Novel hybrid-DIA acquisition strategy combines targeted and discovery proteomics to enable simultaneous quantification of global proteomes and predefined mutated peptides

Ana Martínez-Val[1], Kyle Fort[2], Giulia Franciosa[1], Claire Koenig[1], Ulises Hernández Guzmán[1], Tanveer S. Batth[1], Daniel Lopez Ferrer[3], Andreas F. Huhmer[3], Yasushi Ishihama[4], Yu-ju Chen[5], Yue Xuan[2], Alexander Makarov[2], Jesper V. Olsen[1]

[1]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, DENMARK
[2]Thermo Fisher Scientific, Bremen, GERMANY
[3]Thermo Fisher Scientific, San Jose, USA
[4]Kyoto University, Kyoto, JAPAN
[5]Academia Sinica, Taipei, TAIWAN

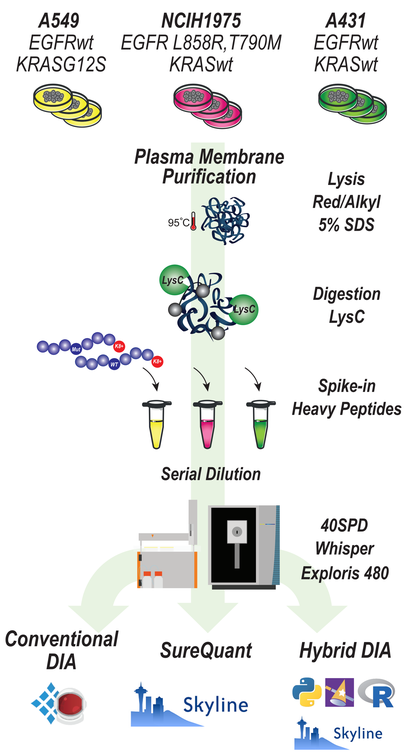
ana.mdval@cpr.ku.dk

OLSEN GROUP

Aims

- ***Hybrid DIA*** *is based on an* ***Application Programming Interface****, which enables the ability to* ***combine targeted and discovery proteomics****, by intercalating DIA scans with accurate triggering of predefined peptide targets.*
- *We have* ***benchmarked the sensitivity*** *of hybrid DIA against purely targeted methods, such as SureQuant.*
- *We have applied the hybrid DIA strategy to analyze lung adenocarcinoma proteomes and* ***cancer-driver mutations in EGFR and KRAS****, which are of high relevance for treatment-decisions and prognosis of this tumor type.*

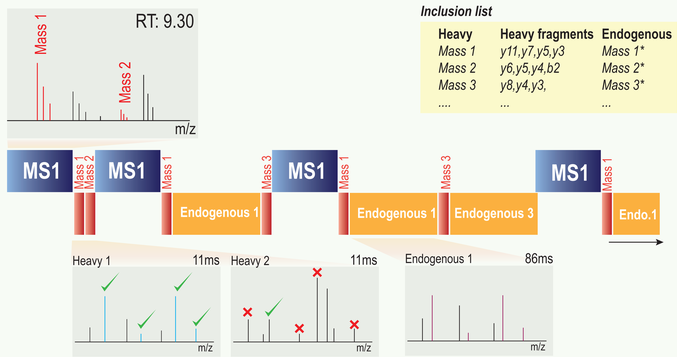
Sample Preparation



Overview of Data Acquisition Methods

Conventional DIA

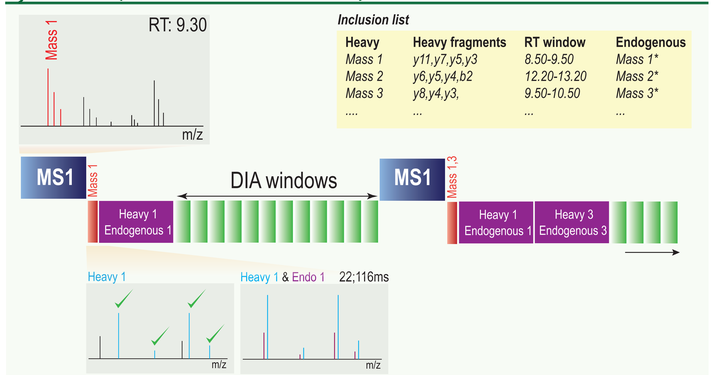
Targeted method (i.e. SureQuant)



Inclusion list

Heavy	Heavy fragments	Endogenous
Mass 1	y11,y7,y5,y3	Mass 1*
Mass 2	y6,y5,y4,b2	Mass 2*
Mass 3	y8,y4,y3,	Mass 3*
....	...	...

Hybrid DIA (i.e. Moonshot workflow)



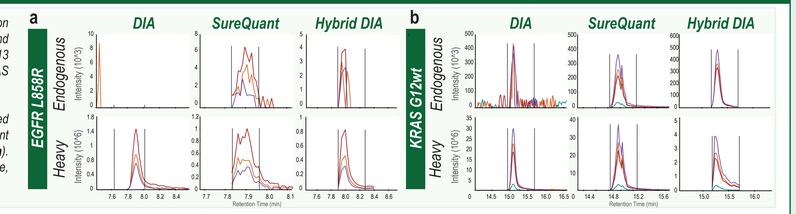
Inclusion list

Heavy	Heavy fragments	RT window	Endogenous
Mass 1	y11,y7,y5,y3	8.50-9.50	Mass 1*
Mass 2	y6,y5,y4,b2	12.20-13.20	Mass 2*
Mass 3	y8,y4,y3,	9.50-10.50	Mass 3*
....	...	...	...

