NEOsphere
BIOTECHNOLOGIES

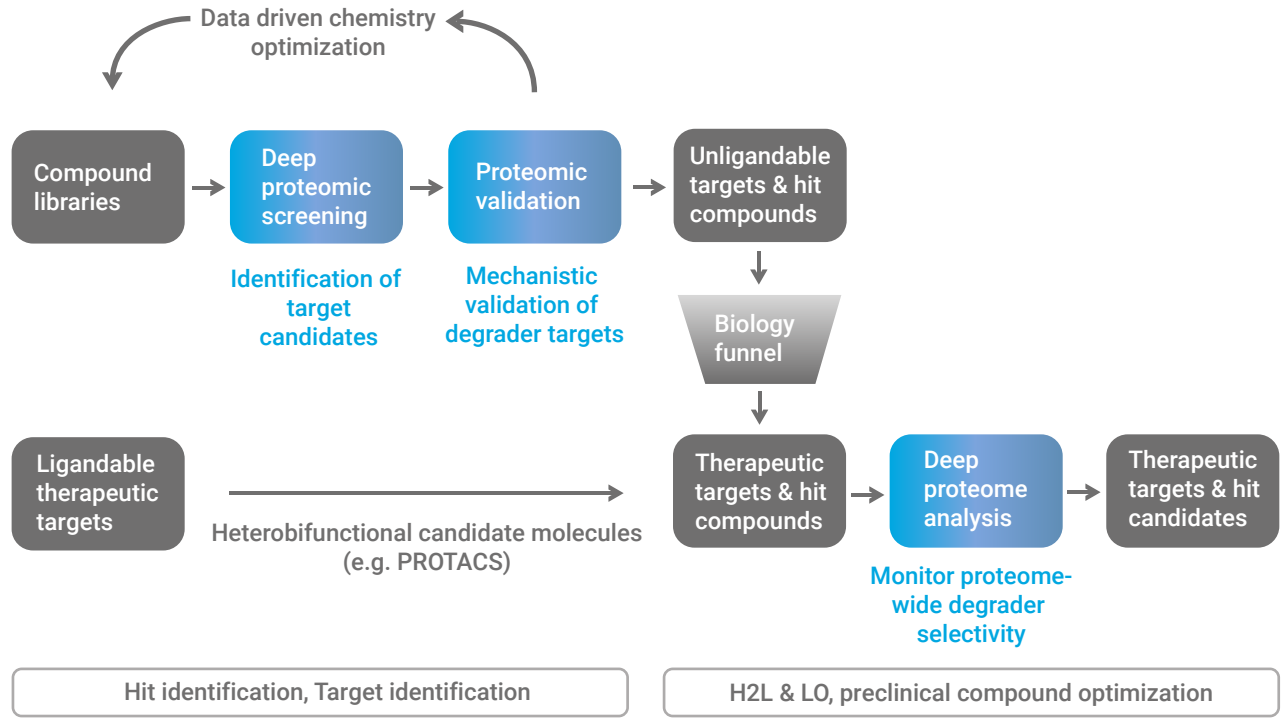
Unlock the therapeutic potential of targeted protein degradation with deep proteomic screening

First in class proteomic screening to systematically identify and validate degrader targets

NEOsphere Biotechnologies combines world-class mass spectrometry with expert data analysis to advance targeted protein degradation (TPD) drug discovery programs at all stages.

Our target and E3 ligase-agnostic **deep proteomic screening** platform characterizes all types of degraders (e.g., PROTACs, molecular glues, monovalent degraders, DUB inhibitors) with high throughput and short turn-around times, supporting systematic identification of novel target candidates and proteome-wide selectivity profiling in drug optimization cycles. Potential degrader targets identified in screening can be rapidly and reliably mechanistically validated using our unique proteomics-based **global ubiquitinomics** and **interactomics platforms**.

Proteomics data can be conveniently reviewed with our comprehensive **data analysis suite** to be used for degrader library optimization and expansion, as well as for computational approaches such as AI-based predictive modeling of degrader drugs.



