SAPIENT

APPLICATION NOTE

Protein Degrader Proteomics: from High Throughput Screening to Deep Proteome Profiling for PROTACs and Molecular Glues

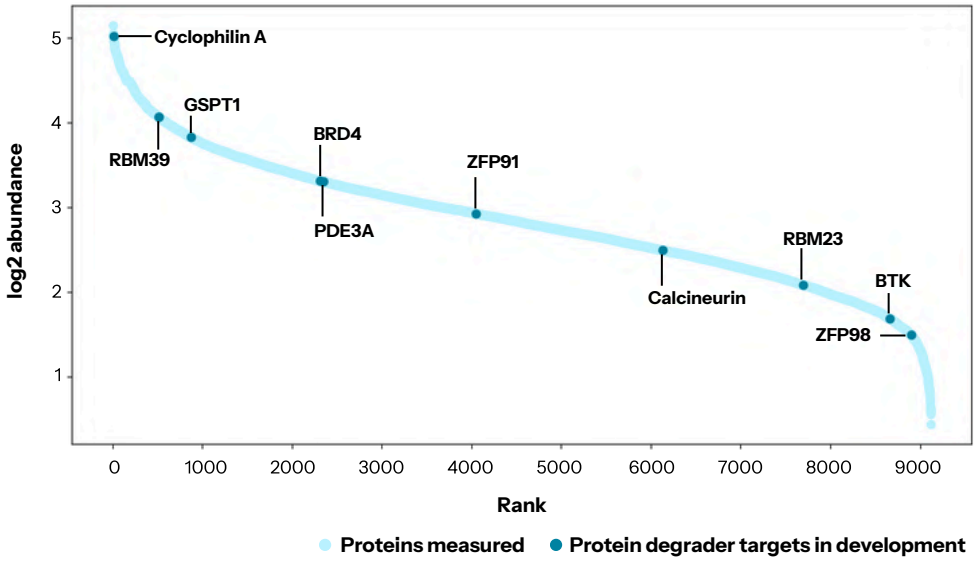
Introduction

Proteolysis Targeting Chimeras (PROTACs) and molecular glues are novel classes of therapeutic agents with potential to transform the treatment landscape for cancer by extending therapeutics to previously 'undruggable' proteins. PROTACs are bifunctional molecules that bind to target sites on protein surfaces and recruit E3 ligases into close contact with the target protein for targeted degradation by the ubiquitin-proteasome system. Molecular glues are small molecules that work in a similar fashion by enabling the interaction of target proteins with E3 ubiquitin ligases for proximity-induced target protein degradation.

Proteomics can help elucidate how these protein degraders interact with their target proteins and the ubiquitin-proteasome system. Analyses can span from broad cellular-based drug screenings of degrader molecules, assessing their selectivity toward a target of interest, to deep profiling of the global proteome for selected degraders to identify potential off-target effects. Understanding the intricate interplay and mechanisms underlying PROTAC and molecular glue action is critical to identify novel targets and for optimizing drug efficacy and safety.

Sapient's mass spectrometry-based **Protein Degrader Proteomics**

Sapient provides a discovery proteomics approach to explore PROTAC-proteome and molecular glue-proteome interactions with **scalable throughput and coverage depth** specifically aligned to support development of these innovative therapeutics.



Our workflow is enabled by Evosep liquid chromatography coupled to state-of-the-art Bruker timsTOF HT mass spectrometers, leveraging label-free, data-independent acquisition (DIA) combined with Parallel Accumulation Serial Fragmentation (diaPASEF) to achieve high confidence protein identification via direct peptide sequencing.

The method features fully automated sample preparation for rapid turnaround and can be tailored for high throughput cell-based screening via **/HT/ Protein Degrader Proteomics** or deep global proteome profiling via **/Deep/ Protein Degrader Proteomics**. Together these approaches deliver essential biological insights to drive timely decision-making in development programs for PROTACs and molecular glues.

/HT/ Protein Degrader Proteomics: optimized for high throughput protein degrader drug screening

Sapient's **/HT/ Protein Degrader Proteomics** workflow provides a high throughput approach to rapidly screen tens of thousands of compounds for their selectivity and specificity towards a protein target of interest.

The short, 5-minute gradient workflow measures >5,000 proteins and post-translational modifications (PTMs) per cellular sample and can be used to process thousands of samples per day. It is an ideal choice to identify viable protein degrader molecules from large chemical libraries which can then be moved forward for additional profiling.