Benchmark of Hybrid DIA vs SureQuant: EGFR and KRAS mutation profiling in cell lines.

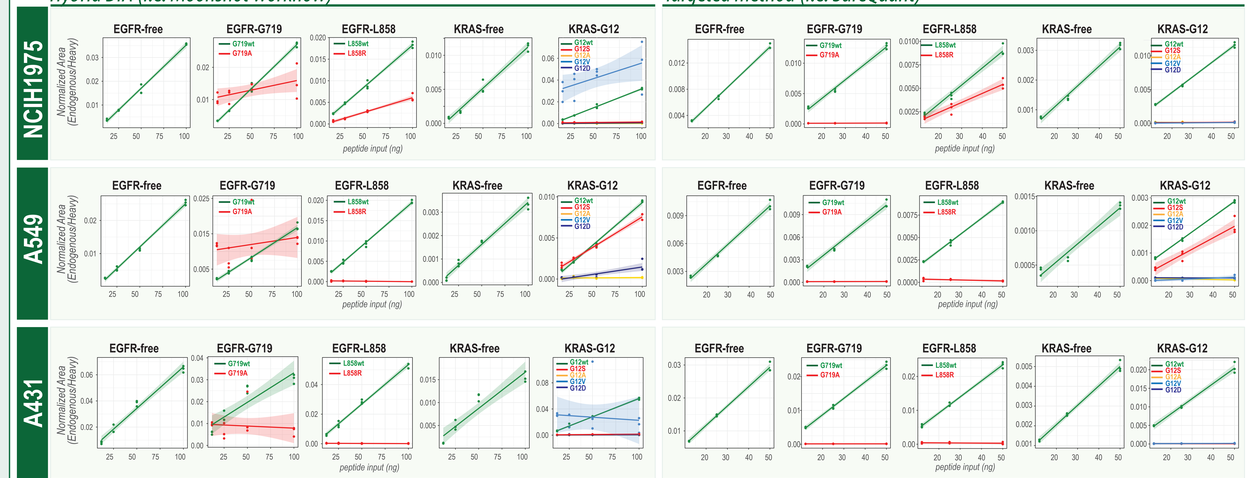
We have evaluated a new hybrid-DIA acquisition method to analyze global protein expression levels, while simultaneously detecting and quantifying predefined sets of mutated and endogenous peptide forms of EGFR and KRAS in cancer cell lines. We targeted a panel of 13 peptides, including wt and mutated forms of EGFR (L858R, T790M and G719A) and KRAS (G12A/S/D/V).

*We benchmarked the performance of the method against a state-of-the-art targeted proteomics approach, SureQuant, and conventional DIA (**fig. a,b**). In contrast to SureQuant and hybrid DIA, conventional DIA failed to detect low abundance mutated peptides (**fig. a**). Moreover, SureQuant and hybridDIA also provided better signal-to-noise and, therefore, quantification for targeted peptides (**fig. b**).*



Hybrid DIA (i.e. Moonshot workflow)

Targeted method (i.e. SureQuant)



EGFR - T790 detection

Presence of T790M mutation in EGFR in Non-Small Cell Lung Cell Carcinoma patients is related to resistance to treatment with TKI (Tyrosine Kinase Inhibitors).

LysC-derived peptide containing T1790 is not suitable for MS. Cys-derivatization with BrEA allows to cleave at C-term of Cys with LysC, resulting in a peptide with better LC-MS/MS properties.

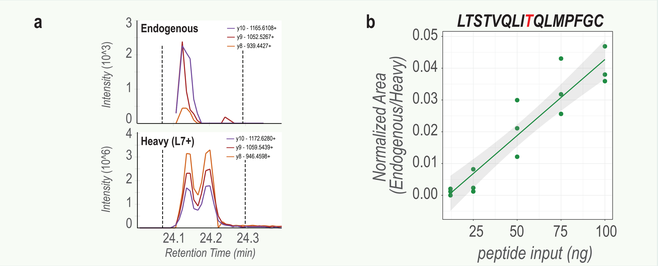
LysC product

modif Cys - LysC product

EILDEAYVMASVDNPHVCRLLGICLTSTVQLI**(T/M)**QLMPFGCLLDYVREHK

Figure a. Extracted Ion Chromatograms of the MS2 fragments specific to the Heavy labelled peptide (Leu 7+) and the endogenous, obtained from the analysis of 50 ng of A431 sample. Data acquired in using SureQuant. Double peak might inicate the co-existence of to isoforms with each one corresponding to the two potential steroisomers resulting from aminoethylation of cysteines, only one of which is susceptible of LysC digestion.

Figure b. Results of quantification of endogenous T790-containing EGFR peptide (as normalized area against the spiked-in heavy counterpart) in A431 cells, using hybrid DIA at different amounts of peptide injected in the LC-MSMS (from 12.5 to 100 ng).



ThermoFisher SCIENTIFIC

EPIC-XS

Novo Nordisk Foundation Center for Protein Research