NEOsphere Biotechnologies: Breaking the Barriers of TPD Drug Discovery

Developing novel TPD drug candidates is a complex process owing to the lack of easily druggable pockets on the protein of interest. Mass spectrometry (MS)-based proteomics is essential in revealing the complete target spectrum of degrader molecules, confirming on-target degradation, highlighting off-target regulation and identifying potential novel targets. Here, high throughput and rapid turnaround are key to accelerating drug development and avoiding significant bottlenecks. NEOsphere Biotechnologies specializes in deep proteomic screening and MS-based mechanistic validation of potential novel targets to advance the development of degrader drugs for previously undruggable targets. This interview explores the technology of NEOsphere Biotechnology and its impacts on TPD drug discovery.

Dr. Jutta Fritz

CBO and co-founder
NEOsphere Biotechnologies

Dr. Fritz is a business development expert with more than 15 years of management experience within the life sciences and diagnostics industries. Prior to joining NEOsphere Biotechnologies, she was co-founder and CBO of the cancer diagnostics company NEO New Oncology, VP of Business Development for Proteomics Services at Evotec and Head of Business Development at the proteomics company Kinaxo Biotechnologies. Dr. Fritz has a PhD in molecular biology from the University of Vienna and an MBA in financial management from the University of Wales.

Dr. Uli Ohmayer

Head of Mass Spectrometry and co-founder
NEOsphere Biotechnologies

Dr. Ohmayer is a leading expert in industrial-scale, deep proteomic screening. He has over a decade of experience in MS-based proteomics and broad expertise in laboratory automation and rapid scaling of proteomics infrastructure. In his previous position at Evotec, he was instrumental in the development of data-independent acquisition MS for single-shot proteomics with unprecedented throughput, depth and sensitivity. Dr. Ohmayer has a PhD in biochemistry from the University of Regensburg and was a postdoctoral researcher in the Mass Spectrometry Core Unit of Helmholtz Zentrum Munich.

Q: Can you give us a brief overview of NEOsphere Biotechnologies? What sets you apart from other companies in the field?

Jutta Fritz (JF): NEOsphere Biotechnologies is a leader in the field of MS-based proteomics for drug discovery, with a focus on targeted protein degradation. Founded in 2022 and located in Munich, we work with pharmaceutical and biotechnology companies to systematically evaluate the true target scope of their degrader compounds in a proteome-wide context.

Our technology combines the highest data quality and proteome coverage with high throughput and fast turnaround time, making it ideal for supporting drug discovery and optimization. To this end, all our laboratory and data analysis processes are automated and scalable.

Our deep proteomic screening platform reveals changes in protein regulation upon compound treatment. The analysis is performed on intact, unmodified cells to monitor compound selectivity in endogenous environments. We routinely quantify up to 11,000 proteins in a single experiment, allowing us to comprehensively evaluate degrader efficacy, assess off-target effects and identify potential new target proteins for degraders. In this regard, our deep proteome coverage combined with reliable protein quantification is critical for the identification of low-abundance proteins that may be of great interest for drug discovery, such as transcription factors. It is also possible to analyze the effect of a degrader on the whole proteome at different time points or concentrations to determine how quickly and strongly it acts and at what point secondary effects may occur. Thanks to our high-throughput capabilities, we can screen degrader libraries of thousands of compounds (for an example of how deep proteomic screening data is presented, see Figure 1).

In addition to deep proteomic screening, we offer MS-based technologies to mechanistically validate potential degrader targets, e.g., by ubiquitinomics or high throughput interactomics. In this way, potentially interesting hits from the deep proteomic screen can be further investigated immediately.

