CROWN BIOSCIENCE

Next Generation Ion Mobility Mass Spectrometry-based Proteomics Services

4D-DIA Quantitative Proteomics and 4D Phosphoproteomics

A JSR Life Sciences Company

FACTSHEET

Next Generation Ion Mobility Mass Spectrometry-based Proteomics Services Overview

Crown Bioscience, in partnership with leading third-party proteomics providers, offers next generation ion mobility-mass spectrometry (IM-MS)-based-proteomics services to global clients.

This will allow clients to assess the biological function of proteins for understanding signalling mechanisms within cells as well as specific biomarkers to diseases, and systematically assess quantitative differences in protein profiles of distinct samples for biomedical and clinical research.

Services Offered

- 4D-DIA quantitative proteomics
- 4D phosphoproteomics
- Bundle package (4D-DIA Proteomics + 4D Phosphoproteomics)

Applications

- Global proteomics profiling of cells or tissues, with/without treatment
- Proteomics biomarker discovery and validation
- Drug mechanism of action and toxicity studies
- Disease mechanism studies
- Target identification and validation
- Complementary analysis and correlation approaches for other omics analysis

Our Advantages

- Better reproducibility: no missing values, reduced batch effect, better data parallelism, and traceability
- Increased sensitivity, wider dynamic range, and improved detection depth: identify more proteins from same or lower amount of sample, significantly improved quantification especially for low abundant proteins
- Lower amount of samples required
- Suitable for large sample cohort studies
- Customized bioinformatic analysis available for large cohort studies

Factors to Consider When Choosing Mass Spectrometry-based Proteomics Services

Liquid chromatography (LC) coupled with MS has become the gold standard in various omics fields. When choosing proteomics services, there are several factors to consider:

- Data completeness and reproducibility
- Sensitivity and identification depth:
  - Number of identified and quantified proteins
  - Low abundant protein identification and quantification
  - Sample amount requirement
- Speed, throughput, robustness and cost
- Data analysis

4D-DIA Quantitative Proteomics: A Combination of 4D Proteomics and DIA Technology

A new generation of 4D-DIA proteomics technology (**Figure 1**) combines 4D proteomics, which added ion mobility as the fourth separation dimension to traditional LC-MS/MS (retention time, mass-to-charge ratio (m/z) and MS/MS fingerprint), with data independent acquisition (DIA) strategy, which avoids data imbalance caused by randomness by realizing "lossless acquisition" of all possible data.

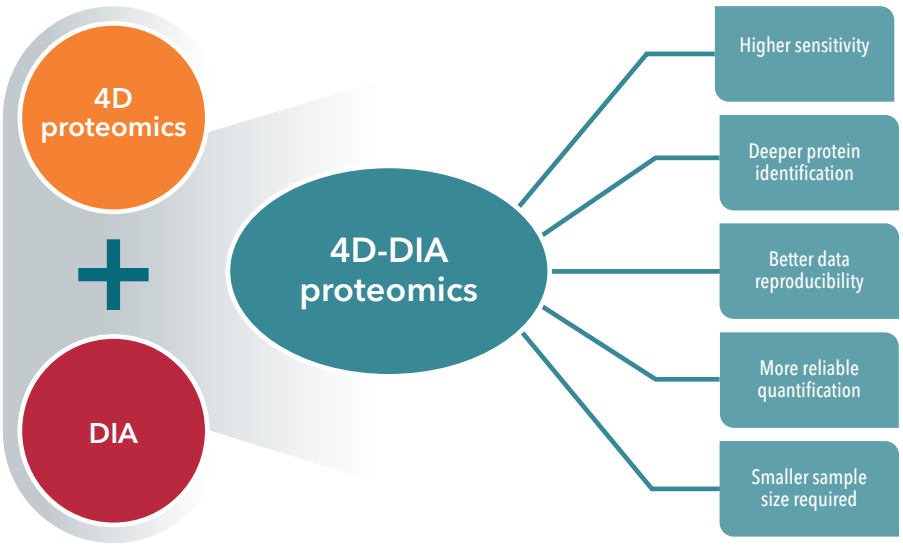


Figure 1: 4D-DIA proteomics

Cutting-edge 4D Proteomics Technology

Classic LC-MS/MS-based bottom-up proteomics separate peptides based on 3 dimensions: chromatographic retention time (RT); m/z and ion intensity.

