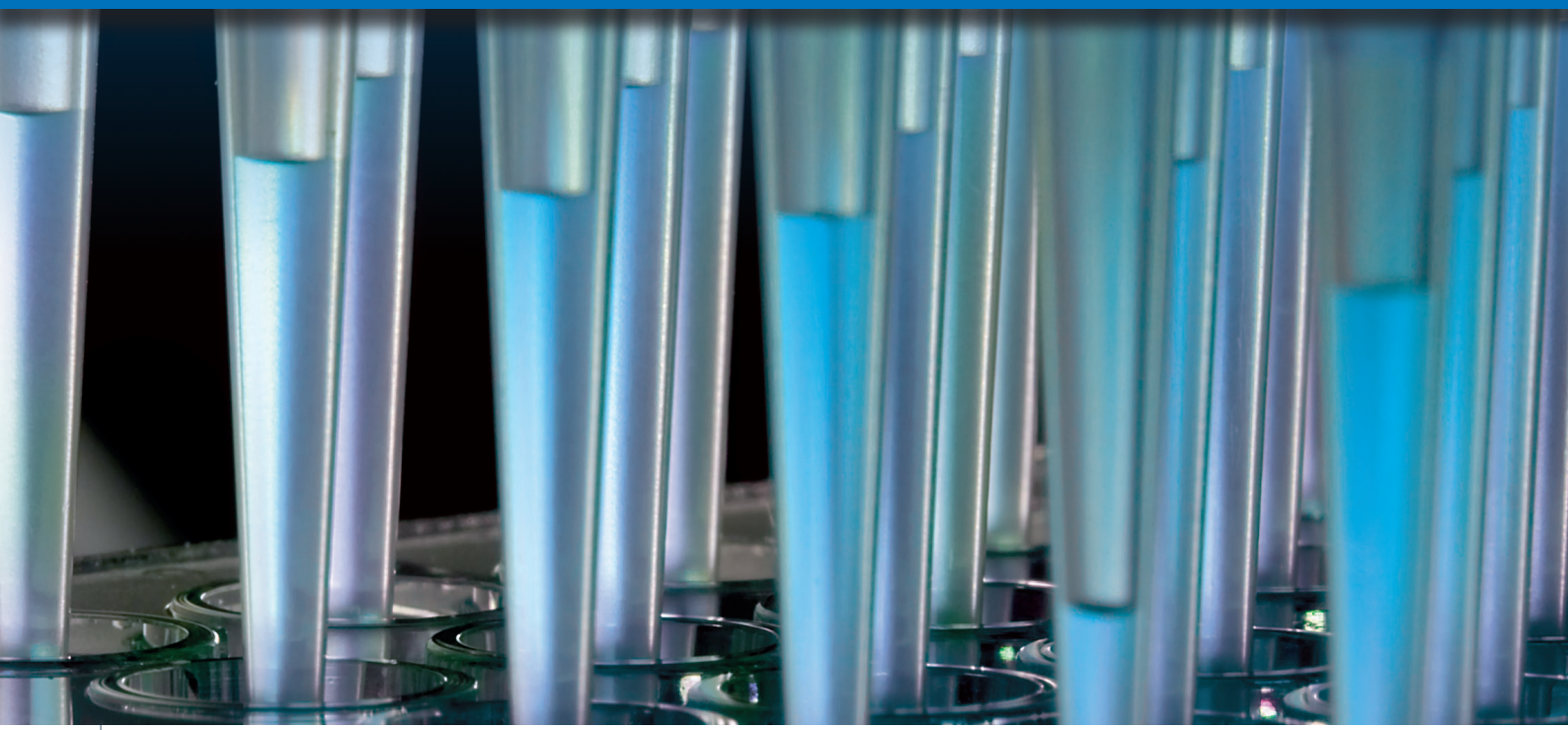

High sensitivity and high-throughput label free interaction proteomics using PASEF® on the timsTOF Pro mass spectrometer

Protein interactions play a crucial rule for functional outcome of the living systems. Here we employ fast and sensitive PASEF technology on the timsTOF Pro and demonstrate high-throughput interaction proteomics studies on mammalian cell systems.