At a Glance

Sapient's /HT/ Protein Degrader Proteomics method enables:

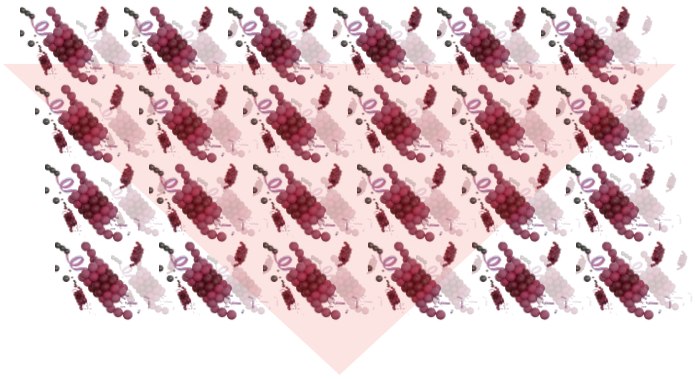
- Measure of **>5,000 proteins and PTMs per cellular sample**
- Processing of **thousands of samples per day**
- Rapid, broad cellular-based protein degrader **drug screening**

/Deep/ Protein Degrader Proteomics: optimized for global profiling of protein degrader-proteome interactions

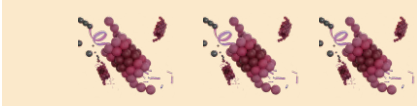
Sapient's **/Deep/ Protein Degrader Proteomics** workflow measures the abundance of >10,000 proteins and PTMs in as little as 20ug protein lysates. This approach is amenable to deeply profile selected or pre-screened protein degraders across different cell lines, drugs, doses, and time points with high accuracy and precision.

Our deep discovery proteomics can be used to confirm protein degrader binding to the intended target and identify putative E3 ligases recruited to ubiquitylate the target protein for degradation. The workflow also enables comprehensive analysis of unintended binding sites and potential off-target effects, thus limiting toxicity and drug failure for protein degraders.

Use **/HT/ Protein Degrader Proteomics** to screen thousands of degrader compounds for a target of interest



Use **/Deep/ Protein Degrader Proteomics** to comprehensively profile degraders with target selectivity



Using stable isotope labeling by amino acids (SILAC) approaches, Sapient can also provide estimation of protein half life, including turnover and resynthesis kinetics, which is central to the design and development of protein degraders.

Additionally, Sapient can monitor for changes in the cellular proteome in longitudinal samples to elucidate cellular mechanisms contributing to development of resistance or adverse events in patient populations over time. This allows for the careful selection of clinically relevant degrader agents.

Conclusion

Sapient's scalable discovery proteomics method provides a robust framework for accelerating the development of PROTACs and molecular glues and allows for broader exploration of novel targets that were once considered undruggable.

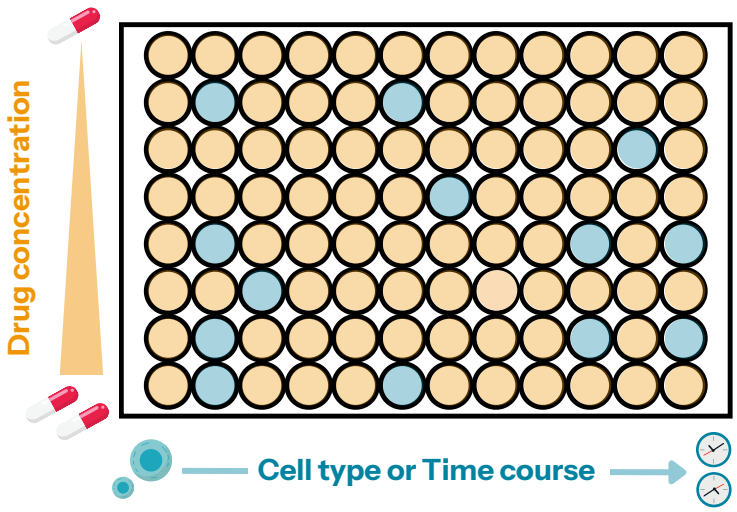
From broad initial cellular drug screenings to elucidating the intricate interactions among degrader agents, selective target proteins, and cellular pathways, we empower researchers to optimize drug efficacy, minimize toxicity, and overcome resistance mechanisms.

Through quantitative insights and kinetics profiling, our platform enables informed decision-making at every stage of the protein degrader development process, ultimately driving innovation and advancement in precision medicine.

At a Glance

Sapient's /Deep/ Protein Degrader Proteomics enables:

- Measure of **>10,000 proteins and PTMs** per cellular sample
- **Quantification** of protein degradation
- Assessment of protein degrader **on-target and off-target effects**
- Estimation of **protein turnover and resynthesis kinetics**
- Monitoring of **proteome changes over time** for resistance and / or toxicity



Sapient's /Deep/ **Protein Degrader Proteomics** can be used to comprehensively profile protein degraders across different cell lines, drugs, doses, and time points.

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