Performed by NEOsphere Biotechnologies

Performed by partner

Reveal the true target scope of targeted protein degradation

- Routinely test degrader compounds against 11,000 proteins per sample in intact cells
- Monitor proteome-wide selectivity at short turnaround times compatible with drug optimization cycles
- Screen entire degrader libraries of 1,000s to 10,000s compounds at high-throughput
- Reliably detect even low abundance target proteins such as transcription factors
- Discover and mechanistically validate novel degrader targets
- Confirm degrader-induced modification with global ubiquitinomics that quantifies > 50,000 ubiquitination sites in a single experiment
- Detect degrader-induced E3 ligase binding by mass spectrometry-based interactomics
- Identify molecules that act through new E3 ligases and emerging TPD mechanisms

High-throughput proteomics in drug discovery compatible turnaround times

Cell treatment and automated sample preparation

- **Automated, scalable workflows** for efficient and reproducible cell lysis, proteolytic cleavage, and peptide purification in 96-well format
- Routine screening of **cell lines and primary cells** in **three replicates** per treatment condition
- **Optimized experimental design** to filter out noise and identify statistically significant downregulations of target candidates

Single-shot liquid chromatography mass spectrometry

- Outstanding performance through leading expertise in **multiparameter optimization** to maximize ion utilization and resolution in data independent acquisition mass spectrometry (DIA-PASEF[1])
- **Screening compatible**. Up to **11,000 proteins per sample** can be reliably quantified in **thousands and tens of thousands of samples**

Automated data analysis & identification of novel target candidates

- Data processing using customized **DIA-NN**[2] and internal **data analysis** for maximum **coverage, accuracy, precision and data completeness**
- **Scoring & reviewing** to identify likely direct degrader targets
- **Comprehensive statistical analysis** including filtering, normalization and batch correction procedures

Mechanistic validation of degrader targets

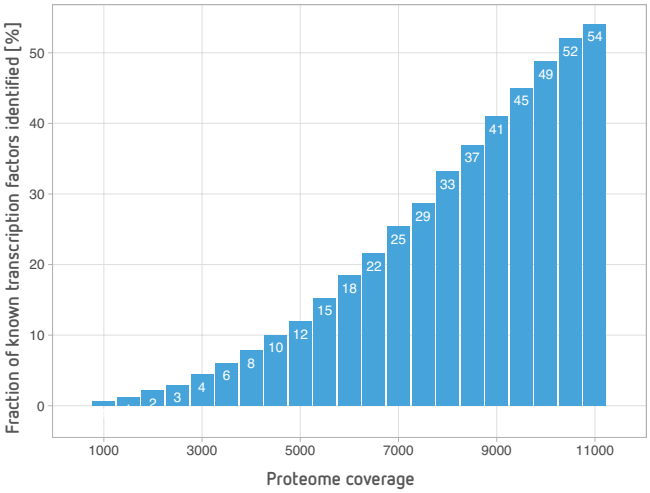
- Verification of **E3 ligase** and **UPS dependency**
- **Fast and reliable validation of E3 ligase - neosubstrate relationships** by global ubiquitinomics, quantifying up to 50,000 ubiquitination sites without proteasome inhibition
- Easy analysis of **compound-induced ternary complex formation** by mass spectrometry-based interactomics (affinity enrichment and cellular proximity labeling)

1 Meier et al, 2020, Nature Methods.
2 Demichev et al, 2020 Nature Methods.

Proteomic degrader analysis at highest sensitivity and proteome coverage

DEEP PROTEOMIC SCREENING - KEY FACTS

- routine proteome coverage and quantification of up to 11,000 proteins per cell line per sample in single-shot MS analysis*
- data completeness on protein level of > 99%
- median protein CVs of ~ 5%
- > 200,000 precursor ions with > 98% data completeness
- 1% false discovery rate **

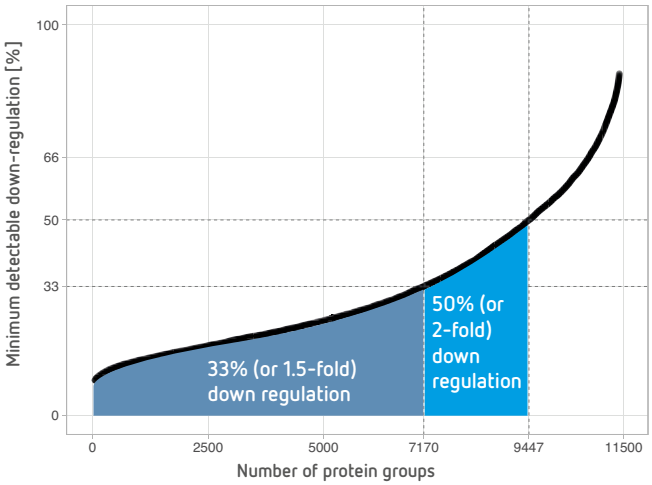


High coverage identifies low abundance target proteins

Deep proteome coverage significantly increases detection of potential low abundance degrader target proteins of therapeutic relevance, such as transcription factors.