Keywords:
timsTOF Pro, interaction proteomics, PASEF

Introduction

MS based interaction proteomics is routinely applied to identify interaction partners using label-based and label-free quantification techniques. In these studies, a so-called "bait" protein is expressed with a "hook" where the hook is a sequence that can be pulled down with standard affinity pulldown methods. The bait protein is then incubated under native conditions with a cell lysate or other complex mixture of proteins. Proteins that interact with the bait protein are then captured along with the bait protein when it is pulled down. Typically, in label-free interaction proteomics, samples are analyzed in at least triplicates for the bait as well as for controls. Global or large-scale interactome studies have been carried out but this is predominantly done in yeast cells that are easier to culture. Global interactome analysis on mammalian cell cultures have also been carried out but

Authors: Nagarjuna Nagaraj[1], Ralph T Boettcher[2], Reinhard Faessler[2];
[1] Bruker Daltonik, Bremen, Germany; [2] MaxPlanck Institute for Biochemistry, Martinsried, Germany

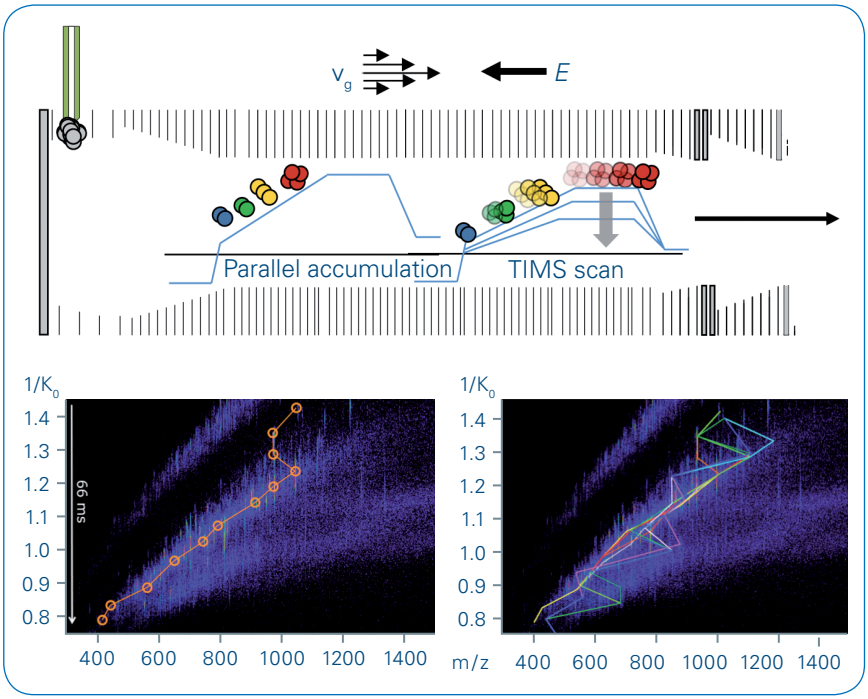

Figure 1: Parallel accumulation serial fragmentation (PASEF). PASEF strategy in a timsTOF Pro instrument where ions are accumulated while the second TIMS region is serially eluting peptide species. The bottom panel depicts a typical PASEF precursor scheduling within one and many frames within a acquisition cycle.

this requires elaborate planning for cell culture and sample preparation. Culturing cells in 6-wells or 12-well formats if not in 96-well format would significantly enhance the feasibility of performing such large-scale interactome studies with multiple experimental conditions. The timsTOF Pro mass spectrometer with the PASEF acquisition method (1,2) brings a two-fold advantage to such measurements. Trapped ion mobility spectrometry (TIMS) provides time focusing of ions in the TIMS tunnel thus enabling a boost in sensitivity. This boost in sensitivity facilitates scaling down the sample amount that in turn enables higher throughput in generating samples. Parallel accumulation and serial fragmentation (PASEF) mode results in full usage of ion beam and can rapidly schedule multiple precursors for fragmentation thus making high throughput analysis in short gradients routinely feasible. Here we show a proof of principle with an interaction proteomics experiment from samples inputs equivalent to a 12-well plate format and demonstrate the improved depth of coverage in half the measurement time previously required in our proteomics platform.

