state distributions can help monitor digestion efficiency, while plotting MS2 injection times can guide the optimization of maximum ion injection time settings.

Conclusion

MetaXtract is a lightweight, user-friendly tool for the extraction and visualization of mass spectrometry metadata from Thermo Fisher *raw* files. With support for both graphical and command-line interfaces, it offers flexibility for researchers performing manual quality control as well as those integrating it into automated workflows. The command-line functionality allows for simple integration into workflow management systems such as SnakeMake or Nextflow, making it adaptable for high-throughput proteomics pipelines. By combining structured metadata extraction with interactive visualizations of MS1 and MS2 scans and potentially contributing this analysis alongside search results, MetaXtract provides intuitive data exploration, helping researchers optimize acquisition methods and troubleshoot experimental problems. The ability to process multiple *raw* files in a single session makes MetaXtract a scalable solution for both small-scale method development and large-scale computational proteomics applications and reinforcing data quality across the field. In future versions, we plan to expand support to additional vendor file formats and integrate MetaXtract as a dedicated node within Proteome Discoverer, further enhancing its utility in diverse proteomics workflows.

Materials and Methods

MetaXtract is a Python-based mass spectrometry data processing tool (19) designed to support both graphical (built with *PySide6* (20)) and command-line usage. It enables reading, parsing, and visualizing Thermo *raw* files by leveraging the Thermo Fisher RawFileReader library (14), which provides direct access to MS1/MS2 scan data, sample metadata, and instrument parameters. The tool adopts a modular architecture, separating data extraction, processing, and visualization into distinct components for improved maintainability and easier integration of future features. The core pipeline includes: (1) *raw* file parsing via the *RawFileReader* C# library, (2) data transformation into structured formats (TXT, CSV, PDF), and (3) visualization of mass spectrometry plots using Matplotlib (21). The demonstration dataset was acquired using Pierce™ HeLa Protein Digest Standard, 50 ng of Hela digested was used as a quality control

(QC) sample was injected for each LC-MS/MS analysis. The LC-MS/MS analysis was performed using a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) connected to an Ultimate 3000 RSLCnano system (Dionex, Thermo Fisher Scientific). Peptides were resuspended in a solution containing 1.6% v/v acetonitrile and 0.1% v/v formic acid, then injected onto a 50-cm EASY-Spray C18 LC column (Thermo Scientific) operating at 50°C column temperature. The mobile phase consisted of water with 0.1% v/v formic acid (mobile phase A) and 80% v/v acetonitrile with 0.1% v/v formic acid (mobile phase B), with peptides loaded and separated at a flow rate of 0.25 µl/min. A gradient was applied, starting with 2% to 9% B over 10 minutes, increasing to 31% B over 30 minutes, further to 44% B over 5 minutes, and finally to 95% B over 2 minutes. Eluted peptides were ionized by an EASY-Spray source (Thermo Scientific) and introduced directly into the mass spectrometer, where MS data was acquired in data-dependent mode. The full scan mass spectrum was recorded with a resolution of 60,000. In each acquisition cycle, the ten most intense ions with a charge state from 2+ to 6+ were isolated and fragmented using higher-energy collisional dissociation (HCD) with a normalized collision energy of 27%. The fragmentation spectra were recorded with a resolution of 15,000. Dynamic exclusion was enabled with a single repetition count and a duration of exclusion of 30 seconds. The data used in this study was acquired on two separate days (19/12/2024 and 20/02/2025, two replicates per date). Protein identification was carried out using MaxQuant (version 2.6.2.0), searching against the human protein sequence database downloaded from UniProt, with the default search parameters.

Acknowledgements

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Tables

Tool	Metadata Extraction (MS1/MS2 scan-level)	LC/MS Method	Visualisation	GUI/CLI	FAIR/Export Formats
RawMeat (7)	Limited	No	Basic static plots	GUI	Proprietary text
MSQC (8, 9)	Yes (search-dependent)	Limited	QC metric plots	CLI	Metrics reports
RawBeans (10)	Limited	No	Basic plots	GUI	.CSV
LogViewer (11)	MS2/log-derived	No	Log summaries	GUI	Text logs
SIMPATIQCO (12)	Yes	Limited	Report-style	Server-based	Reports
QuaMeter (13)	Yes	Limited	None	CLI	.CSV
MetaXtract	Yes	Yes	MS1/MS2 & Multi-sample	GUI & CLI	.CSV, .TXT, .PDF

