PHOREMOST

DRUGGING THE UNDRUGGABLE®

for the next generation of therapies

SITESEEKER® and CRISPR:

Multiplexed perturbation tools to advance novel drug target discovery

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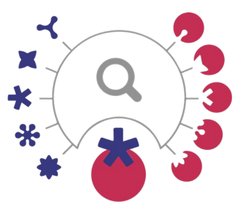
ABOUT US

We use our SITESEEKER® target discovery platform to identify novel targets with druggable sites

Series B Investors



DRUG DISCOVERY PIPELINE

Novel and challenging disease targets

Targeted Protein Degradation | Beyond Cereblon, tissue-selective degradation

Oncology | ICB synergy, IFN pathway and innate immunity

PARTNERSHIPS

SITESEEKER® and discovery alliances

Target Discovery | Partnered for success across therapeutic areas

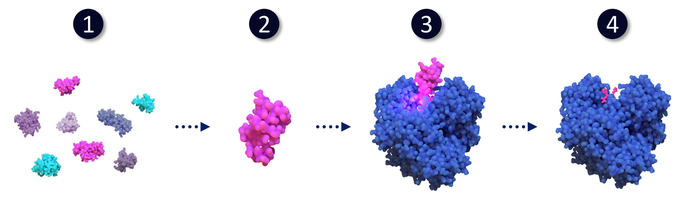
ABSTRACT

PhoreMost have developed a novel technology called SITESEEKER® - a phenotypic pooled screening platform that can rapidly identify novel druggable sites for unmet disease targets. The SITESEEKER® technology uses vast libraries of computationally engineered mini-protein fragments to explore disease-relevant phenotypes and discover novel drug targets in an unbiased manner. The mini-proteins, or PROTEINi®, are introduced into cell models of interest to affect phenotypic responses and identify novel mechanisms that can perturb disease. Crucially, the identification of new phenotypic PROTEINi® interactions astutely defines novel small molecule development strategies to greatly enhance drug discovery with unique chemical biological insights.

PhoreMost have used a comprehensive toolbox to identify the targets through which the bioactive mini-proteins operate. A powerful technique is the use of genetic perturbation methods such as CRISPR to identify the functional relationship and interactions of the mini-protein with the targets of interest. To advance this approach we have recently developed combinatorial tools to massively streamline this approach - **ComboPROTEINi®.**

As a proof-of-concept, we have deployed this approach in the field of Targeted Protein Degradation (TPD) drug discovery. The SITESEEKER® approach was adapted to discover PROTEINi® that are active by degrading disease-associated proteins, however the mechanisms responsible for this degradation were unknown. By applying a combinatorial approach, we have revealed a diverse portfolio of novel PROTEINi® ligands to multiple TPD effector targets, including previously unprecedented E3 ligases. The structural and chemical properties of bioactive PROTEINi® were then used as a springboard for small molecule discovery, resulting in novel bivalent small molecule degrader drugs.

TECHNOLOGY

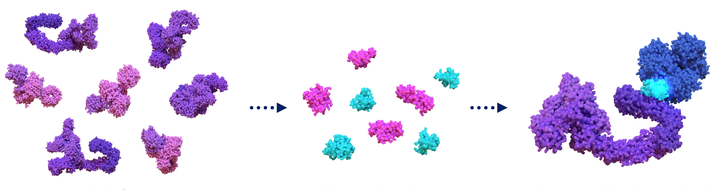


1. **SITESEEKER® Screening:** We screen millions of carefully designed mini-protein fragments we call "PROTEINi®" for phenotypic effects
2. **PROTEINi® Hits:** Hit PROTEINi® are selected through high-throughput cellular assays and evolved to optimise their bioactivity
3. **Drug Target ID:** PROTEINi® are mapped to novel cellular targets, identifying the protein responsible for the therapeutic response AND the pocket docked by the PROTEINi®. Perturbation methods such as CRISPR advance this approach.
4. **Drug Discovery:** The PROTEINi® and target pairing is used as a novel tool to rapidly develop new small molecule drugs that can copy the effect of the PROTEINi®