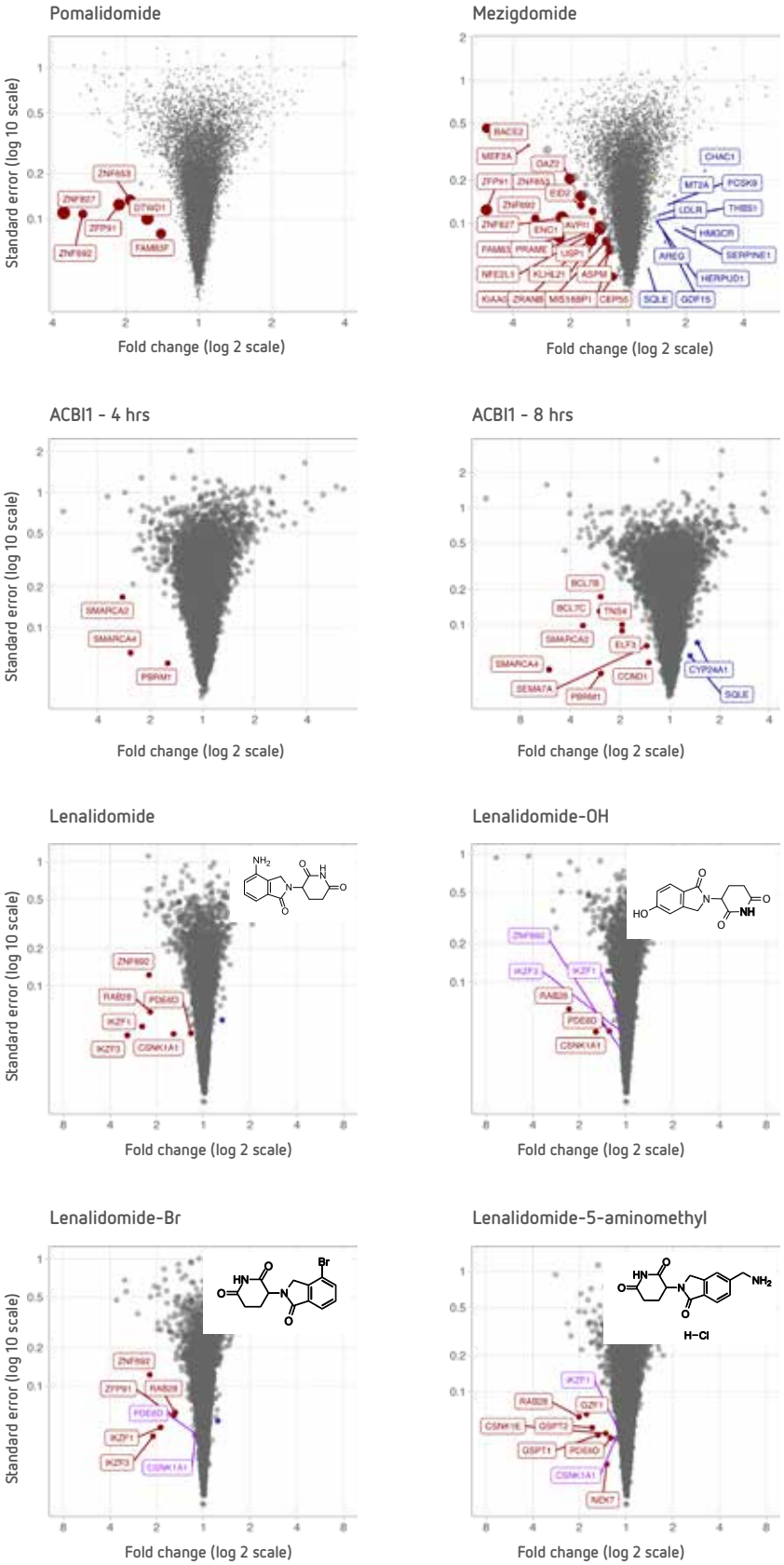
Highest sensitivity to detect even small protein changes

Precise quantification performed in replicate treatments detects significant downregulations of two-fold or less for almost 10,000 quantified proteins.



