



• Search Engine in Real-time

Innovation with Integrity

TIMS-QTOF MS

PaSER 2022

GPU powered Parallel Search Engine in Real-time



From data acquisition to data analysis your lab's productivity is critical. timsTOF platform enables you to run more experiments with greater information content than ever before, but more experiments mean more data to analyze. By introducing real-time search capabilities with PaSER™ you can now break the data analysis bottleneck. PaSER™ brings you Run & Done with smarts, where at the end of every data acquisition you have results in hand, maximizing your labs efficiency and making the most informed acquisition decision.

Proteomics Innovations by Bruker



Bruker innovations in recent years have transformed the proteomics landscape, starting with the timsTOF Pro which ushered in the era of 4D-Proteomics[™]. 4D-Proteomics enabled by PASEF[®] provides MS/MS acquisition at >100 Hz, making high-throughput measurements using **short gradients** possible while maintaining **deep proteome coverage** or high protein depth measurements in **less time**. This gives you the most information rich datasets for your samples in the least amount of time.

The big picture that Bruker is delivering with timsTOF and PaSER

Sequencing of the human genome was thought to have revolutionized the application of not only genomics but also proteomics, because the description for each coded gene could be predicted. Formerly, proteomics struggled to achieve expectations for reasons including lack in proteomic depth of coverage, quantitative capabilities, and throughput. The capabilities of modern mass spectrometers, most notably the timsTOF Pro 2, have changed this outlook. Instead of analyzing tens of samples per day at maximal sample loads (>1 μ g) with ineffective sequencing speeds (<40 Hz), the timsTOF Pro has allowed hundreds of samples with minimal sample load

(< 200 ng) at sequencing speeds exceeding 100 Hz. All of this with uncompromised proteomic depth. This combination has changed the way proteomics can be done, but it also unleashes a new constraint, which is data analysis. Formerly having to process tens of samples in a day was an easy task, but modern researchers with timsTOF technology must process hundreds of samples of information dense content. For most pipelines this creates a bottleneck at the data analysis step, for which we have specifically tried to innovate. Bruker has done so by introducing real-time database search capabilities called PaSER (Parallel Search Engine in Real-time) which removes the data analysis hurdle. Using PaSER, as soon as a LC-MS run is completed, results are in hand or Run & Done.

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Several groups have envisioned the utility of a system that makes use of prior knowledge to make decisions 'on-the fly'. PaSER makes this a reality. PaSER is a complete hardware and software solution that brings intelligent acquisition to the lab and to any current or new timsTOF instrument.

High-throughput 4D-Proteomics

by coupling short gradients to PASEF technology

PASEF delivers the synergistic benefits of sensitivity and speed. This combined with short gradients results in unprecedented proteome coverage without compromise in protein sequence coverage. The sheer speed of PASEF (120 Hz) has pushed the formerly accepted "norm' of two hour gradients to less than 30 minutes. Now proteomes can be measured at a pace of 60, 100, 200 and 300 sample measurements in a single day.



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High proteome coverage with exceptional protein sequence coverage and high quantitative reproducibility. Example base peak chromatogram traces for the different Evosep One gradients for high throughput proteomics at 300, 200, 100, 60 samples per day (SPD). Cuantitative reproducibility in such high-throughput runs.

This paradigm shift in gradient lengths used for proteomics naturally leads to measuring many more samples. This transfers the capability of large-scale proteomic systems biology studies and large cohort clinical research applications to any lab equipped with a timsTOF mass spectrometer.

Introducing PaSER Run & Done

Uncompromised data processing on the fly

Traditionally data acquisition has been the bottleneck for large scale proteomics. This has also remained one of the limitations in leveraging mass spectrometry within the clinic. PASEF and short gradients have facilitated these large-scale studies in any lab but have pushed the bottleneck downstream to data processing. The timsTOF Pro 2 has made the acquisition of thousands of samples in a modest period of time reality. Thus, it is now crucial to have real-time results particularly in terms of identification to monitor the quality of datasets and intervene in a timely manner when required. This is made possible by Parallel Search Engine in Realtime (PaSER). PaSER 2022 is a complete hardware and software solution that has two focuses: Speed and Smarts.

PaSER is powered by a GPU optimized search algorithm and necessary hardware to communicate to the instrument's acquisition PC to identify peptide sequences from the MS/MS spectra as they 'elute' from the acquisition PC. These results are then appended, and actions can be triggered based on the cumulative identification results at the end of the sample measurement.