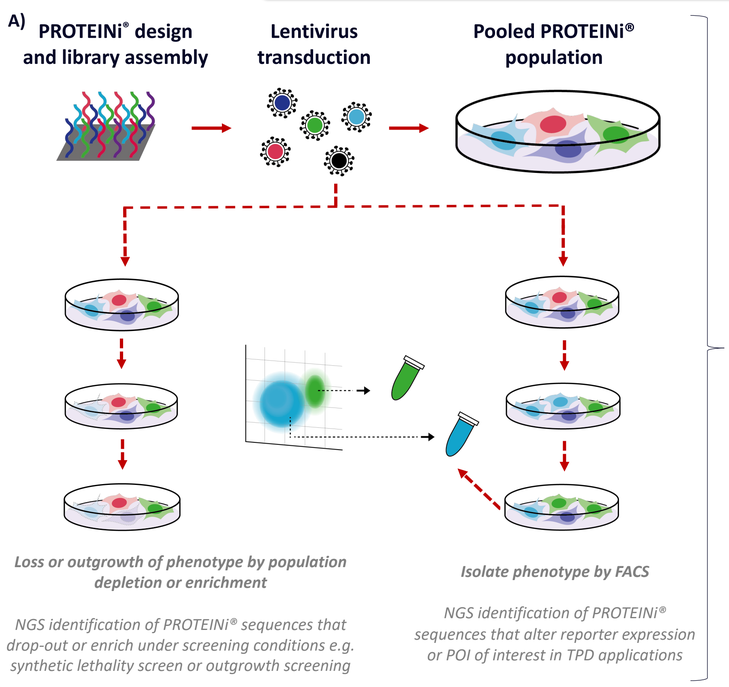
Targeted protein degradation

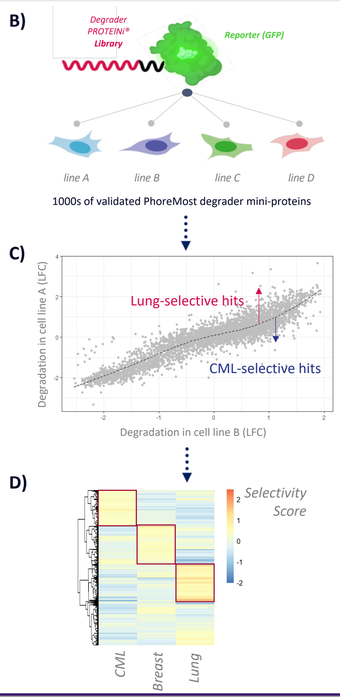
Current Targeted Protein Degradation (TDP) modalities, such as PROTACs, are constrained by their heavy reliance on a limited set of target-degrading E3 ligases (i.e. Cereblon, VHL).

PhoreMost has used its SITESEEKER® platform to study protein degradation, with the aim of identifying phenotypically active PROTEINi® that can act as ligands and bind and recruit E3 ligases to close proximity of target proteins and promote its degradation via the proteasome. This technology also has the potential of the simultaneous identification of new target binding mini-proteins (warheads) and E3 binding ligands for the development of heterobifunctional mini-proteins for the development of new drug discovery programs.



SITESEEKER® Assay Configurations and TPD applications





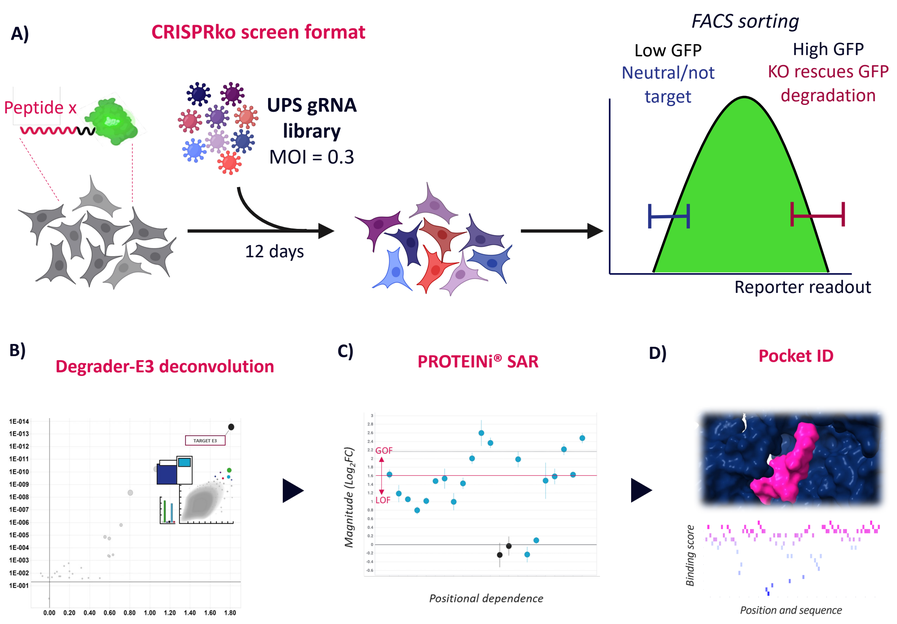
PROTEINi® differs from genome-based target screening technologies (such as CRISPR) in that it operates directly at the protein level, so that new druggable space can be defined as an inherent part of the target-function screening process.