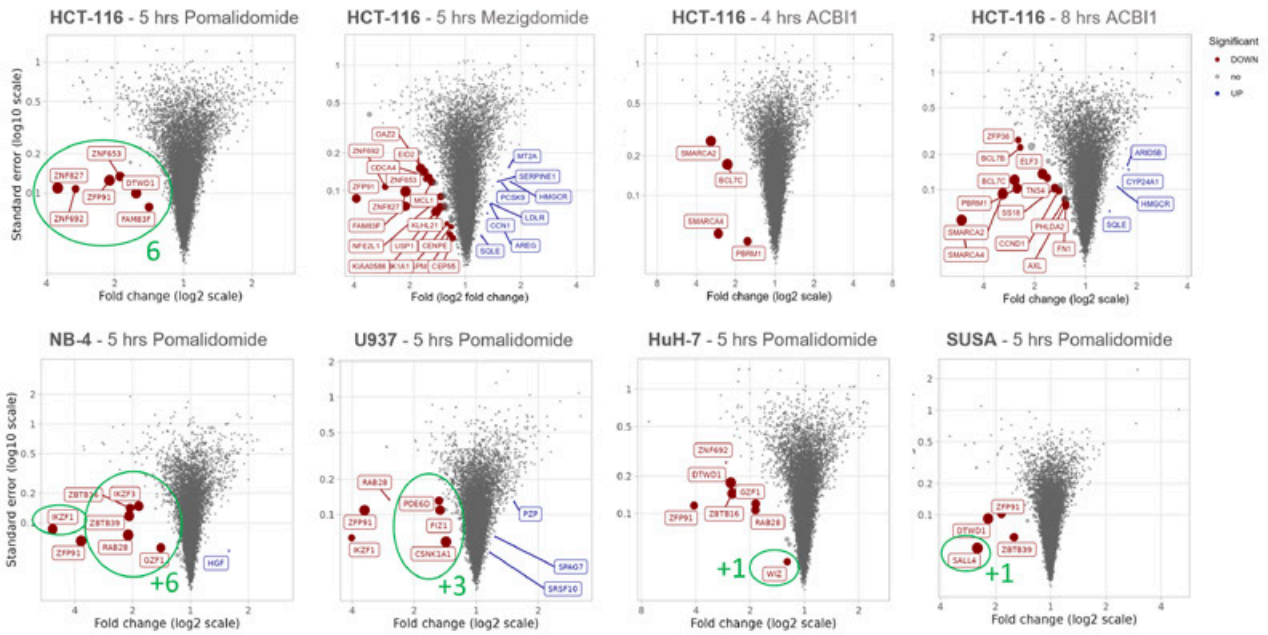

Figure 1. Comprehensive detection of compound-specific and cell line-specific degradation events. HCT-116 cells were treated with two immunomodulatory imides (IMiDs) and one PROTAC as indicated (upper panel) and analyzed by single-shot MS analysis. The volcano plots illustrate significant up- (in blue) and downregulations (in red) induced by the different compounds. The x-axis depicts the fold change (log2) of proteins in compound vs DMSO-treated cells and the y-axis depicts the standard error. Known cellular targets such as zinc finger proteins were detected for pomalidomide and mezigdomide. SMARCA2 and SMARCA4 were downregulated upon 4 hours treatment with the VHL based PROTAC ACBI1, together with two interacting BAF complex members, while secondary regulation was seen at a later 8 hours time point. Additional cell lines were treated with pomalidomide for 5 hours (lower panel) and compared to HCT-116. ZFP91 was significantly down-regulated in all cell lines, other neosubstrates exhibited varying degrees of regulation, reflecting cell type-specific expression (e.g., IKZF1 in U937 and NB-4, or SALL4 in SUSA) or different levels of IMiD responsiveness to commonly expressed neosubstrates. In total, 17 different neosubstrates were significantly downregulated in at least one cell line.

Q: What is your process for screening potential TPD drug candidates?

Uli Ohmayer (UO): To evaluate and quantify the effect of a compound on protein degradation while maintaining very short turnaround times, we have developed scalable and robust laboratory processes and statistical methods. We routinely work in a 96-well plate format and test every compound in triplicate, which enables very powerful statistical analyses. Like compound treatment, sample preparation is largely automated. First, we perform a tryptic digest followed by peptide purification so that the complete cellular proteome is cleaved into peptides, which are then separated by liquid chromatography (LC) and analyzed by MS.

To minimize technical variation, maximize data completeness and significantly reduce measurement time for mass spectrometry, we don't use chemical labeling followed by off-line fractionation, but measure samples in a label-free single-shot approach. For MS analysis, we use dia-PASEF (data-independent acquisition and parallel accumulation and serial fragmentation) on timsTOF instruments from Bruker. Here, ion mobility separation is used to reduce signal interference and increase the sensitivity of proteomics analysis. This results in very deep proteome coverage and detection of more than 200,000 precursor ions in one sample, corresponding to approximately 11,000 proteins. Each mass spectrometry run generates a large amount of highly complex raw data. We use DIA-NN software developed by one of our scientific advisors, Dr. Vadim Demichev, as well as proprietary data analysis and statistical tools developed at NEOsphere for the analysis. To detect statistically significant protein regulation upon compound treatment, a comparison is made with untreated controls.

To determine whether regulation is due to protein degradation or other effects, we can then perform mechanistic validation of all potential hits identified in the screen using additional MS-based tools such as interactomics or ubiquitinomics. Degrader compounds induce proximity between the E3 ligase and the protein of interest (POI) and initiate ubiquitination of the POI, marking it for degradation by the proteasome. Our ubiquitination assay quantifies up to 50,000 ubiquitination sites in a single experiment. Comparing the regulation of ubiquitination sites in treated and untreated cells provides clear clues as to whether the protein regulation observed in the proteomic screen is indeed due to degradation. NEOsphere's ubiquitinomics platform stands out in the field for its depth, speed, precision and throughput.

Q: How do you optimize the assays in your pipeline to ensure maximum efficiency?