Ion mobility analysis is a gas-phase technique allowing the separation of ions based on their mobility through an inert gas (typically helium or nitrogen) under the influence of an electric field. Adding ion mobility as an additional dimension of separation for peptide ions to LC-MS/MS analysis has become increasingly popular for proteomics studies (**Figure 2**). It significantly improves the scanning speed and detection sensitivity, as well as enhances proteomic analysis performance in terms of identification depth, detection cycle, and quantitative accuracy.

Data Dependent / Independent Acquisition (DDA / DIA)

There are two data acquisition strategies in tandem mass spectrometry (MS/MS) data acquisition (**Figure 3**):

DDA mode: The mass spectrometer selects a fixed number of most intense precursor ions. They are then fragmented and analyzed, in the second stage of tandem mass spectrometry.

DIA mode: The mass spectrometer divides the full scan range of mass spectrometry into several windows and selects, fragments, and collects all ions in each window.

DIA strategy avoids data imbalance caused by randomness by realizing "lossless acquisition" of all possible data. However, the "all-acquisition" strategy produces a highly complex spectrogram that poses a great challenge to data analysis.

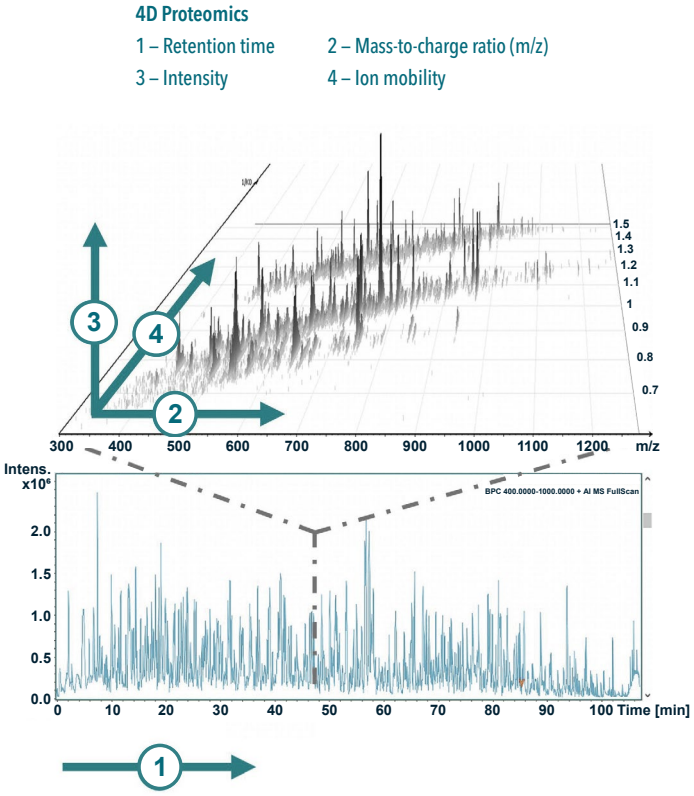


Figure 2: 4D proteomics

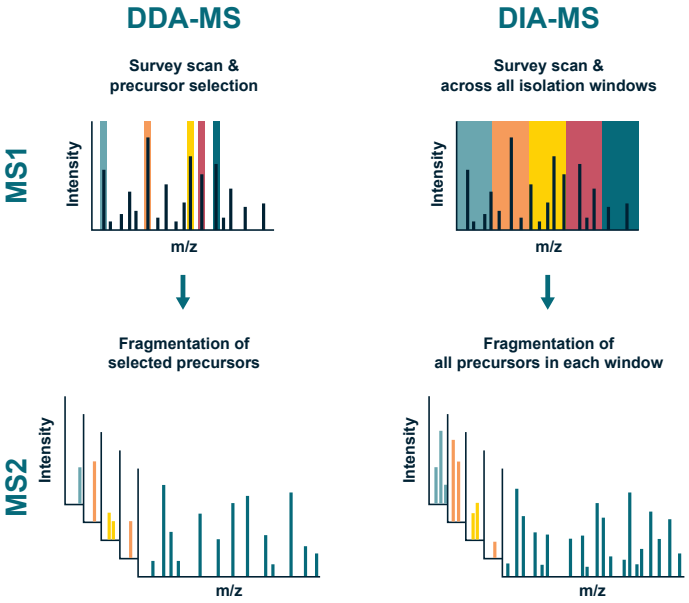


Figure 3: DDA and DIA data acquisition

Technical Strengths of 4D-DIA Quantitative Proteomics

4D Alignment for Better Identification

The greatest challenge in traditional DIA without ion mobility separation lies in the difficulty of reliably analyzing mixed spectra. During quantification, matching is mainly based on chromatographic elution time (retention time). However, due to co-elution, the retention time alone is not enough, and many other interfering signals will affect the detection accuracy.

