Comparing dda-PASEF and prm-PASEF approaches for the quantification of 2000 RAS induced Phosphopeptides

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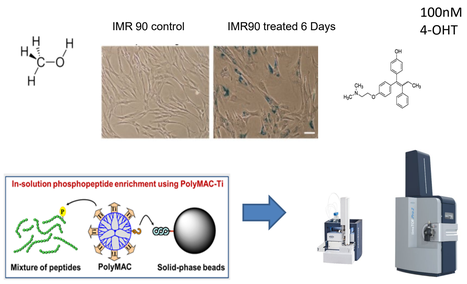
Introduction

Targeted proteomics methods are a traditional choice for protein quantitation of proteins in complex cell samples. Senescence is a tumor suppressive mechanism of cells and acts as a primary layer of protection against the development of cancer. Senescent cells, that lose the ability to divide, are also the underlying mechanism of aging. Therefore, understanding molecular factors and biological processes of cellular senescence provides important insights into the intrinsic cellular mechanisms for cancer prevention, and organismal aging. Herein, we have used dda-PASEF and prm-PASEF for the quantification of RAS induced phospho-peptides. Our analysis is added by the ability of the trapped ion mobility to give greater separation to phospho position specific isomers.

Methods

Human diploid fibroblast strain IMR90 (CCL-186; ATCC, USA) cells were transduced with ER:RAS lentivirus and treated with 100 nM of (Z)-4-Hydroxytamoxifen (4-OHT) for ER:RAS activation and induction of oncogene induced senescence (OIS). Control cells were treated with MeOH. Cells were harvested after 6 days of 4-OHT activation for nuclear extraction, trypsinization, and enrichment of phosphopeptides using Polymer-based Metal-ion Affinity Capture (PolyMAC) spin tips. Pre and post enrichment samples were both run on a nanoElute LC (Bruker Daltonics) using an Aurora nano column (25 cm x 75 µm ID, C18 - IonOpticks, Australia) at 400 nl/min with a 70 min gradient 80min run time, and a longer 120min gradient for global samples. LC-TIMS MS/MS data were obtained from a timsTOF Pro instrument operated in DDA- PASEF mode. Data were analyzed using PEAKS OnLine (Bioinformatics Solutions). PRM PASEF data was analyzed with Skyline.

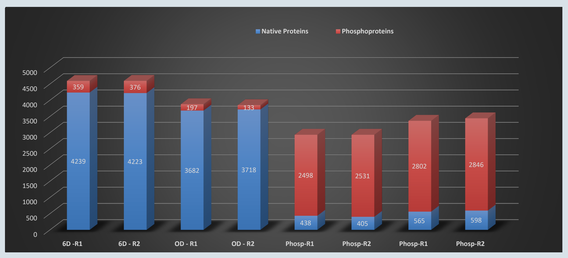
Samples



Results

To investigate the speed and sensitivity of the dda PASEF method for shotgun phospho protoemics, we first analyzed a complex peptide mixture that was enriched for Phosphorylated peptides with 300ng and 450ng on column.

Proteins



Before ER Ras induction
No PolyMAC peptide Enrichment

Post ER RAS induction
with PolyMAC peptide Enrichment

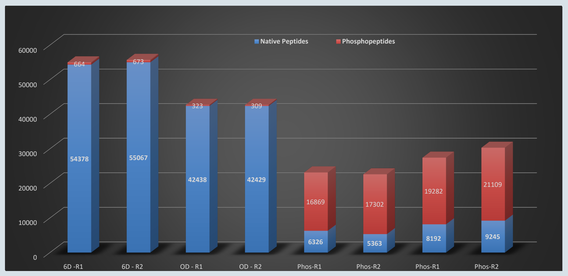


Figure 1: Global proteome analysis **:** Number of Proteins and peptide and proteins identified in ER RAS induced cells

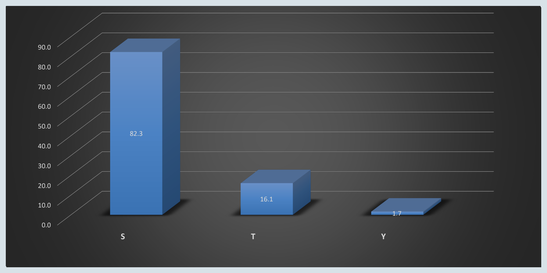


Figure 2: Serine Threonine and Tyrosine % in PolyMac enriched Peptides

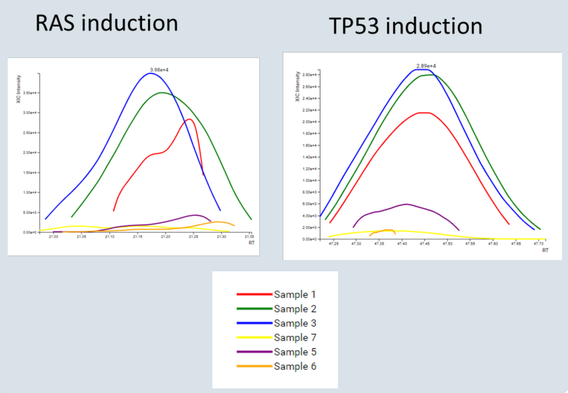
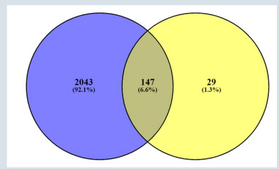