Methods

Cell lysates from 6-well pates (250 µg total protein) were incubated with beads bound to bait-peptide or scrambled-peptide (control) loaded beads. After incubation and washing, proteins were digested on-beads and the peptides were desalted via StageTips. One fourth of total peptides equivalent to starting material from one well off a 12-well cell culture plate were analyzed on a standard 90 minute method using a timsTOF Pro mass spectrometer. In addition, a 45 minutes analysis was carried out in the timsTOF Pro instrument. PASEF measurement were performed using one of the standard methods available in the oTOFControl software, which did not require any further optimization. All

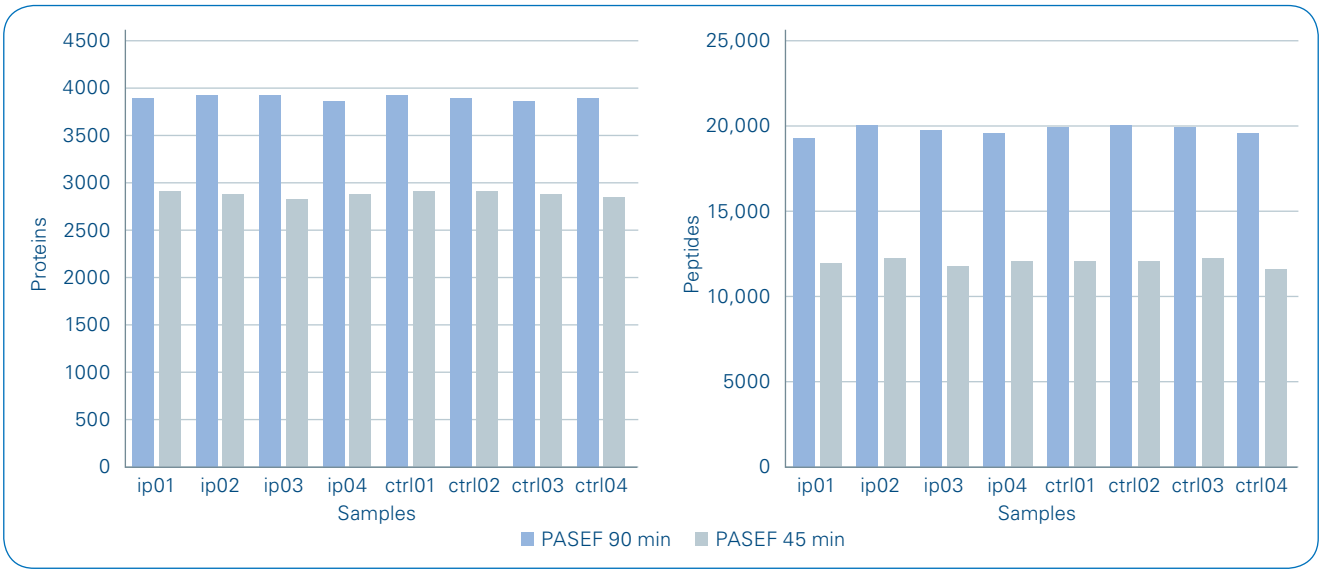

Figure 2: Number of proteins and peptides quantified in different gradient lengths. Proteins and peptides were filtered at 1% false discovery rate.

runs were performed using a Thermo Easy LC 1200 and a 15 cm pulled emitter column (New Objective Inc., USA) in quadruplicates and acquired raw data were processed in MaxQuant and analyzed in the Perseus bioinformatic tool. Protein quantification was performed using the MaxLFQ algorithm in MaxQuant version 1.6.7.0 and match between runs, including 4-D matching using CCS values, was enabled (3). All identifications were filtered at 1% FDR for proteins and peptides.