* Encoded by app. 10,500 genes
** Empirically tested by mixed species approach

Enabling and supporting degrader drug discovery



Comprehensive target identification in native cells

Deep proteomic screening enables unbiased target profiling of degrader compounds in cell lines (adherent or suspension) or primary cells (e.g., PBMCs).

Known and novel target proteins are reliably identified, as shown for molecular glues such as the cereblon based degraders pomalidomide and mezigdomide, or for PROTACS such as the VHL based ACBI1.

Data-driven compound and SAR optimization

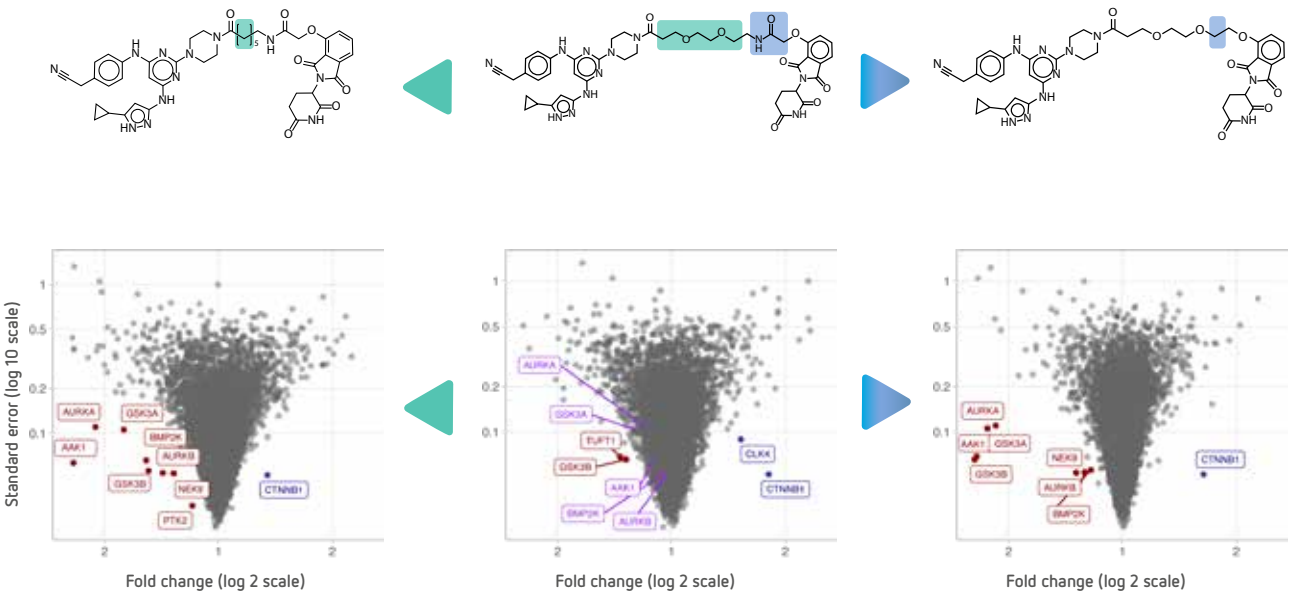
Subtle chemical changes can have profound effects on cellular selectivity that can be revealed by deep proteomic screening.

Presented are lenalidomide derivatives with their different target spectra.

HCT-116 or MM-1S cells were treated with degrader compounds either for 5 hrs or as indicated and analyzed by single-shot MS analysis. The volcano plots illustrate significant up- (in blue) and downregulations (in red) induced by the different compounds. Proteins marked in purple were identified in the respective cell line but were not significantly regulated. The x-axis depicts the fold change (log2) of proteins in compound vs. DMSO-treated cells, the y-axis the standard error.