Figure 2a DDA LFQ Results
3,984 Phospho-peptides were quantified in at least 2 of the 3 replicates Ras had a 13-fold induction and 20-fold for TP53B

RAS induced LFQ Phospho



# Protein Groups	#Peptides	# Precursor	#PSM	Phospho Sample name
2478	14889	97305	44508	ER Ras activated all
1587	8601	27861	14276	ER Ras activated R1
1485	7095	29315	13079	ER Ras activated R2
1720	9479	40129	17153	ER Ras activated R3
658	2346	45361	4020	Control IMR90 cells total
609	2230	14744	3087	IMR90 Control R1
156	394	14095	603	IMR90 Control R2
99	226	16522	330	IMR90 Control R3

Area Phosphopetide Area comparison DDA PASEF LFQ

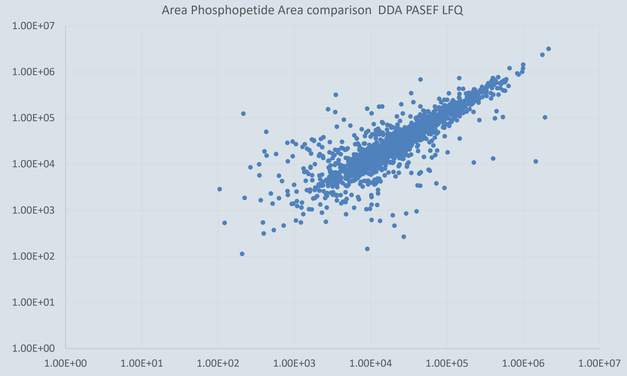
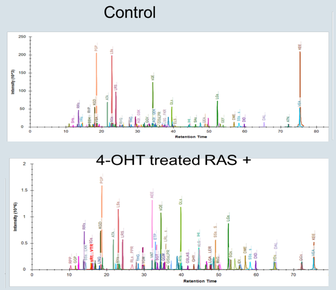
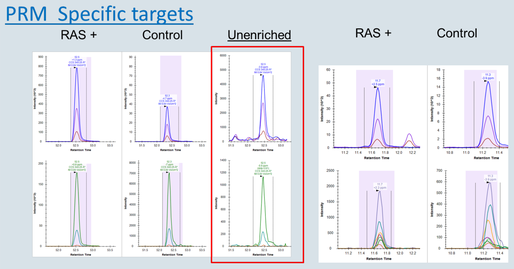


Figure 3 and Table 1: Reproducible and accurate quantification
A) LFQ intensities of ER Ras induced Phosphorylated Peptides

PRM in Skyline



PRM Specific targets



FLGS[+80]FGSITR- FLNC-FilaminC

GGPGKLS[+80]PR TP53 p53

Table 1 dda PASEF LFQ

	Sample Name	#PSM	#Peptides	# Proteins	# Protein Groups
RAS+ PolyMAC	All (3 samples)	44508	14889	6465	2478
	Rep 1	14276	8601	4533	1587
	Rep2	13079	7095	4192	1485
	Rep3	17153	9479	4703	1720
	Phosphorylated		8246		1789
Control PolyMac	All (3 samples)	4020	2346	1324	658
	Rep 1	3087	2230	1252	609
	Rep2	603	394	340	156
	Rep3	330	226	283	99
	Phosphorylated		856		339

Figure 4 PRM Quantitation
Table 2 dda PASEF LFQ

Conclusions

- High depth of phospho proteome coverage even with low sample amounts
- High reproducibility in identification and label free quantification
- PASEF provides accurate label free quantification over a high dynamic range and high depth of proteome coverage
- Phospho enriched IMR90 control cells had 1,789 phospho proteins
- ER Ras induced treated cells had a greater number of protein groups at 3,035 when compared to the control treated group
- 1,890 of these protein groups overlapped between the treated and control samples
- Using Label free quantification in DDA 2,391 phospho-peptides were able to be compared across the two groups and 2000 had reproducible area.
- 243 most significantly differentiated Phospho Peptides between the Control and 4-OHT treated cells were acquired in a targeted PRM approach
- This allowed for an impressive 234 to be identified and quantified in both non phospho enriched sample and enriched samples.
- timsTOF ion mobility allows for many phospho peptides to be both identified and quantified in DDA and PRM.

timsTOF Pro 2