4D technology provides ion-mobility separation and adds an extra dimension for calibration. It can accurately discriminate the specific peptide signals from the mixed spectra of DIA, effectively reducing spectra complexity and improving the detection accuracy and reliability of DIA (**Figure 4**).

Nearly 100% Ion Utilization, Maximized Detection Sensitivity

In the 4D-diaPASEF scanning mode, the ion-mobility-related CCS value correlates well with m/z. This feature allows the Quadrupole to scan gradually to collect nearly 100% of the ion signals, greatly enhancing the sensitivity and depth of detection (**Figure 5**).

In comparison, in the traditional LC-MS/MS-based proteomics without ion mobility separation, only limited signals can be collected.

Significant Improvement in Detection Depth

4D-DIA identifies and quantifies low-abundant proteins more accurately than traditional methods, thereby increasing the depth of proteomics detection (**Figure 6**).

Better quantitative integrity

4D-DIA technology can further push the limits of sensitivity with advanced instrument performance and upgraded acquisition methods. Even at this trace level, 4D-DIA showed 85% data integrity for protein quantification (**Figure 7**).

4D-DIA Technology Effectively Reduces the Complexity of the Spectrum

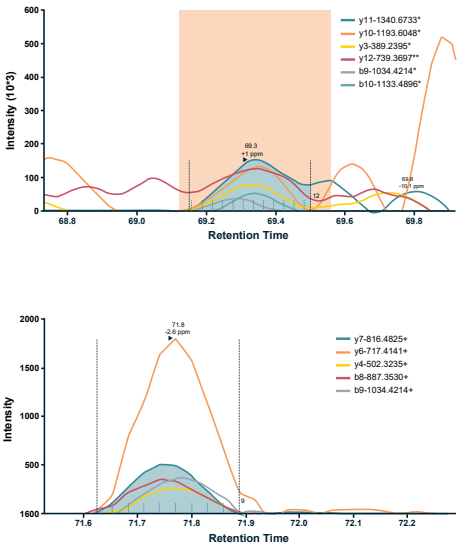


Figure 4: The chromatograms of EVGSHFDDFVTNLIEK peptide after calibration in traditional DIA (top) and 4D-DIA (bottom). There is a high interference signal in the traditional DIA method (top), while the signal background in the 4D-DIA (bottom) is much cleaner.

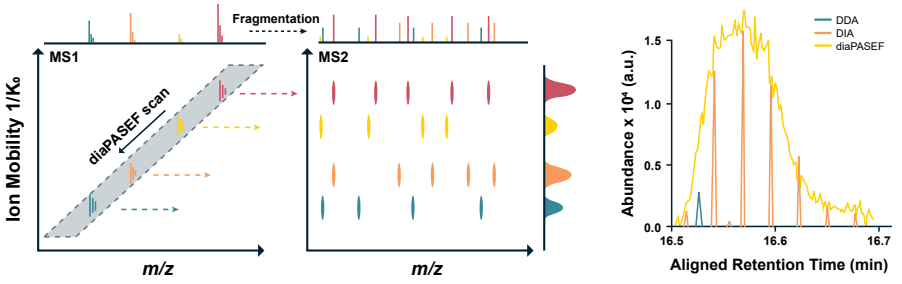


Figure 5: 4D-DIA based on the 4D platform can reach nearly 100% ion utilization. Left–Data acquisition in 4D-DIA mode; Right–Comparison of ion utilization among 4D-DIA, DDA, and DIA. (Source: Florian Meier, et al. Nature Methods.)