Optimization of compound efficacy and selectivity

Deep proteomic screening driven linkerology



The effect of compound treatment on cellular regulation
Volcano plots show the effect size (x-axis) versus observation uncertainty (y-axis). Epithelial human breast cancer cells (MDA-MB-231) were treated for 6 hours each, subsequently deep proteomic screening was performed. Proteins significantly downregulated upon compound treatment are shown in red, upregulated proteins are shown in blue. Proteins marked in purple were identified in the respective cell line but were not significantly regulated.

PROTACs with the same kinase ligand and the same cereblon warhead, differing only in the linker regions, were analyzed by deep proteomic screening to reveal the effects of linker modification on proteome wide cellular regulation.

The reference compound in the middle induced weak degradation of GSK3B as the only protein kinase affected. The absence of the peptide bond and an adjacent methylen in the linker (in blue) increased potency for GSK3B and resulted in broader selectivity towards kinases such as AAK1 and AURKA.
Absence of two ether groups (in green) altered both compound potency and selectivity, with AAK1 and AURKA being more strongly degraded than GSK3A/B. Both linker modifications also led to weaker downregulations of other protein kinases.

SGC
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Collaboration with the Structural Genomics Consortium at the Goethe University Frankfurt (SGC Frankfurt)

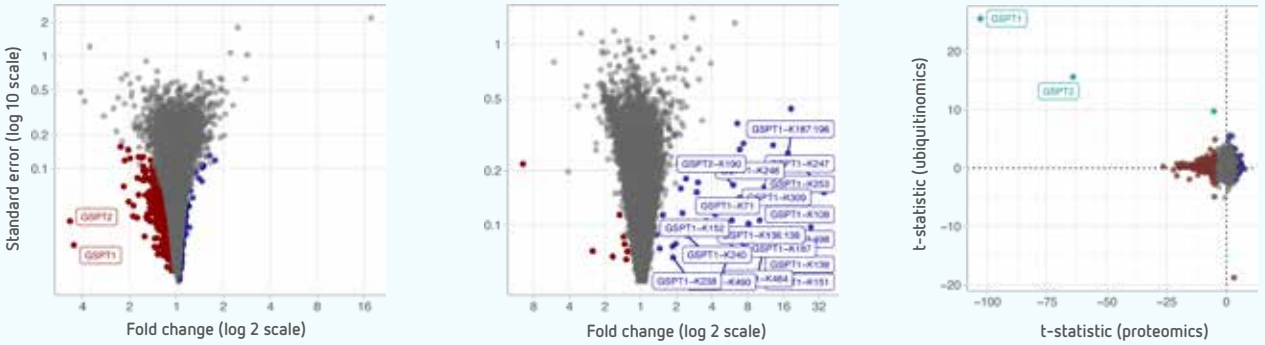
Global ubiquitinomics is a powerful tool to mechanistically validate degrader targets

Deep proteomic screening
5hrs compound treatment

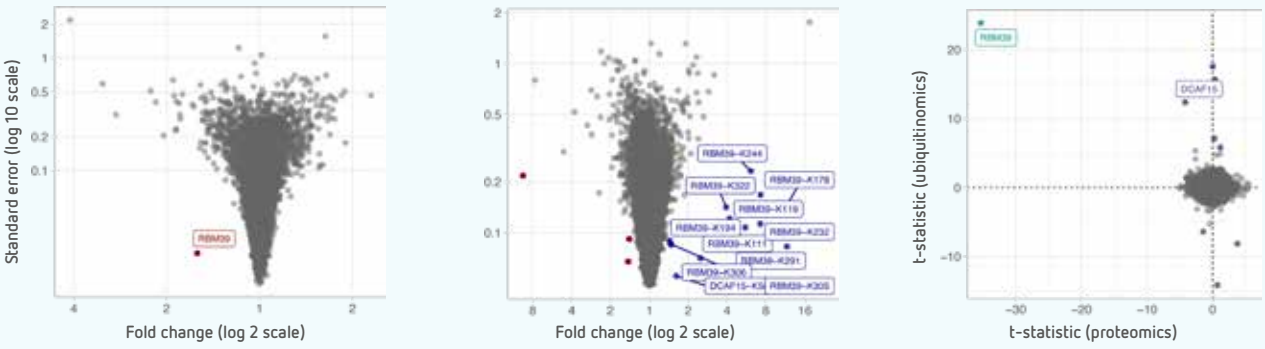
Global ubiquitinomics
30mins compound treatment

