Phenotypic & Targeted: Novel approaches to drug discovery

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Overview

o2h Discovery has enhanced its biology expertise across therapeutic areas in Cambridge, UK, and Ahmedabad, India, utilizing advanced platforms to support all stages of preclinical drug discovery, from target validation, hit identification through to lead optimisation and IND enabling. Our Integrated Drug Discovery (IDD) approach combines biology, chemistry, and ADME capabilities to accelerate the design-make-test-analyze cycles. Our experienced biologists develop customizable assays to support potency-to-efficacy translation and mechanism-of-action characterization, with seamless collaboration between our UK and India teams. Specializing in small molecule drug discovery, we bring in-depth expertise in target biology, providing valuable insights that drive progress in complex biology projects across a broad range of therapeutic areas.

Scan for our blogs!

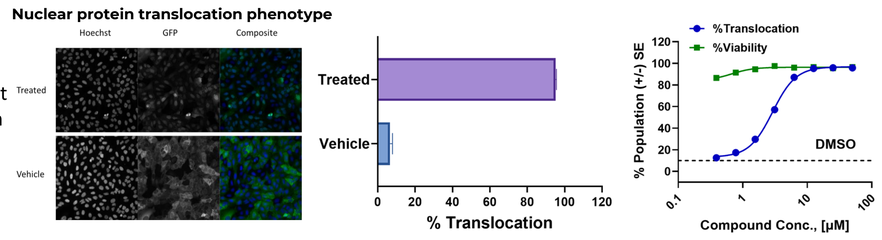


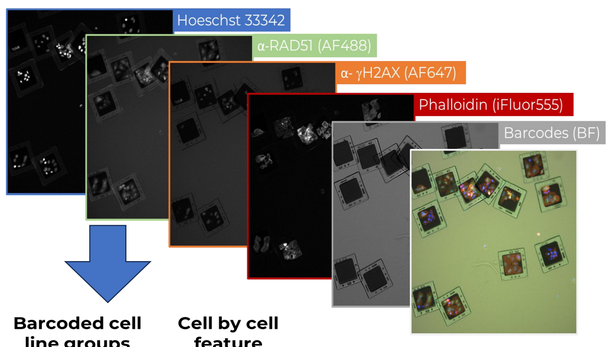
Phenotypic

Multiplexed High Content Imaging

o2h has developed a bespoke high-content imaging assay to measure the translocation of a target protein from the cytoplasm to the nucleus in response to test compounds, enabling quantitative assessment of changes in subcellular localisation.

Nuclear protein translocation phenotype



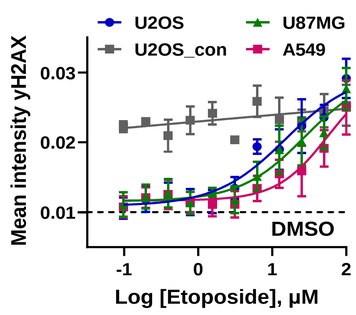


In collaboration with Semarion, o2h Discovery has enhanced the HCI approach for DNA damage analysis using the innovative SemaCyte® microcarrier system, optimizing cell monolayer management for more efficient drug discovery.

Assay Parameters

- 3 cell lines (U2OS, U87MG, A549)
- Each assigned SemaCyte barcodes
- Revived following frozen storage
- Pooled for assay
- Drugged 2hr with EP DRC same day
- Fixed and stained for subsequent HCIC

Cell line-specific etoposide dose response curves



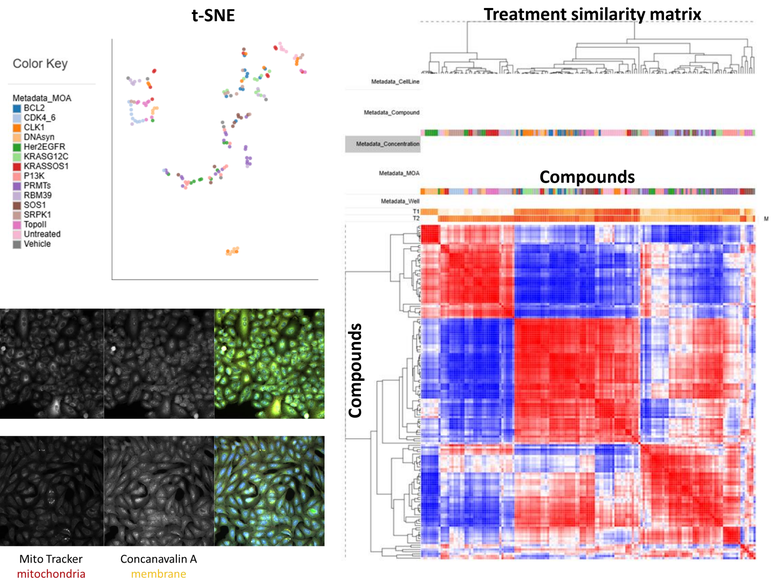
Experimental Workflow

1) Cell line handling
2) High-content imaging
3) Image processing I – deconvolution
4) Image processing II – quantitation