Table 1. Comparative features relevant to QC, metadata extraction, and FAIR export in LC-MS/MS tools, including MetaXtract. *GUI: Graphical User Interface; CLI: Command Line Interface.*

Figures

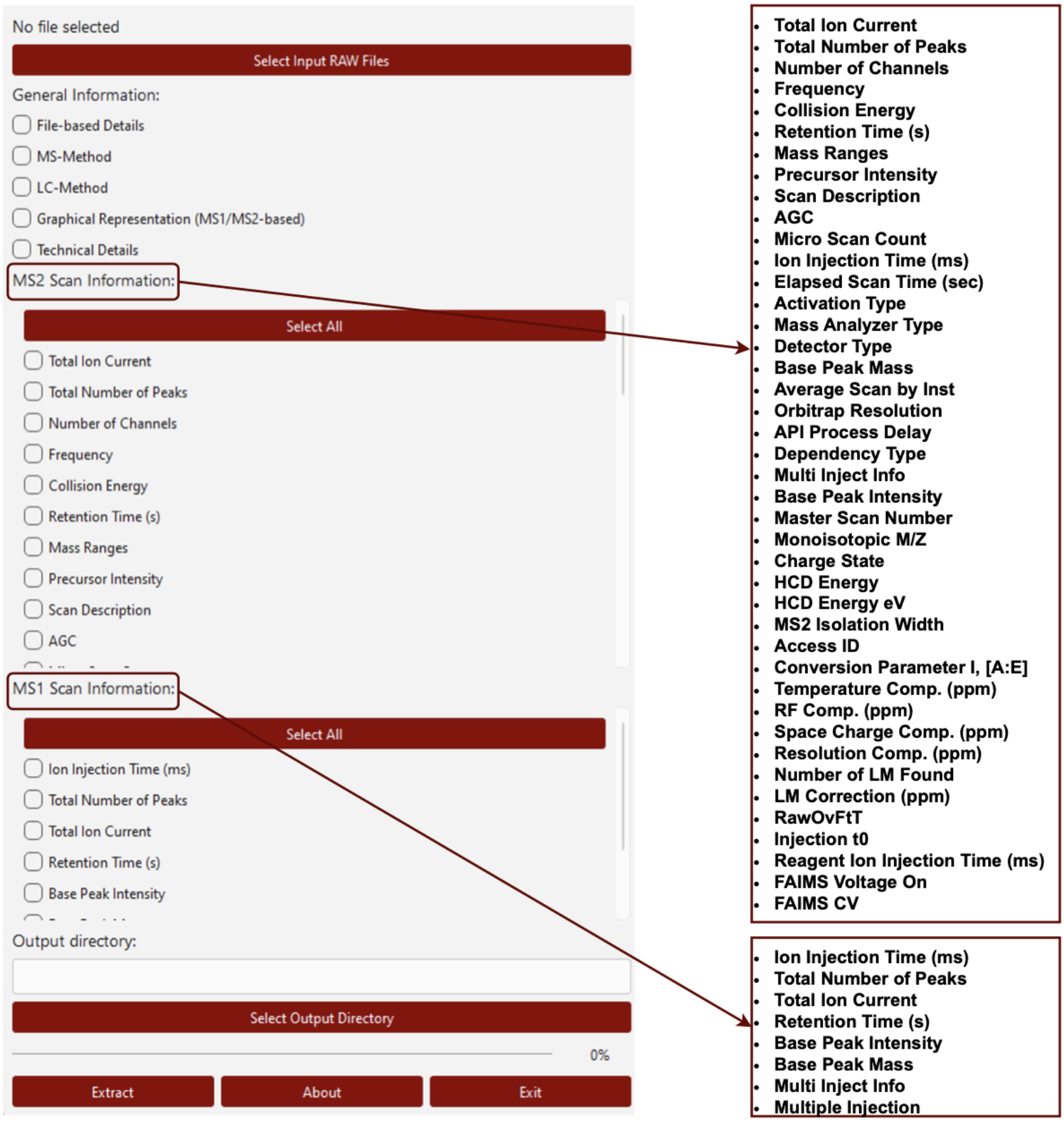

Figure 1. Graphical User Interface (GUI) of MetaXtract. The interface enables users to select specific mass spectrometry parameters for extraction and visualization. Parameters are grouped into MS1 and MS2 scan information.