The PaSER workflow – simple, fast and smart: Users interface with the acquisition PC to setup an acquisition table. In addition to standard parameters, such as injection volume and LC-MS methods, users would also specify a PaSER method and qualifiers for each injection. As data is acquired on the acquisition PC, the data is simultaneously streamed to the PaSER box. This 4D data is processed by PaSER in real-time providing results as soon as acquisition ends enabling Run & Done. These results are utilized to verify if the user defined qualifier was met and if acquisition should continue.

No more worrying about injecting those precious samples. No more worrying of wasted instrument time. No more worrying about those samples with expensive reagents and sample prep, only to inject on a poor performing system. No more waiting for the QC runs to determine system suitability. No more waiting for search results during method development.



Precious samples
Instrument time
Analysis time

Bioinformatics

Full-fledged database search in less than 5 ms

PaSER provides consistent results obtained from the real-time search, identical to offline search of the same data. Since the database search algorithm is run on the GPU, the search times are negligible compared to CPU based searches. In addition to real-time search the opportunity to search a larger space for specific applications, including peptidome studies, becomes reality.



Blazing search speed. A single injection of a human cell lysate analyzed against the forward and reverse sequences of the Uniprot Human fasta file with up to 6 PTMs using either ProLuCID or ProLuCID-GPU search algorithm. B PaSER can process MS/MS spectra with an average search time ~ 1 ms. PaSER search results are nearly identical whether done in a real-time manner or offline.

TIMScore – Machine Learning and CCS exploited for better FDR

The first database search algorithm, developed in the Yates lab circa 1994, revolutionized proteomics and utilized the cross-correlation (XCorr) scoring function for identifying peptide MS/MS spectrum. XCorr is still widely utilized or adapted in many popular algorithms including Comet, Crux Tide and of course ProLuCID. TIMScore extends that concept to the mobility dimension. TIMScore is the measure of the correlation between the experimentally measured CCS for a peptide versus predicted CCS of that peptide. To predict the CCS of peptides, deep learning was utilized to build a transformer model, which utilizes self-attention to differentially weight the significance of each part of the input data, providing a fast and robust model of CCS values when provided with the amino acid sequences.



Utilizing TIMScore is easy

The PaSER search algorithm is run as normal, and the search algorithm automatically compares the predicted and measured CCS values and calculates TIMScore for the top 5 peptide candidates for each spectra. The true benefit of TIMScore can be realized during the peptide-validation and False Discovery Rate (FDR) estimation steps of the proteomics pipeline. In a non CCS-enabled algorithm, only two dimensions can be utilized to estimate the FDR rate, and so a discriminate line is fit to a 1 % error (Panel A) to distinguish forward and reverse peptide candidates. With TIMScore, and the extra CCS dimension, the peptide-candidates can be vectorized in 3 dimensions (Panel B) allowing a discriminate contoured plane to be applied to achieve the same 1 % error. Applying a discriminate plane provides increased accuracy and precision, helping to validate formerly poorly scoring PSMs in the standard two dimensions. Thus, the key effect of TIMScore is derived from the additional dimension of CCS it provides in assigning true positives from decoy peptide sequences as shown above.



Bioinformatics

TIMScore increases proteins, peptides, PSMs and sequence coverage

TIMScore when applied to the published data set from the laboratory of Prof. Yasushi Ishihama titled "Effect of Phosphorylation on the Collision Cross Sections of Peptide Ions in Ion Mobility Spectrometry" adds more than 110,000 PSMs and doubles the number of peptides observed from 42,930 to 98,949. The > 98,000 peptides observed for this dataset is a 3.5 times increase in what was reported in the published data and shows good overlap with regards to protein groups and peptides identified (Panel B and C). TIMScore significantly boosts protein sequence coverage which helps provide quantitative accuracy and more expansive libraries for

TIMScore increases phosphopeptide identification and phosphosite localization

More identifications are meaningless if the site of the PTM cannot also be determined. Luci-PHOr, a tool to assess the number and accuracy of PTM sites via false localization rate (FLR) [Fermin 2013] was used to determine the percent confidence interval of correct assignment of phosphorylated peptides. Consistent with label free quantitation (LFQ), data independent acquisition (DIA) and parallel reaction monitoring (PRM) experiments.

Similarly, when TIMScore is applied to an unpublished AP-MS study from the laboratory of Prof. Stanley Stevens Jr. the number of peptides identified increases by >30% in all 4 control and all 4 treated samples (see graph below). Without TIMScore 24,664 phosphopeptides were identified across all 8 samples, which was doubled to 49,423 phosphopeptides with TIMScore. Furthermore, 19,159 of these phosphosites were uniquely identified in the treated samples and not the control samples.

the increased sensitivity TIMScore provides, it improved the number of localized phosphopeptide PSMs at the 5 % FLR by 10,854 or 57 %. These percent improvements using TIMScore held consistent (57-62 %) across both PSMs and peptides at both the 5 % and 1 % FLR.