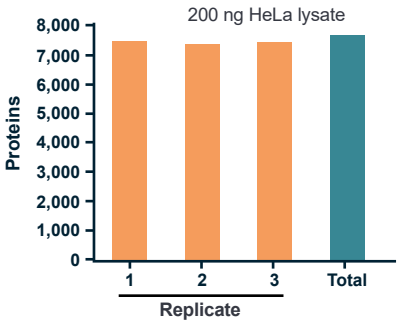


Figure 6: >7,500 proteins can be identified from a single injection of 200 ng of HeLa lysate (120-minute run time), and 6,974 proteins can be quantified with 96% data completeness in triplicate runs. In comparison, conventional proteomics usually requires µg-level samples to detect about 5,000 proteins.

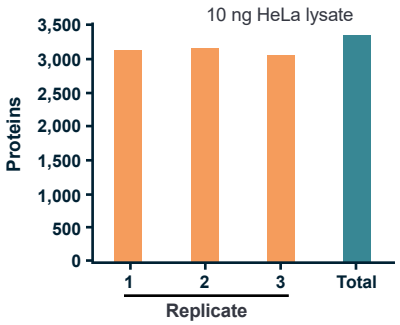


Figure 7: >3,000 protein can be identified from a single injection of 10 ng HeLa lysate (120-minute run time), and 3,323 proteins can be identified by triplicated runs. While under the same study conditions, the 4D-DDA mode could only identify 2,723 proteins.

Isomerization Brings Site Identification Challenge for Phosphoproteomics

Phosphorylation is a common type of post translational modifications (PTM), with more than 30% of proteins in cells phosphorylated. Phosphorylation is the process of transferring phosphate groups from ATP or GTP to specific sites (usually Ser, Tyr, Thr) of proteins, catalyzed by phosphorylating kinase (**Figure 8**). It is one of the most fundamental, prevalent, and important mechanisms to regulate and control protein activity and function, and is involved in various physiological and pathological processes, regulating cellular activities such as proliferation, development, differentiation, and apoptosis.

Phosphorylation on different sites on the same peptide can lead to phosphopeptide isomers co-elution on chromatography (**Figure 9**). These co-eluted phosphopeptide isomers cannot be separated on conventional LC-MS/MS platforms. According to available statistics, at least 26% of phosphopeptides have isomers, 50% of which cannot be effectively differentiated on chromatography, making it difficult to identify phosphorylation sites.