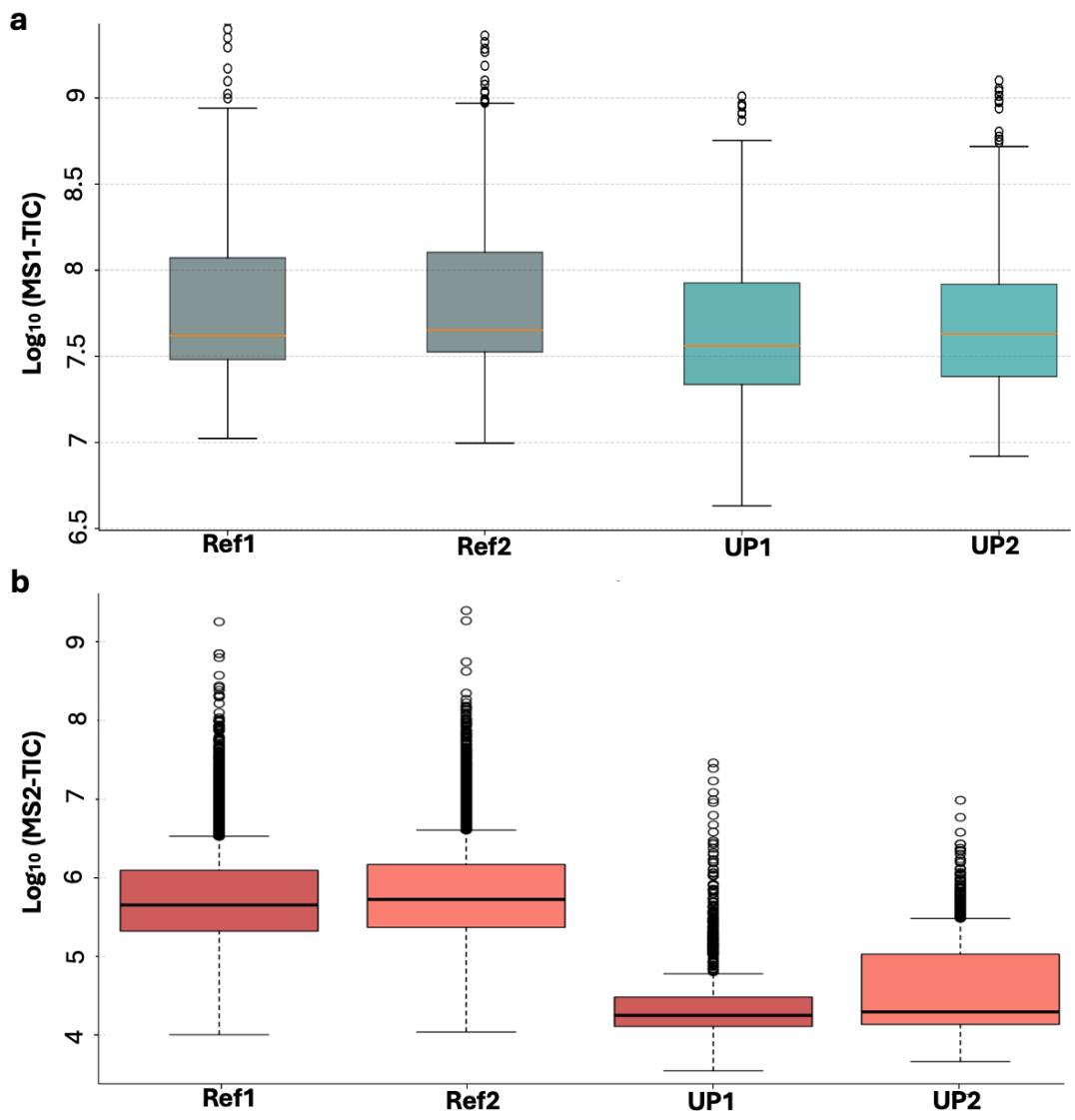

Figure 2. MS1/MS2 total ion current (TIC) across reference and underperforming runs.

(a) Boxplots of per-scan MS1 TIC reveal lower overall values in the underperforming datasets (UP 1, UP 2) compared with the references (Ref 1, Ref 2). **(b)** Boxplots of per-scan MS2 TIC show a pronounced decrease in UP 2 relative to the references and UP 1, indicating substantial MS2 signal loss and suggesting compromised data quality. *(TIC, total ion current; Ref, reference; UP, underperforming.)*