Proteomics vs ubiquitinomics
t-statistic comparison

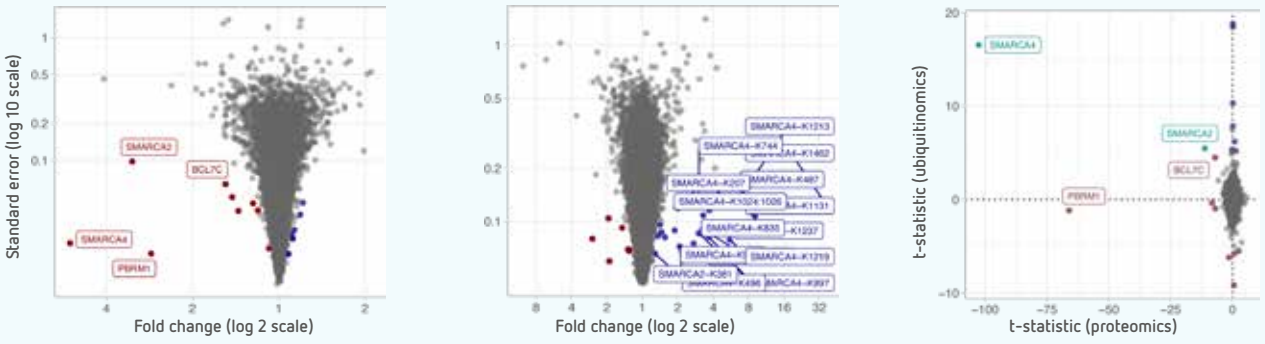
CC885 - molecular glue recruiting cereblon



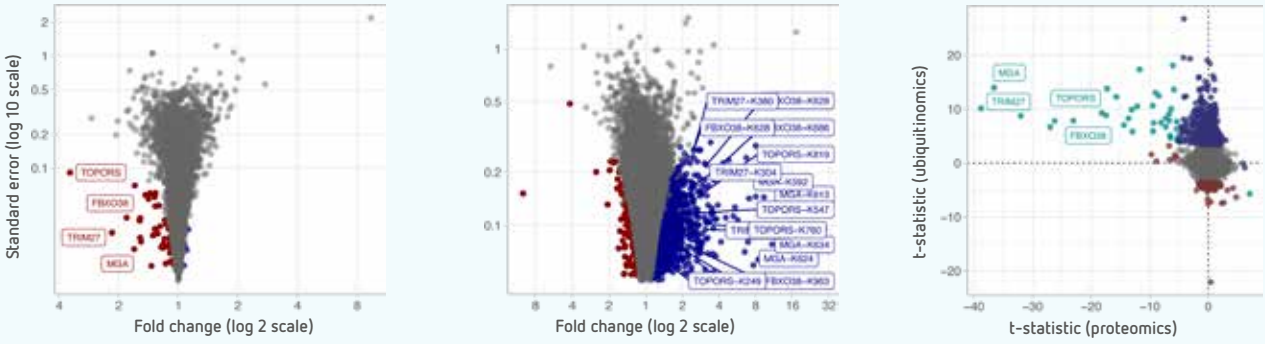
Indisulam - molecular glue recruiting DCAF15



ACBI1 - PROTAC recruiting VHL



FT671 - DUB inhibitor targeting USP7



Quantification of up to 50,000 individual ubiquitination sites in one experiment

GLOBAL UBIQUITINOMICS – KEY FACTS

- strong mechanistic validation of cellular downregulation due to E3 ligase-target interaction
- based on KGG-remnant profiling[1] followed by single-shot DIA-MS technology
- short treatment times (e.g., 30mins) detect rapid ubiquitination events
- ubiquitination profiling to a depth of 50,000 sites, comprising the highest coverage, sensitivity and precision of quantification ever reported
- tailored neural network-based data processing further enhances identification of ubiquitination sites through AI-driven data analysis
- fast analysis of degrader drugs in endogenous cellular systems without the need for proteasome inhibition or genetic modification

Identify true degrader targets at a glance

Deep proteomic screening and global ubiquitinomics were performed in parallel without proteasome inhibition on HEK293 cells with degrader molecules recruiting different E3 ligases. Comparison of the t-statistics of the two experiments allows immediate assessment which proteins are both degraded and ubiquitinated upon treatment and are thus most likely direct degrader targets.

