MS Bioworks

Protein Mass Spectrometry Services

Mass spectrometry-based proteomics for accelerating PROTAC and molecular glue drug discovery -

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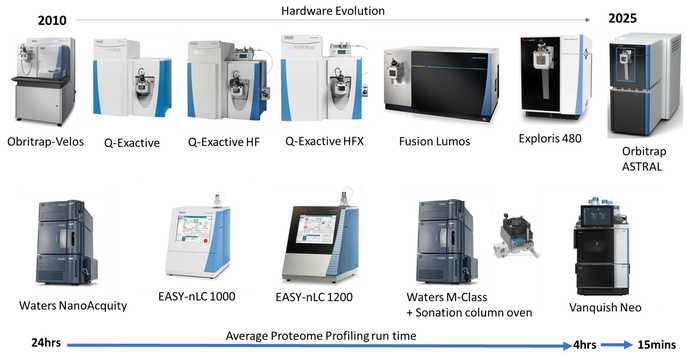
1. Background

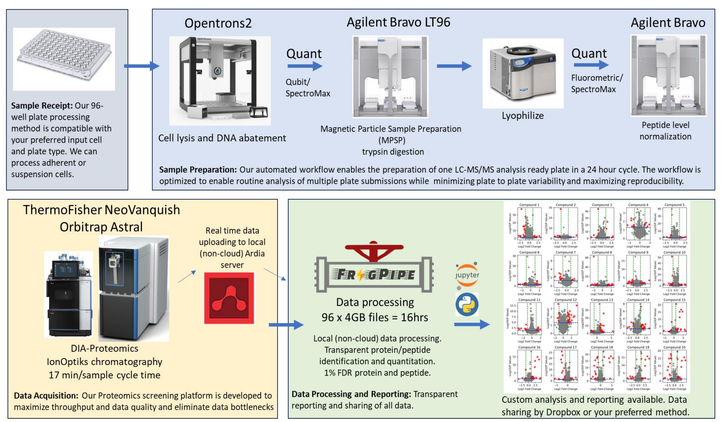
Targeted protein degradation (TPD) using PROTACs and molecular glues represents a transformative strategy in drug discovery. Unlike traditional small molecules, these degraders co-opt the ubiquitin-proteasome system to eliminate disease-driving proteins, offering new therapeutic avenues for previously "undruggable" targets. Mass spectrometry-based proteomics plays a pivotal role in this paradigm, enabling unbiased, high-throughput identification and quantification of proteome-wide changes. Here, we highlight how advanced proteomics workflows accelerate degrader optimization by providing insights into selectivity, mechanism of action, and off-target effects in a single experiment.

Advances in mass spectrometers and nano-scale chromatography have decreased acquisition times for whole proteome profiling while meeting or exceeding expectations for sensitivity. The figure below catches this evolution in our lab.



From its roots in the mid 1980's to present day the workflow for mass spectrometry based proteomics has also evolved. After almost half a century of development most labs would agree that the basic pipeline is described the figure below.

Our goal was to automate the sample preparation workflow and take advantage of the throughput and sensitivity of the Orbitrap Astral mass spectrometer to develop a commercial platform suited to screening degrader molecules. The optimized workflow is described in the figure below.



The platform is capable of processing 96 samples in a 3 day period. A 96 well plate of drug treated plated cells is processed through to mass spec compatible peptides in a single workday. Data are acquired in a little over 24h. Data processing takes 16hrs.

