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Combining µSPE Chip-based CE with PRM-LIVE and Dynamic Targeting Scheduling for Warp-speed Selectivity Profiling of Deubiquitinase Small Molecule Inhibitors

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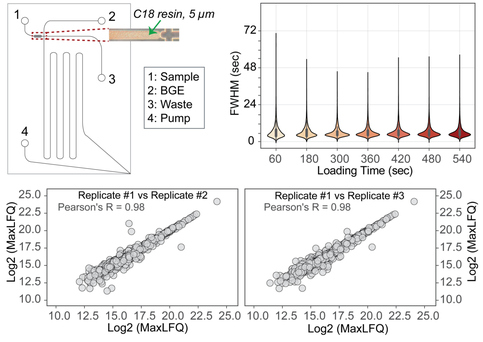
INTRODUCTION

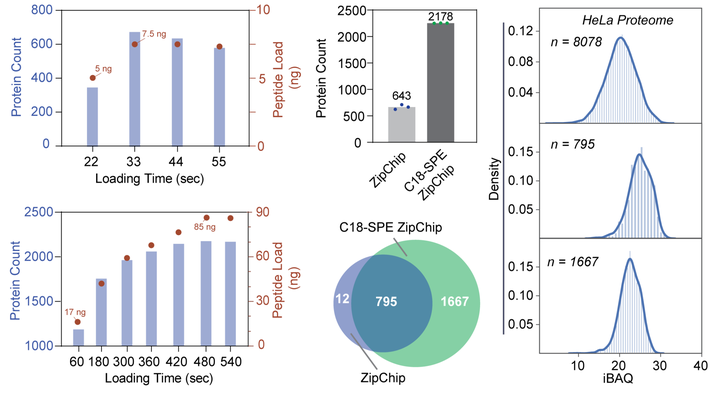
Deubiquitinases (DUBs) comprise ~100 enzymes that cleave ubiquitin from substrates to regulate critical aspects of human physiology. Pharmacologic inhibition of DUBs can have therapeutic benefits in autoimmune disorders, oncology, neurodegeneration, and other indications. Similar to the kinase field ~25 years ago, there are currently no approved DUB-targeting drugs, and most preclinical small molecules are low-potency and/or multi-targeted. To facilitate high-throughput identification of new small molecule inhibitors that target the subset of ~85 cysteine protease DUBs, we developed a novel CE microchip (ZipChip) containing an on-chip C18 bed for sample preconcentration. We coupled this rapid separation platform to our PRM-LIVE acquisition on a timsTOF Pro to enable warp-speed activity-based selectivity profiling (ABPP) of novel small molecule inhibitors against endogenous DUBs.

CHARACTERIZATION OF ON-CHIP PRECONCENTRATION

❖ SPE-ZipChips were constructed by 908 Devices as shown in the nearby scheme. All channels on the device are 10 µm deep × 70 µm wide; the serpentine separation channel is 22 cm long. A SPE bed with a length of 1 mm in the flow path just after the sample well is incorporated to allow packing of a reversed phase bed for on-chip preconcentration. A vacuum source connected to the waste well was used to load peptide samples onto C18 bed.



❖ Across a series of loading time for 1mg/mL HeLa tryptic digest, we observed consistent median peak width across all conditions tested as showed in violin plots. Also, with three replicate injections at a loading time of 480 sec, we found good quantitation correlation between replicates.



❖ Using the same HeLa digest samples, we determined the number of proteins identified by DDA-PASEF as a function of sample loaded onto a commercial ZipChip.

The number of identifications quickly plateaus at 643 proteins corresponding to ~7.5 ng of loaded peptides. We performed a similar analysis with the preconcentration chip SPE-ZipChip and observed a maximum of 2,175 proteins at a loading time of 8min.

❖ Replicate injections (n = 3) using the optimized conditions for each chip architecture further confirmed the performance improvement provided by the preconcentration bed (Bar Plot). Taking the union of these replicate data from each chip configuration we observed that the preconcentration chip captured >99% of all protein identifications, with only 12 proteins identified exclusively by the open channel chip (Venn Diagram).

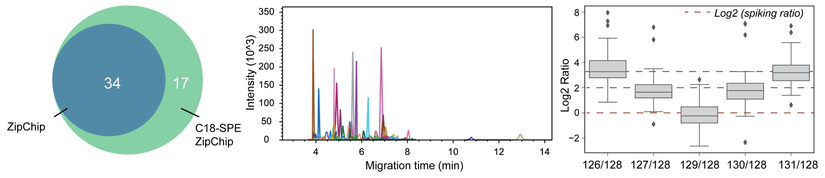
❖ Furthermore, the set of identifications common across both chip configurations was biased towards high abundance proteins relative to the cellular HeLa proteome or the set of proteins detected only with the preconcentration chip (Density Plot).

QUANTIFICATION OF DUBS WITH CE-PRM-LIVE

❖ We used ubiquitin activity probes (ABPs) to enrich DUBs from HEK293T cell extracts. Following tryptic digest and TMT labeling, we performed DDA-PASEF analyses using the SPE-ZipChip to create a peptide spectral library spanning 51 cysteine proteases; some 17 DUBs were not detected on the open channel ZipChip (Venn Diagram).

❖ We established an CE-PRM-LIVE assay to reproducibly detect 175 TMT-labeled peptides spanning 49 DUBs in 14min.