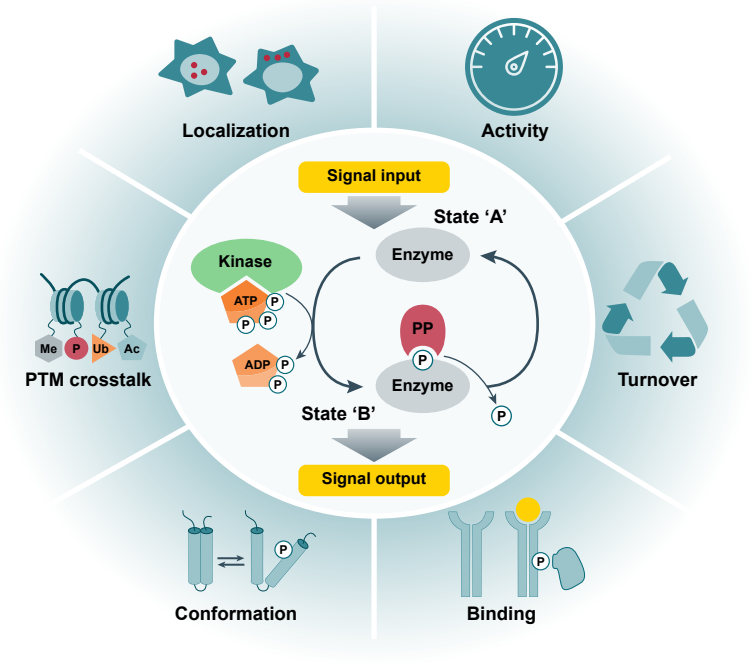


Figure 8: Phosphorylation

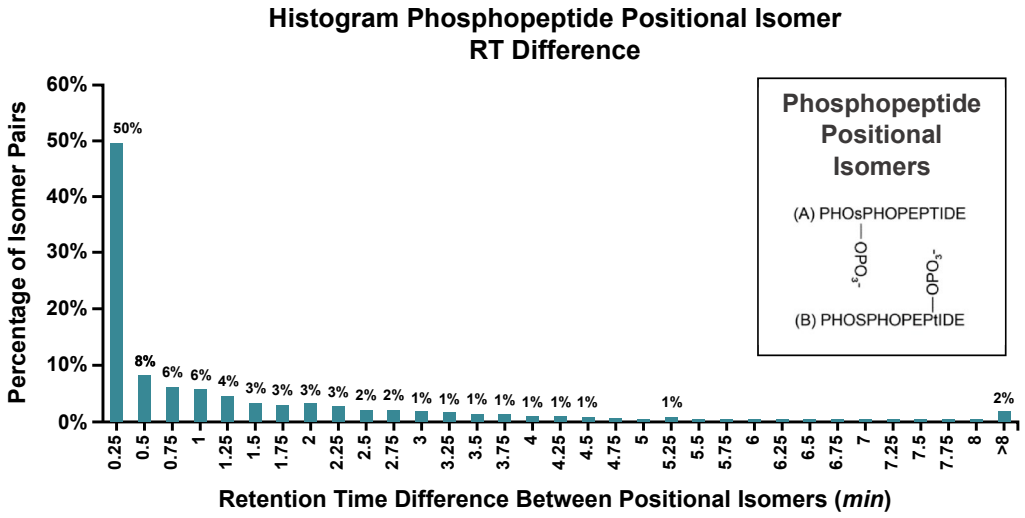


Figure 9: Isomerization brings site identification challenge for phosphoproteomics

4D Phosphoproteomics

Features

- Immobilized metal affinity chromatography (IMAC) strategy: using proprietary targeted antibodies to enrich phosphopeptides, to reduce sample complexity
- Additional ion mobility separation enables more reliable and deeper coverage for phosphorylation
- Strict dual quality control to remove low confident data: false discovery rate (FDR, 1%); false localization rate (FLR, 0.75)
- Upgraded bioinformatic analysis available: kinase prediction, signaling analysis, and data mining

Technical Strengths of 4D Phosphoproteomics

Deeper Coverage of Phosphorylation Site Identification

4D technology brings a dramatic increase in detection depth and sensitivity. The timsTOF Pro system can acquire up to 100 MS/MS spectra per 1.1 second scanning cycle.

>10,000 phosphorylation sites (localization probability > 0.75) were identified with high confidence in various cell tissues, 50% higher than the traditional method (**Figure 10**).

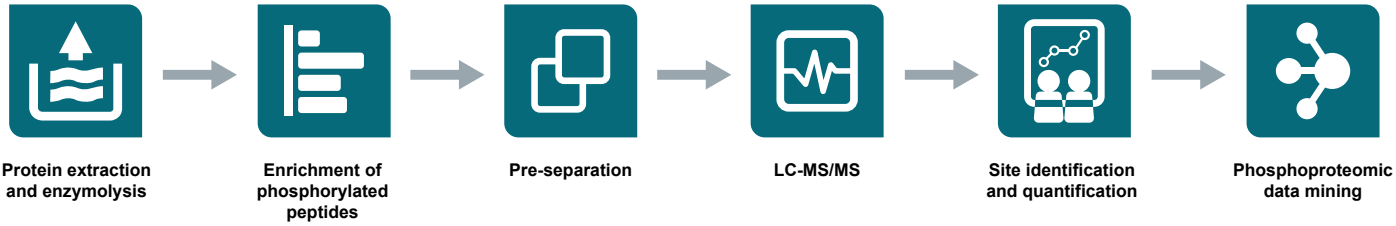
More Reliable Modification Identification Results

4D phosphoproteomics resolves the issue of isomerization in PTM through ion mobility separation, which ensures more reliable identification of PTMs.

As shown in **Figure 11**, the elution time and m/z ratio of many phosphorylated peptide isomers are identical, but the structural differences of the phosphorylation locations can be effectively detected by ion mobility.

The separation of both ion mobility and HPLC can maximize the identification and differentiation of these co-eluted modified peptide signals.