This poster represents the results of a 3 day period of sample preparation, data acquisition and data analysis

2. Test Compounds

Cmpd.	Identification	Expected Degradation Event
1	CC-885	GSPT1
2	Avadomide	ZKSCAN5 (KYSE-30 cells)
3	Pomalidomide	ZFP91
4	Thalidomide	Unspecified
5	Lenalidomide	ZFP91
6	BET degrader	BRD2, BRD4
7	SK575	PARP1
8	NAMPT	NAMPT
9	FC-11	PTK2
10	PT-65	GSK3A, GSK3B
11	TL-12-186	Polypharmic kinase, AURKA, CDK's, others
12	BSJ-03-204	CDK4, CDK6
13	Indisulam	RBM39
14	Kymera SMARCA bispecific	SMARCA2

3. Cell Culture and Treatment

HCC-1806 (breast cancer) and KYSE-30 (esophageal cancer) cells were cultured and harvested at passages 7 and 10, respectively. Cells were trypsinized, centrifuged, and resuspended in warm RPMI-8226 media supplemented with L-glutamine, HEPES, 10% FBS, and antibiotics. To ensure a single-cell suspension, samples were filtered through 35-µm meshes and diluted to 10 mL before counting. Both lines showed high viability (>97%).

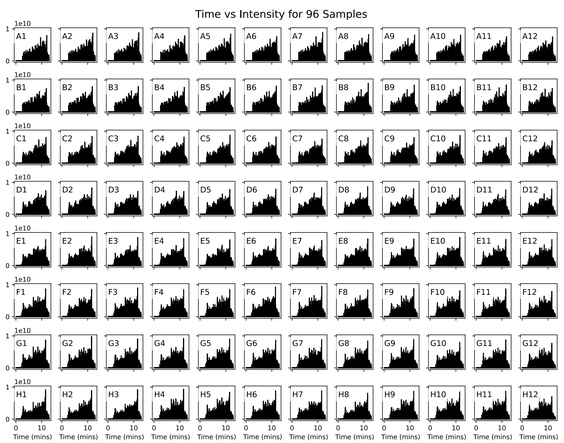
Cells were plated at 25,000 viable cells per well in 96-well plates using a Multidrop dispenser and incubated for 18 hours to allow attachment. After confirming proper cell morphology and confluency, compounds (10 mM in DMSO) were dispensed using a D300e digital dispenser. Plates for the 24-hour treatment were dosed in two phases to manage timing. DMSO volumes were normalized across all wells. After incubation, media was removed, wells were washed with PBS, and plates were sealed and frozen at -80°C for proteomics sample preparation.

4. Total Protein Yields

Total protein amounts are cell line and treatment dependent. The data below are µg total protein per well as determined by Qubit fluorometry.

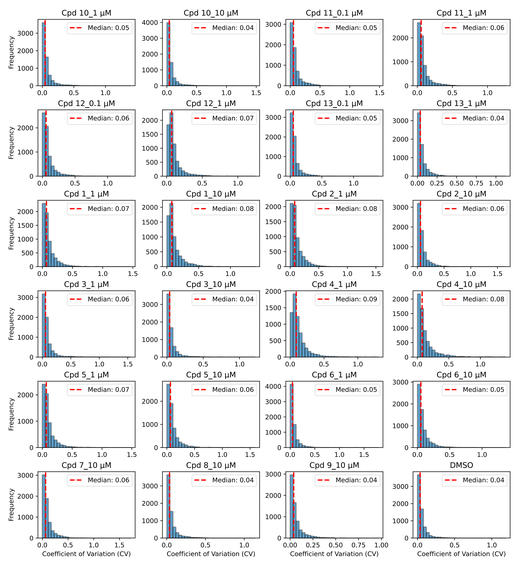
8.8	9.125	8.625	9.35	7.525	8.125	8.1	7.675	8.4	8.325	8.225	7.225
8.6	9.25	8.725	8.725	7.925	7.975	7.425	7.775	8.375	8.3	7.125	7.05
9.95	8.725	8.325	9.375	8	8.125	8.025	7.475	7.675	9.025	7.8	7.475
9.325	8.125	7.525	7.725	8.175	8.15	8.275	7.95	8	8.6	8.275	7.575
8.75	8.1	8.125	8.025	8.875	8.775	9.025	8.4	8.725	9.275	8.825	7.175
8.125	7.5	8.2	7.95	8.35	8.65	8.875	9.325	8.925	8.375	8.1	6.675
8.4	7.8	7.675	7.8	8.275	8.55	8.25	9.175	8.425	8.55	8.275	6.025
7.9	7.75	7.175	7.925	8.05	8.025	8.15	8.3	7.65	8.175	6.925	6.425

5. LC-MS/MS



Robust fast chromatography and cutting-edge Orbitrap-Astral mass spectrometry generate a dense matrix rich in deep proteomics data.