For example, treatment with the molecular glue CC885 leads to significant downregulation and ubiquitination of its known target proteins GSPT1 and GSPT2. Similarly, the known targets RBM39 of the DCAF15 recruiting molecular glue indisulam and SMARCA2 and SMARCA4 of the VHL based PROTAC ACBI1 could be confirmed. Treatment with the USP7 inhibitor FT671 leads to downregulation and ubiquitination of known USP7 substrates such as MGA.

1 Steger et al, 2021, Nature Communications, link to PDF on the right

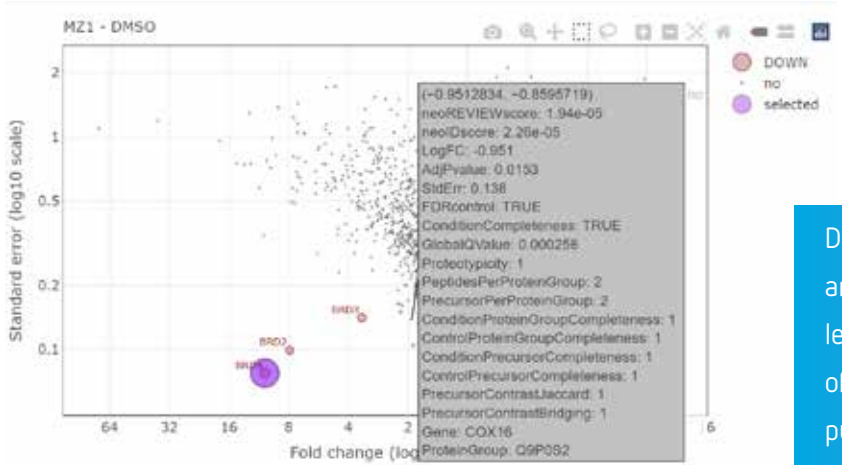
Data analysis suite

NEOsphere Biotechnologies combines DIA-NN software with a powerful proprietary biostatistical pipeline for rapid, automated analysis of large-scale proteomics and ubiquitinomics data, achieving unparalleled precision of quantification, data completeness, and sensitivity.

Routine data processing and statistical analysis include extensive quality control of protein identification and quantification, sophisticated data filtering, proprietary data normalization and batch correction, and highly sensitive differential abundance testing based on refined linear models.

Our proprietary data analysis suite enables user-friendly data evaluation and in-depth meta-analyses to make proteomics data immediately accessible for SAR-optimization, target identification, compound optimization, library expansion, and drug discovery decision making.

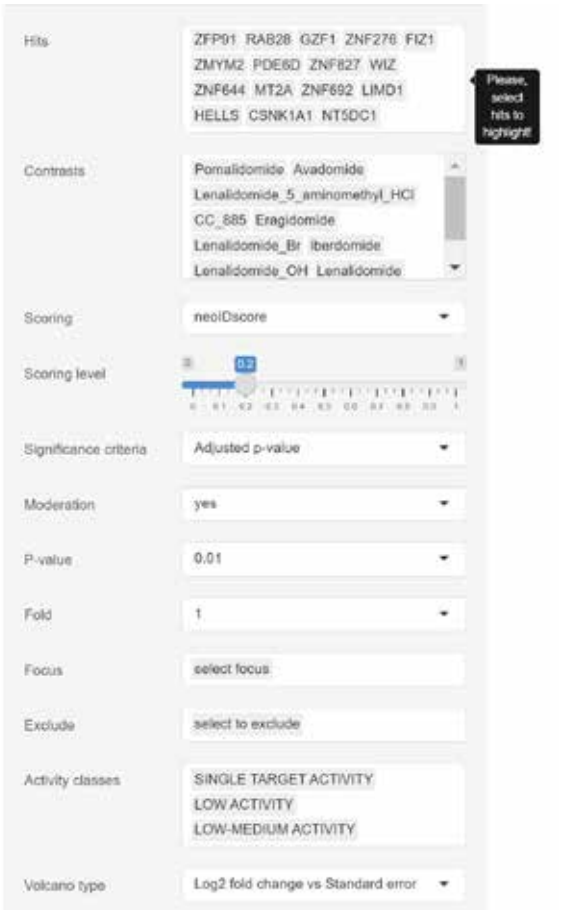
Intuitive dashboard for complete project overview or display of selected parameters