"CCS values and 4D-Proteomics[™] have become indispensable in our application of mass spectrometry-based proteomics. TIMScore shows promise to transform how DDA searches are performed, identifying many more meaningful peptides and proteins allowing us to go deeper into the proteome using CCS."

Professor Stanley Stevens Jr. at the University of South Florida

TIMS Viz – Effortlessly identify isobaric masses resolved by mobility



TIMS Viz provides an effortless way to visualize timsTOF data. Since its launch in 2017 the timsTOF has provided mobility measurements for all peptide, lipid and small molecule analytes which have proven to be reproducible across instruments and laboratories. Until the introduction of TIMS Viz multi-dimensional visualization was difficult. Using TIMS Viz the data acquired on PaSER can be displayed, parsed, and interrogated allowing you to finally see co-eluting, isomeric or near-isomeric ions which collapse onto a single spectrum and remain hidden without mobility separation. TIMS Viz can also overlay the realtime search results from a PaSER run, presenting identified peptide sequences by retention time, m/z and mobility values.

One of the most powerful features within TIMS Viz is the MOMA viewer. Complex protein digests contain hundreds of thousands and even millions of peptides. In running them by LCMS it is common that ions of a similar m/z co-elute in time. When this happens a traditional mass spectrometer is unable to deconvolute these ions as two species, resulting in co-fragmentated MSMS spectra. This is where the power of TIMS shines, when those two co-eluting ions have even the smallest difference in their CCS then they will be represented by different mobility (CCS) values and interrogated as separate species. This advantageous feature is called mobility offset mass aligned or MOMA. The TIMS Viz example above is a 10 second retention time extraction where within this time window many MOMA groups (yellow dots) have been detected. The zoomed window displays a phosphopeptide positional isomer, where both the serine and threonine sites have been positively identified by MS/MS, co-eluting in time and resolved only by their CCS which are different.

dia-PASEF workflows with TIMS DIA-NN

PaSER 2022 is capable of processing dia-PASEF workflows utilizing a customized version of the popular DIA-NN (DIA by Neural Networks) software from the Lilley, Rasler and Demichev labs. TIMS DIA-NN enables reliable, robust, and quantitatively accurate large-scale experiments. TIMS DIA-NN provides stringent statistical control, flexible modelling (AE) of data and automatic parameter optimization.

PaSER utilizes the same stream mechanism for dia-PASEF workflows, streaming in real-time MS and DIA frames from the acquisition PC to the PaSER box. These dia-PASEF data are processed and stored on the PaSER box. At the end of the acquisition a spectral library search is triggered, and the results recorded. TIMScore powered DDA search results can be easily utilized to build spectral libraries or spectral libraries can be imported from other popular tools. At the end of the project the users can trigger match-between-runs analysis of the full project (or selected acquisitions) to view a quantitative profile across the project. This provides users an integrated environment for PASEF and dia-PASEF data analysis.

Nearly 9000 protein groups identified from 200 ng of K562 lysate in 35 min gradient (~ 30 samples per day) with TIMS DIA-NN and a TIMScore spectral library.



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"Our ongoing collaboration with Bruker to tailor DIA-NN to a streamlined processing tool for dia-PASEF data with a CCS focus has been really rewarding. It simplifies and accelerates identifying and quantifying thousands of proteins in even very short gradients. We are pleased that within our close collaboration with Bruker, the vendor-integrated version of DIA-NN called TIMS DIA-NN now becomes part of the PaSER bioinformatics platform."

Prof. Dr. Markus Ralser, Einstein Professor of Biochemistry at Charité, Berlin, Germany

PaSER Data Viewer

Examine your data however you like

PaSER is complimented with a full featured, LIMS based analysis platform and data viewer. All realtime search results are stored on the PaSER box. Users may examine the results in as much detail as they desire. Start with a simple overview of the results, dig deeper into a specific protein, or visualize an individual MS/MS spectrum. Users can

Width: 75g

also re-search past acquisitions, perhaps with an updated fasta file, a new PTM or simply apply a new FDR threshold. Search results from multiple samples can be combined and comparative analysis can be conducted. PTM localization scores and their MS/MS spectra can be visualized. The PaSER Data Viewer affords you the capability to decide.

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