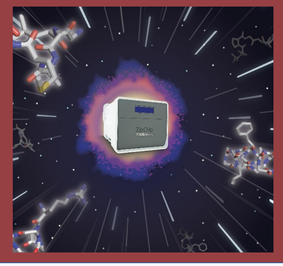

# Combining µSPE ZipChip CE with PRM-LIVE on a timsTOF Pro for Warp-speed Selectivity Profiling of Deubiquitinase (DUB) Small Molecule Inhibitors


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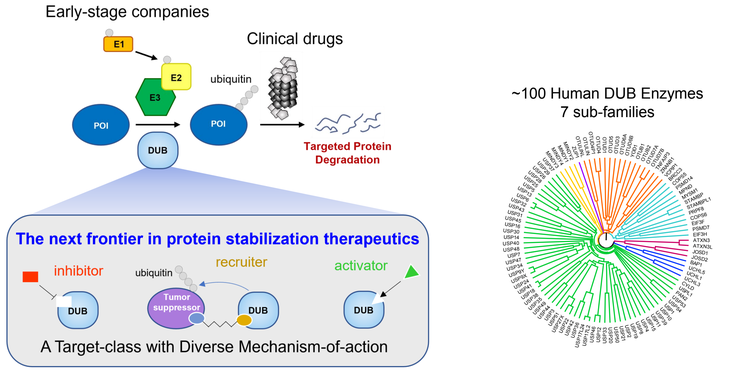
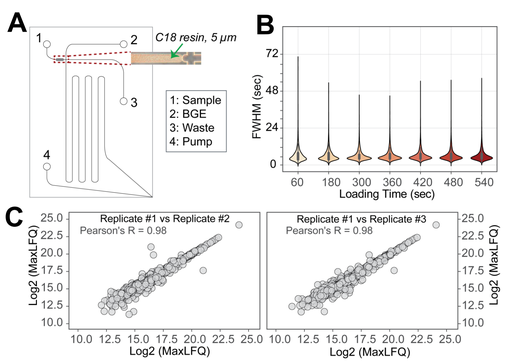
## INTRODUCTION

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.

## THERAPEUTIC TARGETS IN THE UBIQUITIN-PROTEASOME SYSTEM (UPS)

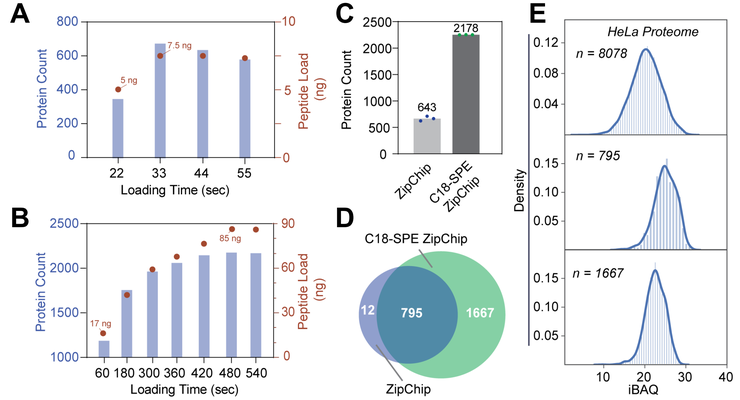



## CHARACTERIZING SPE-ZIPCHIP PERFORMANCE



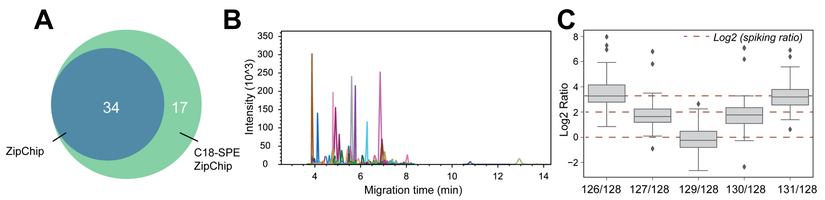

❖ (A) Architecture of SPE-ZipChip. (B) Across increasing sample loads from 1mg/mL HeLa tryptic digest, we observed consistent median peak width for peptide migration. (C) Label free quantification showed excellent reproducibility across replicate injections.

## CHARACTERIZING SPE-ZIPCHIP PERFORMANCE

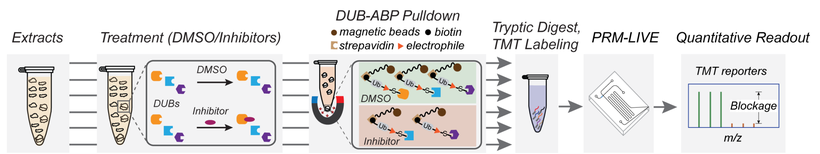



❖ (A) For the standard ZipChip, the number of protein identifications quickly plateaus at ~650 proteins corresponding to ~7.5 ng of loaded peptides. (B) The new SPE-ZipChip enables a 10× improvement in loading (85 ng) along with a significant increase in protein identifications (~2,200). (C) Replicate injections (n=3) using the optimized conditions for each chip architecture further confirmed the performance improvement provided by the SPE preconcentration bed. (D) Proteins identified with the SPE-ZipChip are a superset of those identified on the standard chip. (E) The set of protein identifications common across both chip configurations (n=795, Venn diagram) was biased towards high abundance proteins relative to the cellular HeLa proteome or the set of proteins detected only with the SPE-ZipChip.