A) The library is transduced into a screening cell line of choice and transduced cells are selected. At the endpoint of the screen, cells are pelleted, DNA is extracted and PROTEINi® identified via NGS detection of a unique barcode.

One recent application of our libraries is in the TPD space, using GFP-linked PROTEINi® to identify Tissue selective E3-ligases. Here the PROTEINi® library is fused to GFP via a linker and applied to different cancer cell models **(B)**. PROTEINi® that act as E3 binders and recruit E3 ligases to cause degradation and be inferred via loss of GFP and will have a positive degradation signal. **(C)** To infer selectivity, the difference in degradation is calculated as distance from the trend line and in this way assigned a selectivity score **(D)**.

Unbiased ID of novel E3 ligases

*Perturbation screening for the identification of new E3 targets (such as in the Tissue-Selective TPD programme) using a cell line expressing a **single PROTEINi®** fused to GFP*

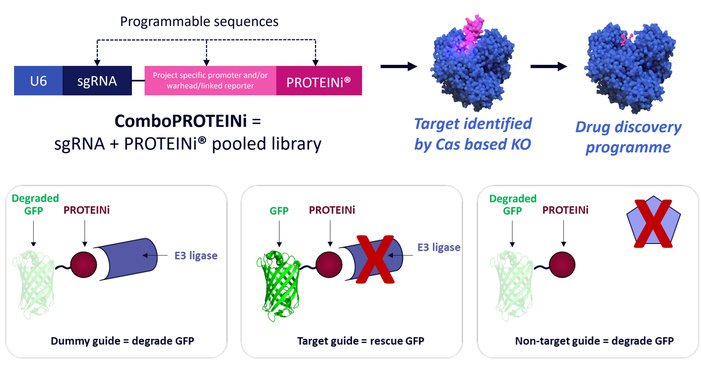


(A) Schematic representation of a CRISPRko screen using a sgRNA library against all the UPS and key interactome genes. Co-expression with cells expressing a validated PROTEINi® and collection of low and high GFP populations for identification of functional dependencies for induced GFP degradation. **(B)** For one of our GFP-degradation inducing PROTEINi®, CRISPR screening deconvolution revealed a single key E3 responsible for degrader function. **(C)** Structural Activity Relationships (SAR) were determined via mutational analysis, to identify key residues and build a binding model. **(D)** This allows simulation of E3 ligase binding and *de novo* identification of an *in silico* validated PROTEINi® binding pocket from the peptide SAR, to kickstart our small molecule development pipeline.

Combinatorial CRISPR and PROTEINi® libraries to streamline Target ID

*Modular cloning for **readout agnostic** TargetID in a **promoter flexible** context allowing linkage to versatile readouts including fluorescence and different CRISPR systems*