Results and Discussion

timsTOF Pro powered by PASEF technology provides extremely fast sequencing speed with smart utilization of all ions and positioning the quadrupole for the precursors as they elute from the TIMS device. As mentioned above the time-focusing effect of the TIMS device boosts the sensitivity of measurement thus enabling low sample input analyses. This enhanced sensitivity enables us to scale down our experiments for example to 6- or 12-well plate formats instead of culturing cells in 10 cm dishes which in turn opens the opportunity to test multiple conditions in our pulldown experiments. A direct comparison with sample input equivalent to about 12-well plates showed significant improvement in the number of proteins and peptides in the timsTOF Pro compared to the previous platform used in our lab. Interestingly, even in half the measurement time better protein coverage is observed on the timsTOF Pro. In comparison to our previous standard measurement PASEF provides about 24% and 15% improvement in the median sequence coverage of identified proteins in the 90 minute and 45 minute measurement time respectively. This increased sequence coverage would enable efficient PTM searches on the bait and interacting proteins (such a search not presented here). We used the dataset from the shorter gradients to further look into the interaction partners for our bait. Label free quantification using MaxLFQ showed very high correlation between the replicates and across the samples. We could identify five out of 10 direct binding partners and found additional novel candidate interactors. Further experiments using alternative biochemical assays would be necessary to verify these interactions. Interestingly, we also find indirect interactors e.g. proteins recruited to the bait through binding to direct interactors. This opens the possibility to make use of the timsTOF Pro mass spectrometer low input and fast sequencing speed to analyze interaction samples with different binding and wash buffers to discriminate between direct and indirect bait binders.

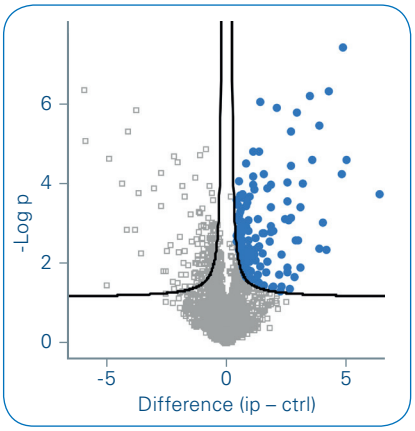

Figure 4: Volcano plot for proteins that are significantly enriched in pulldown or background sample. In addition to known interactors, few novel interactor candidates were also derived from the study that requires further follow up investigation using alternative techniques.

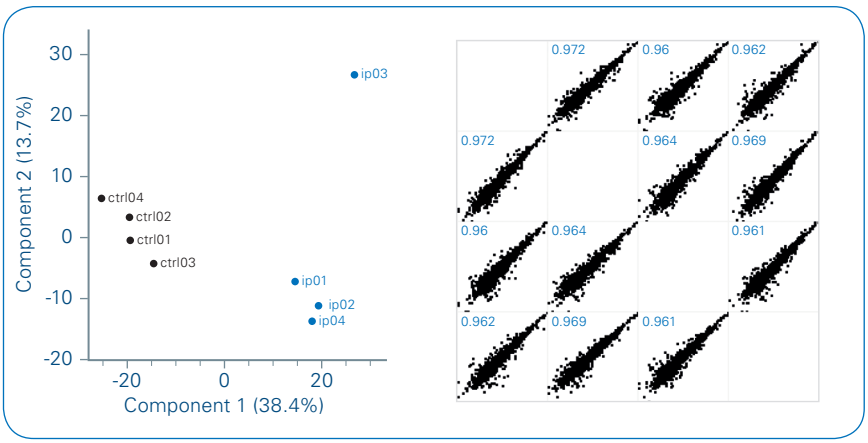

Figure 3: Protein quantification correlation across all samples measured in 45 minute gradients. Principle component analysis plot shows a clear separation of control and bait pulldowns. The one distant data point is attributed to the variations arising from biological replicates.

Conclusion

Our measurements show that timsTOF Pro mass spectrometer is very sensitive for low amounts of samples for routine measurements. Given the depth of coverage that is obtained using the current methods, it should be very feasible to perform data acquisition in an even faster cycle. For example, by using short 21-minute gradients on the Evosep platform coupled to timsTOF Pro should yield data throughput of 60 samples per day, which translates to 15 interactomes per day in quadruplicates. This really opens the possibility of performing multifaceted global interactome studies in a high-throughput manner utilizing very low sample amounts.

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Bruker Daltonik GmbH

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com