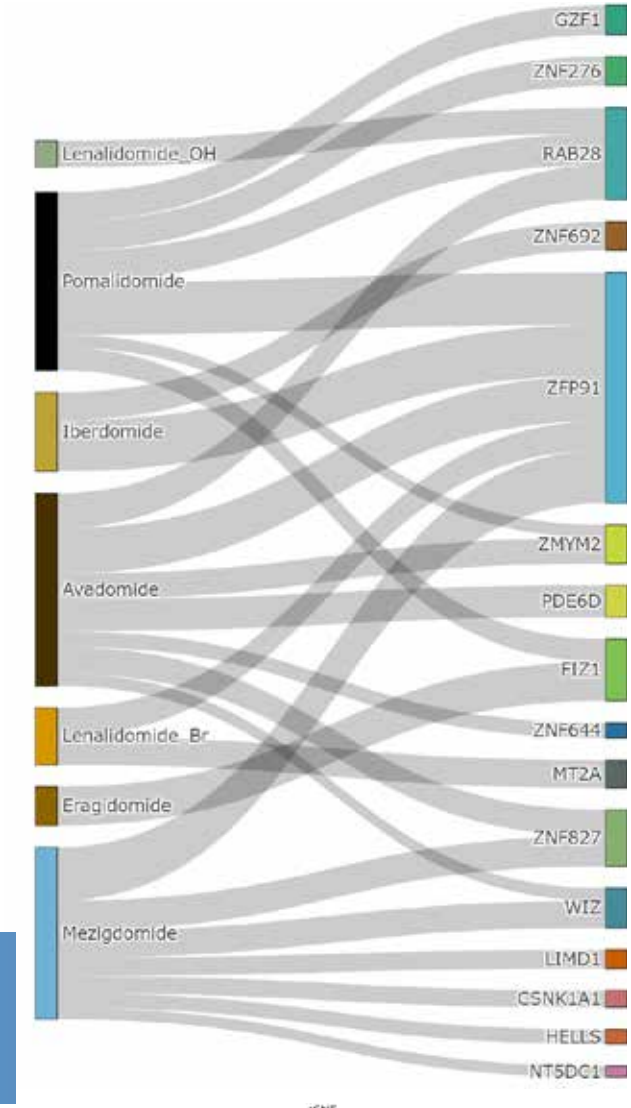


Detailed statistical and activity analysis at single compound level as well as direct comparison of all compounds tested at the push of a button

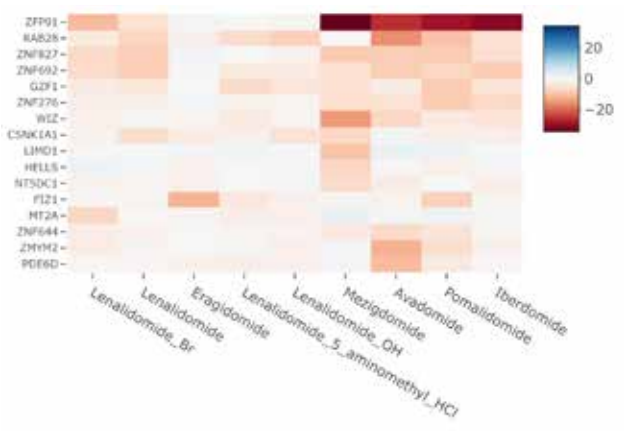
NEOsphere Biotechnologies hosts its data analysis suite on its internal servers. Online access is granted only to our partners to review proteomics data generated in joint projects. An exemplary selection of analytical tools is shown. All data were obtained with published and publicly available compounds and cell lines.

Harness the power of proteomics data for informed drug discovery decisions





Interactive display of drug-target interactions to create a biological activity map of your library



Meta-analysis of significantly regulated features and compound activity profiles

About NEOsphere Biotechnologies

We are a high-end proteomics partner for pharmaceutical and biotechnology companies to build broad and unique degrader pipelines. Our platform combines outstanding mass spectrometry capabilities for high-throughput proteomic screening of entire degrader libraries and mechanistic target validation with unprecedented sensitivity, precision and turnaround to identify novel degrader targets and systematically unlock the previously undruggable therapeutic target space.

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