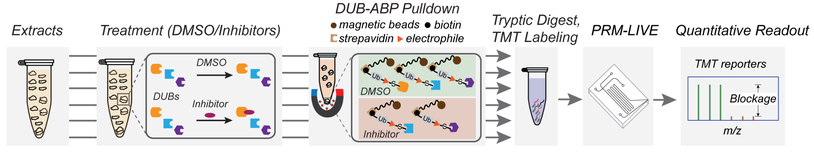
❖ To evaluate the TMT quantitation accuracy of CE-PRM-LIVE, we prepared calibration samples with a known spiking ratio of 10:4:1:1:4:10. The results demonstrated that our TMT version of PRM-LIVE implemented SPE-ZipChip provided average relative error of less than 12%.



SELECTIVITY PROFILING OF DUB INHIBITORS BY CE-PRM-LIVE

➢ Workflow

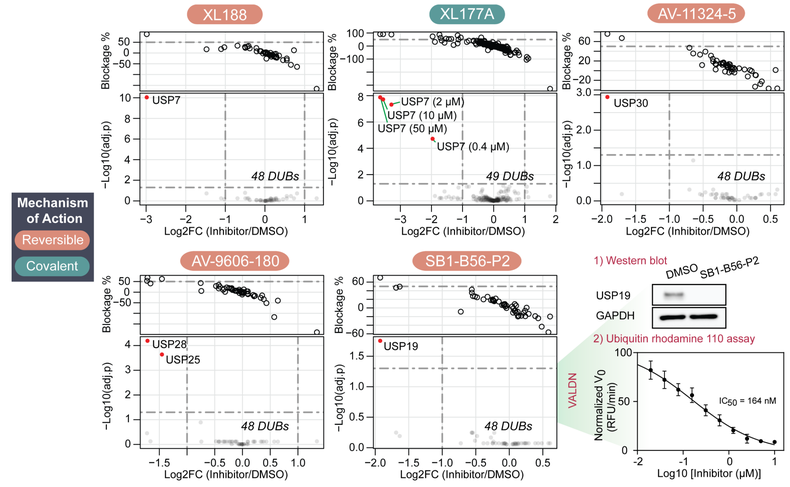
❖ Treatment with small molecule DUB inhibitor is performed in cell extracts level.
❖ DUB enrichment is performed with ubiquitin activity-based probes (ABPs).
❖ DUB peptides were labeled with 6-plex TMT reagents and combined.
❖ Competitive binding of inhibitor vs. ABP was quantified by TMT ratios with CE-PRM-LIVE.



➢ Targeted assay for selectivity profiling of DUB inhibitors

❖ CE-PRM-LIVE assay of DUBs: ~175 peptides/49 unique DUBs @ 14min.
❖ Sample preparation: each TMT 6-plex contained 2× bioreplicate DMSO channels and either a single inhibitor at 4 different concentrations or 4 different inhibitors each at a single concentration. Each TMT set was then prepared with three biological replicates.
❖ Data analysis: Skyline, MSFragger, and MSstatsTMT.
❖ We tested PRM-LIVE using well-studied inhibitors XL188, XL177A, AV-11324-5, and AV-9606-180, and observed selectivity consistent with previous characterization data.

❖ Additionally, we provided the first selectivity against endogenous DUBs for a new USP19 inhibitor. We used biochemical assays to validate our CE-PRM-LIVE data.



SUMMARY AND FUTURE DIRECTION

- The on-chip preconcentration stage opens the door for analysis of complex proteome samples on the ZipChip platform. The SPE-ZipChip exhibited excellent reproducibility (quantification, peak width, and migration time) for identified peptides or proteins.
- The integration of 6-plex TMT labeling and CE-PRM-LIVE provides a high throughput of assay along with accurate quantitation.
- The combination of ABPs for DUB enrichment and CE-PRM-LIVE on the timsTOF Pro enabled quantification of ~175 unique peptides spanning 49 unique DUBs with 6 different treatment conditions in 14 min. CE-PRM-LIVE provides a warp-speed platform for small molecule selectivity profiling against endogenous DUBs.
- We are making efforts to develop PRM-LIVE 2.0 to enable dynamic scheduling of peptide targets of interest, which allows us to schedule more targets while maintaining accurate quantification (more data points across the peak).

ACKNOWLEDGMENTS

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REFERENCES

1. Mevissen, T. E. T.; Komander, D. Annu. Rev. Biochem. **2017**, 86, 159-192.
2. Evans, M. J.; Cravatt, B. F., Chem. Rev. **2006**, 106 (8), 3279-301.
3. Mellors, J. S.; Black, W. A.; Ramsey, J. M. US010403488B2, **2019**.
4. Zhu, H.; Ficarro, S. B.; Alexander, W. M.; et al. Anal. Chem. **2021**, 93 (41), 13791-13799.
5. MacLean, B.; Tomazela, D. M.; Shulman, N.; et al. Bioinform. **2010**, 26 (7), 966-8.
6. Yu, F.; Haynes, S. E.; Teo, G. C.; et al. Mol. Cell Proteomics **2020**, 19 (9), 1575-1585.
7. Huang, T.; Choi, M.; Tzouros, M.; et al. Mol. Cell Proteomics **2020**, 19 (10), 1706-1723.
8. Lamberto, I.; Liu, X.; Seo, H. S.; et al. Cell Chem. Biol. **2017**, 24 (12), 1490-1500 e11.
9. Schauer, N. J.; Liu, X.; Magin, R. S.; et al. Sci. Rep. **2020**, 10 (1), 5324.
10. Kluge, A. F.; Lagu, B. R.; Maiti, P.; et al. Bioorg. Med. Chem. Lett. **2018**, 28 (15), 2655-2659.
11. Varca, A. C.; Casalena, D.; Chan, W. C.; et al. Cell Chem. Biol. **2021**, 28 (12), 1758-1771 e13.
12. Rountree, J. S. S.; Whitehead, S. K.; Treder, A. P.; et al. WO2019150119A1, **2019**.