4D Phosphoproteomics Quantitative Analysis Workflow



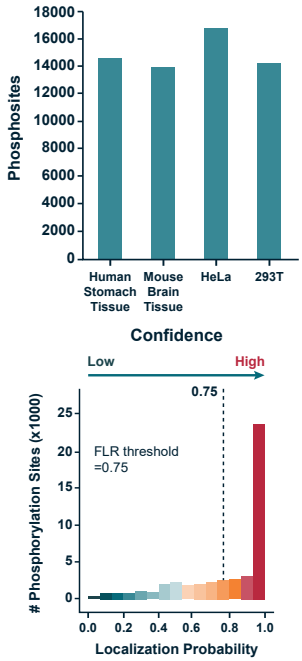


Figure 10: Deep coverage of phosphorylation site identification

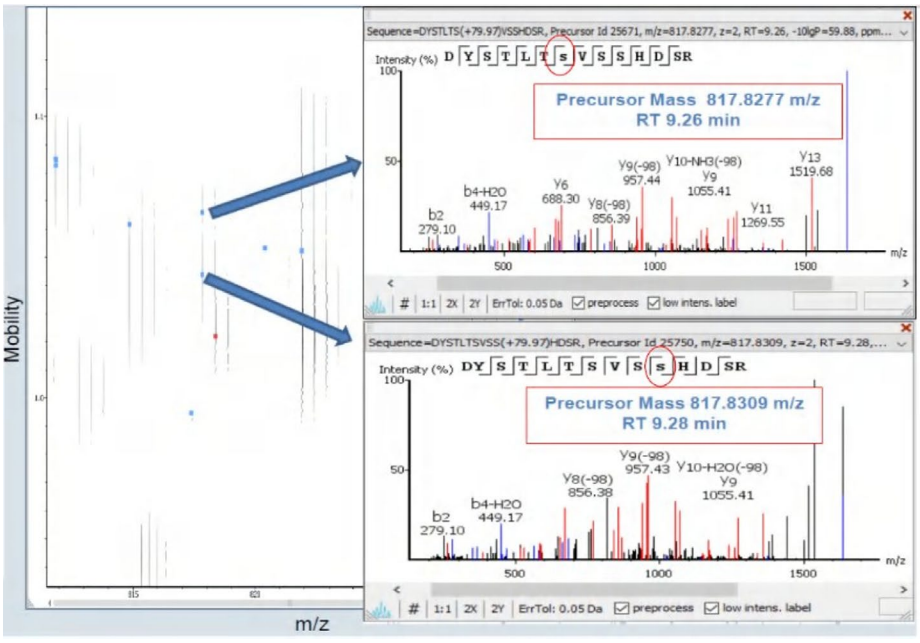
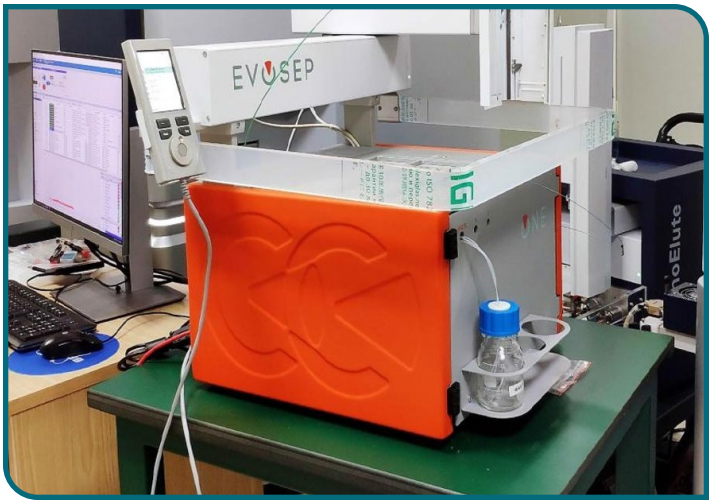


Figure 11: Adding ion mobility as the 4th separation dimension help to identify co-eluted modified peptides

Mass Spectrometry Core Facility

- Top mass spectrometers such as Bruker timsTOF Pro/ timsTOF Pro 2 series, and Thermo Fisher Scientific Orbitrap Exploris™ 480
- Additional separation dimension by ion mobility separation to traditional LC-MS/MS based proteomics
  - Bruker Trapped Ion Mobility Spectrometry (TIMS)
  - Thermo Fisher Scientific FAIMS
- Unique diaPASEF technology to dramatically increase data acquisition speed
- Evosep One provides robust and excellent chromatographic separation
- Continuous analysis of thousands of samples to provide stable performance without cleaning, enhancing the stability of batch analysis



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