## BUILDING A TARGETED DUB ACTIVITY-BASED PROFILING ASSAY

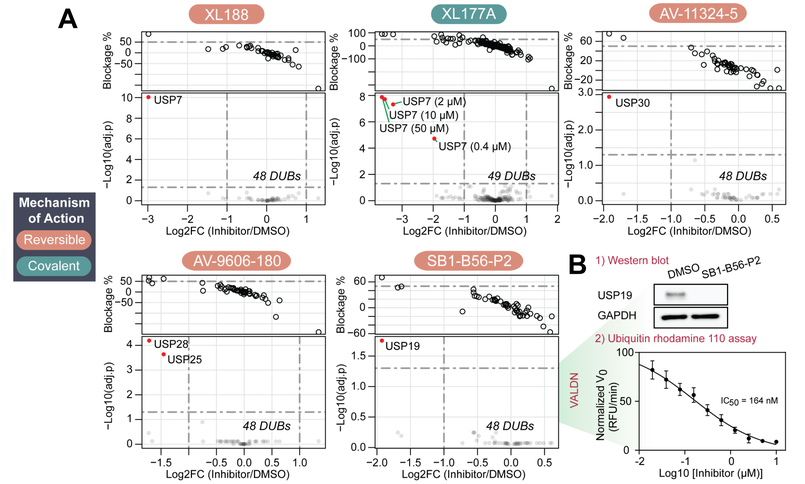



❖ We used ubiquitin activity probes (ABPs) to enrich DUBs from HEK293T cell extracts. (A) Analysis of tryptic peptides demonstrated the expanded DUBome coverage provided by the SPE-ZipChip (B) SPE-ZipChip-PRM-LIVE analysis of a DUB peptide spectral library from these data demonstrated reproducible detection of 175 TMT-labeled peptides spanning 49 DUBs in ~14-min. Samples with known ratios show that our TMT version of SPE-ZipChip PRM-LIVE provided average relative error <12%.



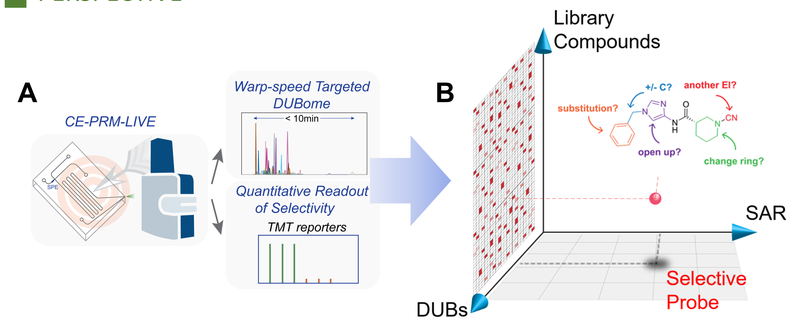

❖ Cell extracts are treated with vehicle or small molecule DUB inhibitor and then co-incubated with a ubiquitin activity probe in a competitive binding assay. DUB enrichment is performed with ubiquitin activity-based probes (ABPs), followed by tryptic digest, TMT-labeling, and SPE-ZipChip PRM-LIVE on a timsTOF Pro mass spectrometer.

## WARP SPEED SELECTIVITY PROFILING OF DUB INHIBITORS BY PRM-LIVE




❖ (A) We used SPE-ZipChip PRM-LIVE to assess binding selectivity of well-characterized DUB inhibitors, including XL188 (USP7), XL177A (USP7), AV-11324-5 (USP30), and AV-9606-180(USP25/28), in addition to a recently reported inhibitor of UPS19 (SB1-B56-P2). These experiments span competitive binding assays performed at a single dose, dose-response, as well as reversible and covalent inhibitors. (B) Biochemical validation assays for competitive binding by western blot and enzyme inhibition using a ubiquitin rhodamine assay.

## PERSPECTIVE




❖ (A) The combination of SPE preconcentration, rapid ZipChip separation, and acquisition speed on the timsTOF Pro provides broad coverage of endogenous DUBs and accurate quantification of competitive binding in a high-throughput ABPP assay. (B) The ability to consolidate initial hit identification with counter screen against the broader target-class in a single high-density chemoproteomic primary screen promises to accelerate the early stages of DUB drug development.

## ACKNOWLEDGMENTS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