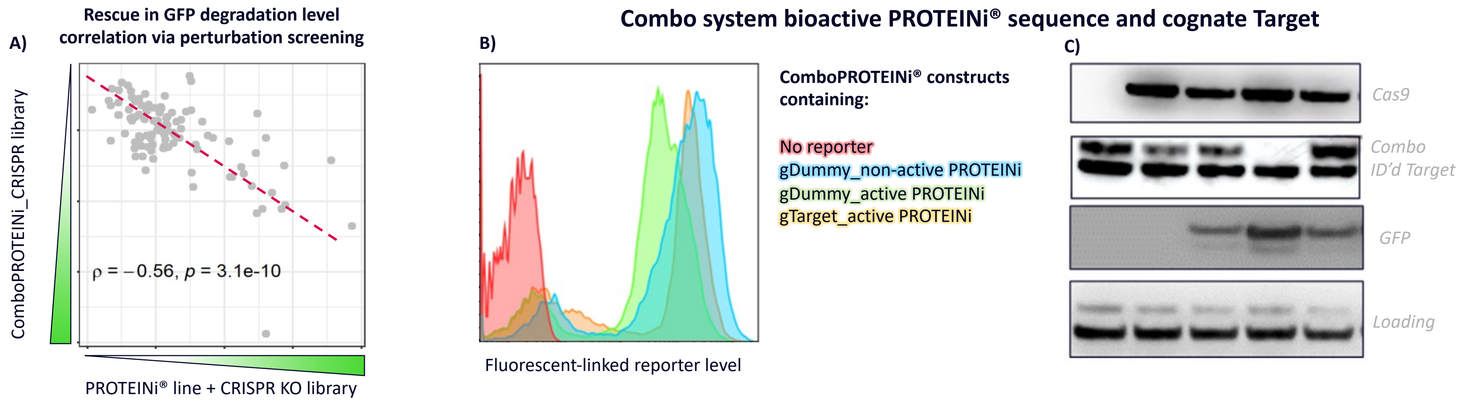
PROTEINi® microshape in druggable pocket



Recently we have sought to streamline the process of validating many PROTEINi® and identifying their cognate targets. For this, rather than applying an sgRNA library to a cell line expressing a single PROTEINi®, **ComboPROTEINi®** uses a paired plasmid expression of the DNA-encoded sequence and sgRNA element in a combinatorial pool of thousands of multiplexed guides and mini-protein sequences. This enables large scale validation of PROTEINi® phenotypes and functional genomic identification of the cognate target within the same pooled screen format.

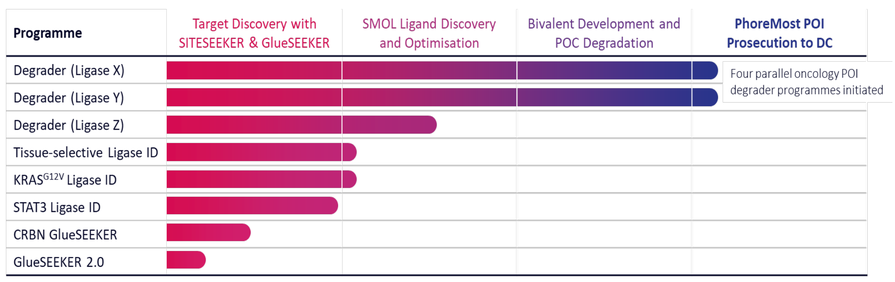
We have applied this technology to our TPD GFP-degradation screening campaigns, but the modular nature of our technology allows us the versatility to apply different guide subsets and different PROTEINi® phenotypes, across all assay configurations.

(A) positive correlation was observed when comparing the level of GFP rescue for PROTEINi® multiplexed with the guide against its target, between ComboPROTEINi® and non-combinatorial perturbation pooled screening. Furthermore, exploring ComboPROTEINi® vectors in a non-pooled format revealed depleting the E3 ligase by which the bioactive mini-protein caused GFP-degradation reverted the phenotype by **(B)** FACS and **(C)** western blot



SUMMARY & TPD PIPELINE

- PhoreMost uses a novel **phenotypic screening** method based on **protein interference** in the context of a collaborative drug discovery model to rapidly identify novel druggable sites for unmet disease targets and inform the design of novel small-molecules.
- Using our mini-protein libraries, we have identified several new targets, and present work in TPD as an example project here. TPD is a core focus, although both our SITESEEKER® and Target ID tools are versatile and target/phenotype agnostic.
- CRISPR and other such perturbation methods are powerful tools in identifying the target by which the mini-protein acts, complementing SITESEEKER® by highlighting the genes and pathways involved and the latter identifying the exact target binding site and most important biochemical properties.
- Development of **ComboPROTEINi®** allows us to streamline and multiplex our hit validation and target validation activities and in this way expedite the drug discovery process.



Our partnerships

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