A microscale proximity-dependent biotinylation procedure for low cell input samples using protease-resistant streptavidin on a magnetic substrate

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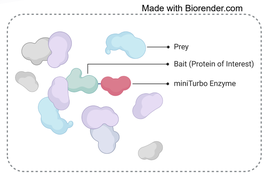
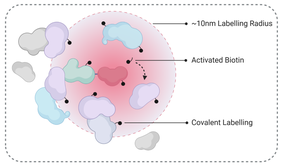
What is BioID?



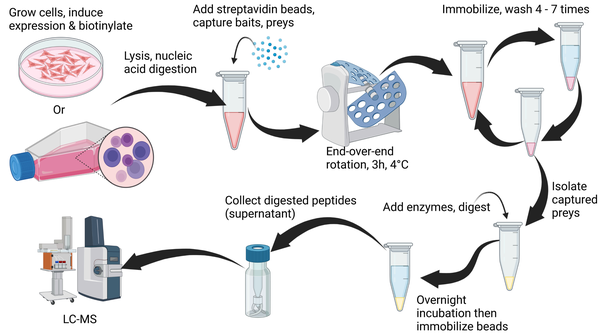
Gene modification to add labelling enzyme to protein of interest



Addition of biotin causes biotinylation on proteins in close proximity to target

BioID shows us where proteins localize in cells, what they may interact with

The BioID method



Limitations:

Conventional method uses 10s of millions of cells

Limits sample types, ease of automation

Digestion off beads leads to streptavidin contamination

Microscale modifications:

Biotinylated sample divided into 50k, 10k cells via FACS

Superparamagnetic particles as opposed to sepharose

Minimal volumes, low binding plasticware

Trap-and-elute LC-MS on IonOpticks Aurora Ultimate column with Bruker timsTOF SCP, 1 hour gradient

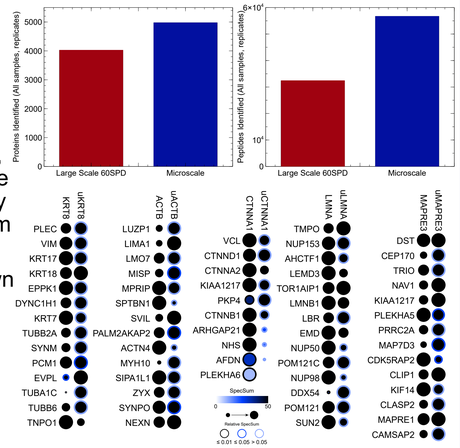
Our focus is to shrink BioID to broaden its applications

50,000 cells vs. bulk samples

From HeLa cells tagged with miniTurbo to various baits, acquired with DDA, spectral counting, searched via MSFragger, proximity interaction analysis by SAINTexpress

Despite increase in identifications, most preys are not statistically significant from background

<20% of known interactors detected

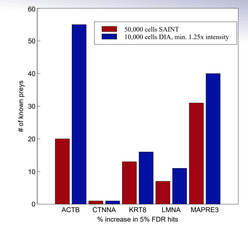


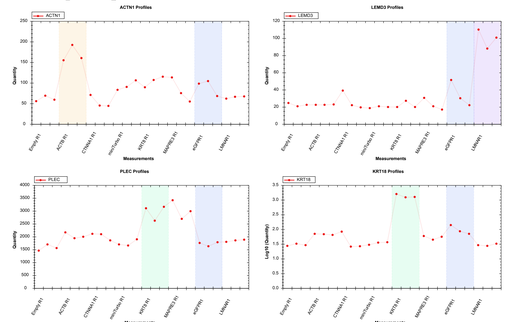
DIA of 10,000 cells

DIA-PASEF of 10,000 cells, searched via spectral library using Spectronaut 17

Compare bait sample vs. eGFP only

Increased sensitivity of DIA results in more known preys detected.

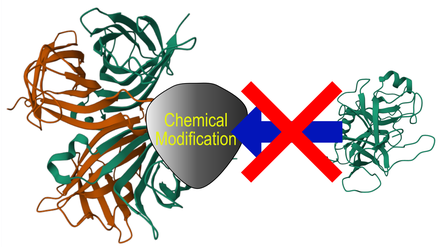




Spectronaut allows for easy comparisons with built in visualization

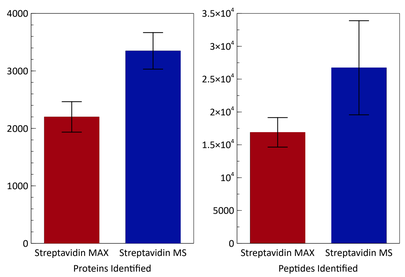
Protease resistant streptavidin magnetic particles

ReSyn Biosciences chemically modifies streptavidin magnetic particles (Streptavidin MS)



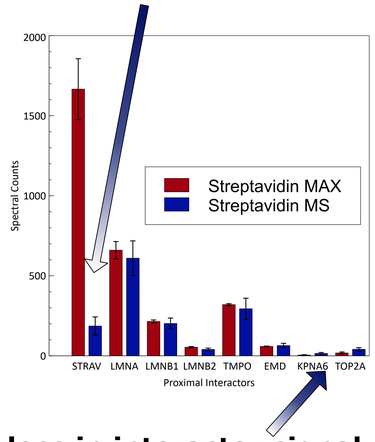
Blocks lysine, arginine residues from attack by trypsin

Reduction of streptavidin digestion increases total # proteins, peptides detected



50,000 HEK293 cells, LMNA tagged with miniTurbo enzyme, plots show mean with standard error, n =3

Large reduction in residual streptavidin



No loss in interactor signal, gains at low level preys

The automated future

10-50,000 cells is approx. amount found in 96-well plate wells

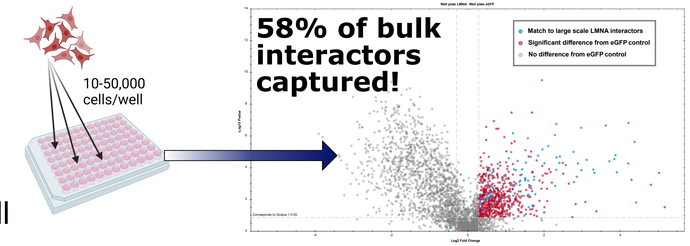
- We can now consider automation with common liquid handler formats

- Additionally, FACS derived proteome differs significantly from well cells

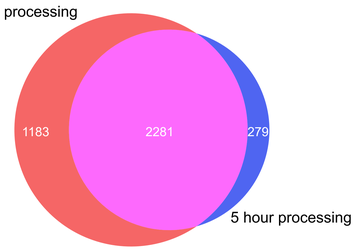
Further modifications allow fast processing

- Reducing capture time to 1h, accelerated digestion at 47°C

Well plate analysis



Accelerated processing - protein identification overlap



Unique hits for each conditions are largely weaker intensity, single peptide IDs

Acknowledgements

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