Cell Painting

Developed and implemented an in-house Cell Painting analysis platform capable of processing large, high-dimensional imaging datasets. The pipeline's robustness was evaluated using compounds from established mechanism-of-action classes profiled in U2OS and A549 cell lines, enabling automated extraction, analysis and clustering of cellular and nuclear phenotypic features.

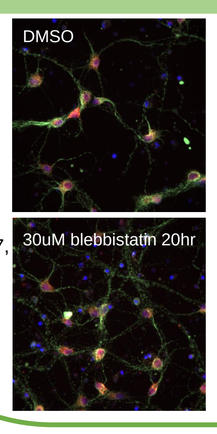
The pipeline successfully identified key morphological features that drove the phenotypic responses and grouped compounds according to their known mechanisms of action. These features showed strong correlations and anticorrelations with previously published biological profiles, demonstrating the platform's ability to reproduce known biology and support the characterisation of novel compounds.



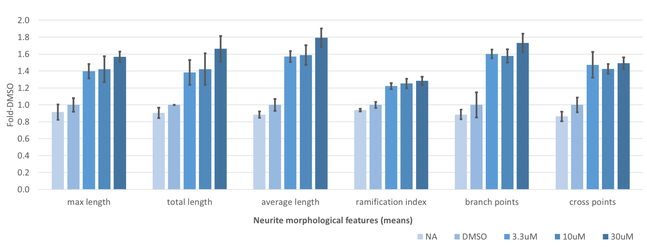
Neurite Outgrowth

Nuclei
MAP2
HuC/HuD

Primary rat cortical neurons, neurobasal media + B-27, grown on poly-D-lysine



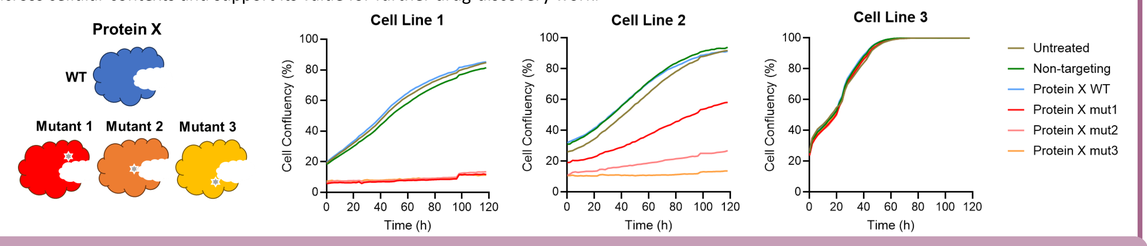
Neurite outgrowth assays can serve as a model for neuronal networking and plasticity. o2h has developed neurite outgrowth assays using high-content microscopy to support drug discovery. These assays can leverage the information-rich image data derived from suitable cell lineages.



Targeted

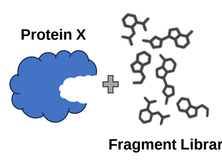
Target validation

Target validation at o2h Discovery is used to confirm that a proposed target is relevant to the biological response of interest and to show whether modulating that target produces the expected cellular effect. In the figures, lentiviral delivery of target protein X and three mutant variants was used to test target dependence across multiple cell models. Using live-cell analysis, o2h was able to show target sensitivity in susceptible cell lines 1 and 2, while cell line 3 showed resistance. Together, these examples demonstrate how the target behaves differently across cellular contexts and support its value for further drug-discovery work.