OU: We have systematically tested and optimized a variety of parameters for all the

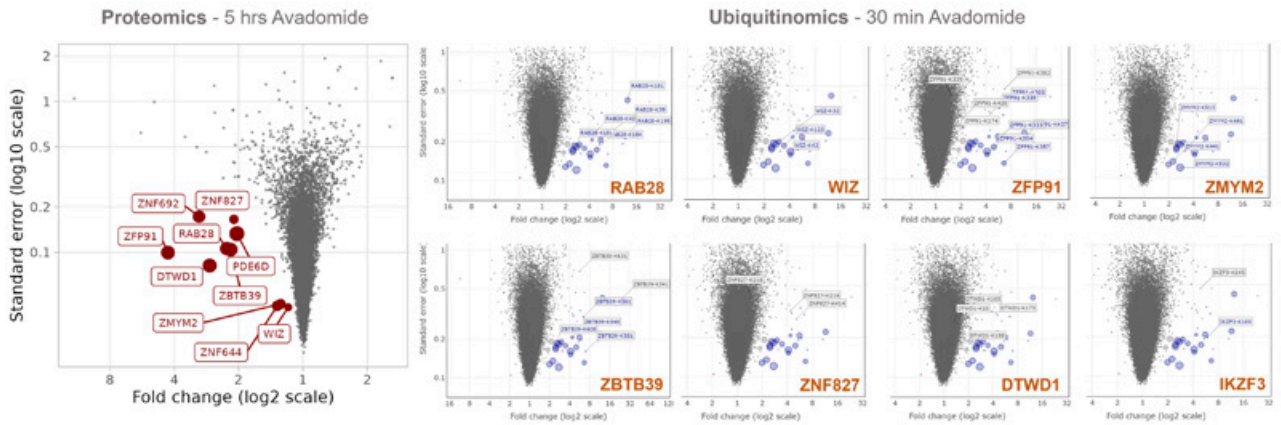

Figure 2. Combined analysis of proteomics and ubiquitinomics reveals and validates primary degrader targets. HEK293 cells were treated with the cereblon modulator avadomide. Ubiquitinomics was performed using MS-based K-GG remnant profiling, enabling the quantification of up to 50,000 ubiquitination sites in single DIA-MS runs without cellular proteasome inhibition. Ubiquitinomics revealed induced ubiquitination sites for almost all neosubstrates found to be degraded upon 5 hrs treatment of HEK293 cells. These included sites on IKZF3, a protein not detected in the proteomics experiment due to its extremely low expression in HEK293 cells. Ubiquitinomics allows the rapid validation of cellular downregulations due to E3 ligase-neosubstrate relationships, by analyzing degrader drugs in endogenous cellular systems without the need for pharmacological intervention or genetic modification.

steps of our workflow, from cell treatment and sample preparation to data acquisition, data analysis and statistical evaluation. We have also adjusted all instrument settings to simultaneously achieve very deep proteome coverage and precise protein quantification. At each stage there are many details that can be optimized, and although each of these individual changes may have a rather small effect, they multiply and eventually lead to a highly efficient process.

JF: Continuous optimization is very important – our workflows are state-of-the-art, but it's a constant process to maintain them as such and to keep pushing the technological boundaries. All our processes are designed to best support the requirements of drug discovery. We can analyze all types of degraders such as molecular glues, PROTACs, DUB-inhibitors and monovalent degraders. The technology can also be used to measure the effect of protein stabilizers on the proteome. In terms of material, we are very flexible and can use, for example, adhesive and suspension cell lines or primary cells.

Q: How do you validate your results? What kind of measures do you take to ensure that they are reliable and reproducible?

UO: Our technology is quite sophisticated and complex, so strict quality control must be performed on every sample. For example, we continuously check parameters such as digestion efficiency, mass accuracy or peak widths in near real-time to ensure that the performance is always at the required level. By testing samples in triplicate, we can calculate a coefficient of variation and determine reproducibility and consistency between replicates. Our automated data processing includes numerous stringent control mechanisms to ensure the highest data quality. For example, filtering is applied to further enhance data completeness and allow precise quantification of even low abundance proteins, and numerous biostatistical tests are routinely performed to improve statistical power.

Q: What are some of the biggest challenges you face when screening degraders and how do you overcome these challenges?

UO: Apart from turnaround time, one of the biggest challenges in deep proteomic screening is throughput, especially when testing large libraries of tens of thousands of compounds. Screening therefore requires a platform that can measure multiple samples in parallel while keeping the time to analyze a set of samples as short as possible. To meet this requirement, we have built our platform so that each step is scalable. If a project requires screening of large number of compounds, we can thus meet that demand very quickly.

Q: How do you see the field of TPD evolving over the next few years? What role do you think MS will play in shaping the future of this industry?

UO: The structure–activity relationship is very steep particularly for molecular glues – even changes by one atom can cause significant changes in compound potency and cellular target selectivity. MS is the key technology to identify these changes and potential off-target effects. The proteomic data we generate is also very useful for developing the chemistry of degrader drug candidates. Chemists can use our data to further optimize the chemical structure of the degraders and implement a more rational approach to degrader design, making drug discovery much faster and more reliable than current methods.

JF: Targeted protein degradation holds enormous potential to address many urgent clinical needs. It's still a young but rapidly growing field, with several promising compounds currently in clinical trials. In our opinion, MS-based proteomics will become one of the key factors for successful development of degrader compounds and thus will gain much importance in the future.

Excerpt from the e-book "Navigating Targeted Protein Degraders" available in full length at: TIMS: A Powerful Tool for TPD Drug Discovery and Development | Bruker