6. Coefficient of Variation

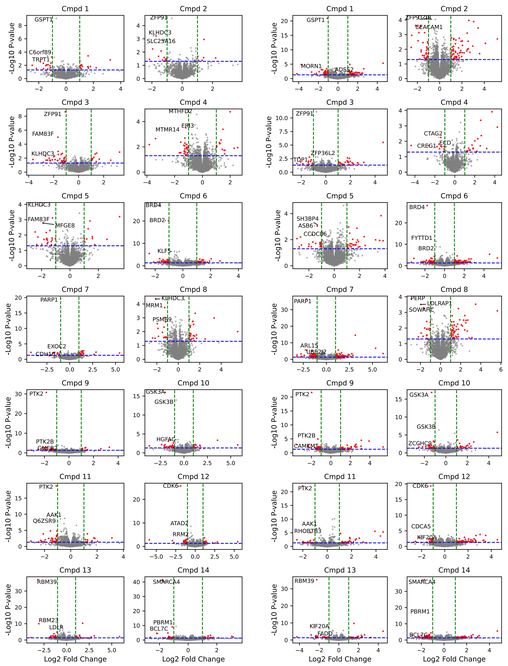


Representative CVs from a typical plate, n = 4. Median CV is indicated.

7. 8hr Treatment

HCC1806

KYSE

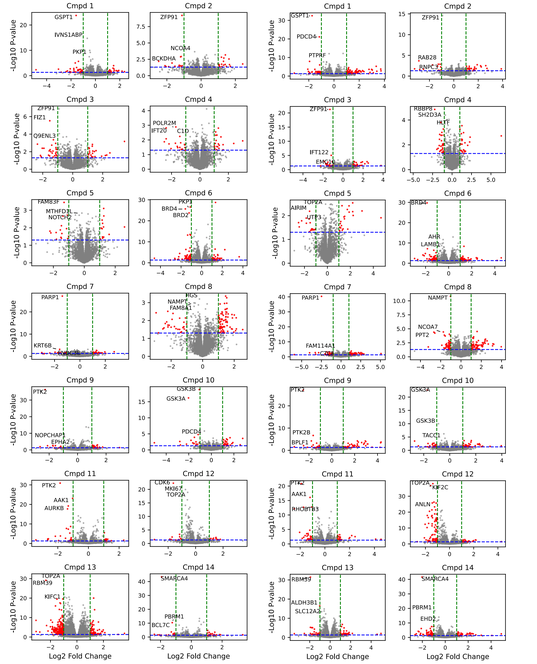


Volcano plots for the 8hr treatment points for two test cell lines. The three most significant degraded proteins are labeled.

8. 24hr Treatment

HCC1806

KYSE



Volcano plots for the 24hr treatment points for two test cell lines. The three most significant degraded proteins are labeled.

9. Summary

We describe an automated, high-throughput proteomics workflow designed to accelerate the discovery and profiling of molecular glues and PROTACs. Utilizing the Orbitrap Astral mass spectrometer, our platform achieves deep proteome coverage with exceptional speed and sensitivity, enabling robust quantitation from minimal input material. Integrated automation, from sample preparation to data processing, ensures consistency and scalability across large sample cohorts. Outputs include differential expression analysis, volcano plots, and funnel plots for rapid identification of degradation targets and selectivity assessment. This streamlined workflow supports early-stage drug discovery by delivering high-confidence insights into compound mechanism and potency.

We continue to refine this platform and develop supporting workflows to meet the evolving needs of degrader-based drug discovery. Our ongoing focus is to push throughput boundaries without compromising data quality—enabling researchers to move from screen to insight faster than ever before.

10. Contact Details

Feel free to contact the authors via email at the following address: info@msbioworks.com.