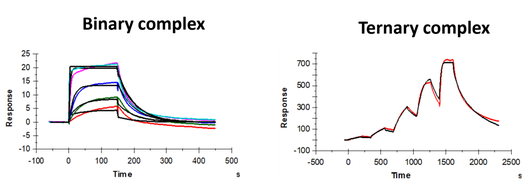


Target engagement

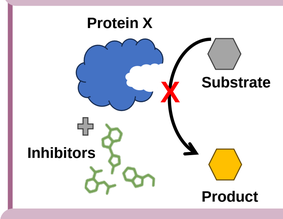
Biophysical (SPR)



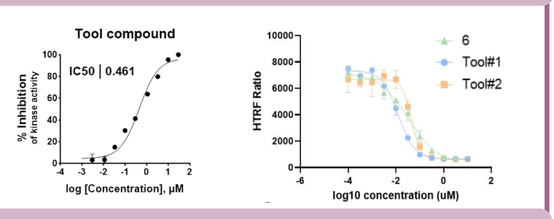
Biophysical SPR assays can screen proprietary fragment libraries to identify compounds that directly bind protein X, then progress hits into small-molecule programmes or degrader strategies using novel E3 ligases or our standard PROTAC toolbox. Example data also demonstrated PROTAC binary and ternary complex formation, highlighting SPR's utility for hit identification, target-engagement confirmation and mechanistic validation across different modalities.



Biochemical



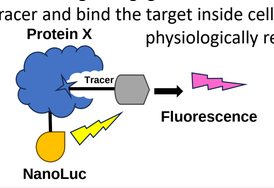
Biochemical assays were used to measure enzyme activity in the presence of inhibitors (or activators to increase product formation), allowing direct assessment of compound effects on catalytic function.

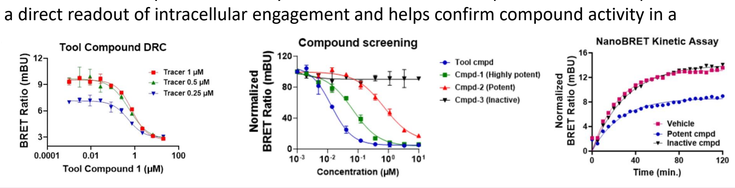


Cellular

Tracer displacement (NanoBRET)

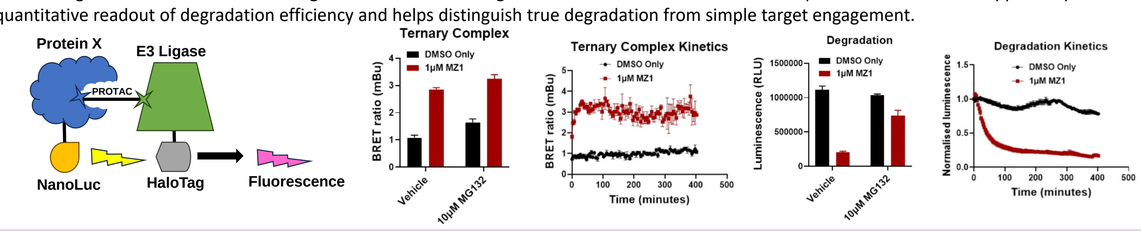
Cellular target engagement was assessed using a NanoBRET tracer displacement assay to measure whether compounds could compete with the tracer and bind the target inside cells. This provides a direct readout of intracellular engagement and helps confirm compound activity in a physiologically relevant context.





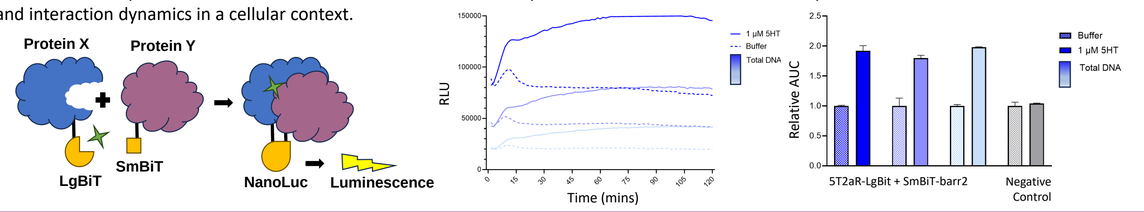
Protein degradation (NanoBRET)

Protein degradation was monitored using NanoBRET to track target loss in live cells over time after compound treatment. This approach provides a quantitative readout of degradation efficiency and helps distinguish true degradation from simple target engagement.



Protein interactions (NanoBiT)

Protein–protein interactions were measured using NanoBiT, with custom clone vectors encoding the interacting proteins to enable live-cell detection of complex formation. The 5-HT2A and beta-arrestin example shown here illustrates how this system can be used to monitor recruitment and interaction dynamics in a cellular context.



Targeting RNA

Current RNA-targeting approaches are largely limited to antisense oligonucleotides, but structured RNA motifs offer opportunities for selective small-molecule binding. We developed SPR and fluorescence dye-displacement assays to characterise RNA–small-molecule interactions and combined them with cellular RNA and protein readouts to demonstrate the functional effects of RiboTAC molecules.

