The Sygnature CHARMD Platform

Combinatorial High-throughput Assembly and Review of Molecular Degraders

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SYGNATURE DISCOVERY

Introduction

Conventional single-compound synthesis and analysis is time-consuming and poses challenges for selection of degrader components with predictive SAR and ADME properties of the assembled bifunctional compounds. Here, we demonstrate an integrated platform that incorporates high-throughput combinatorial chemistry, live-cell kinetic degrader screening and assessment of *in vitro* DMPK properties. Alongside of this, we have in place computational methods for ternary complex prediction and linker selection. Altogether, our approach facilitates rapid screening and identification of bifunctional lead compounds.

Bifunctional Degrader Platform Workflow

High throughput generation and characterization of bifunctional degraders

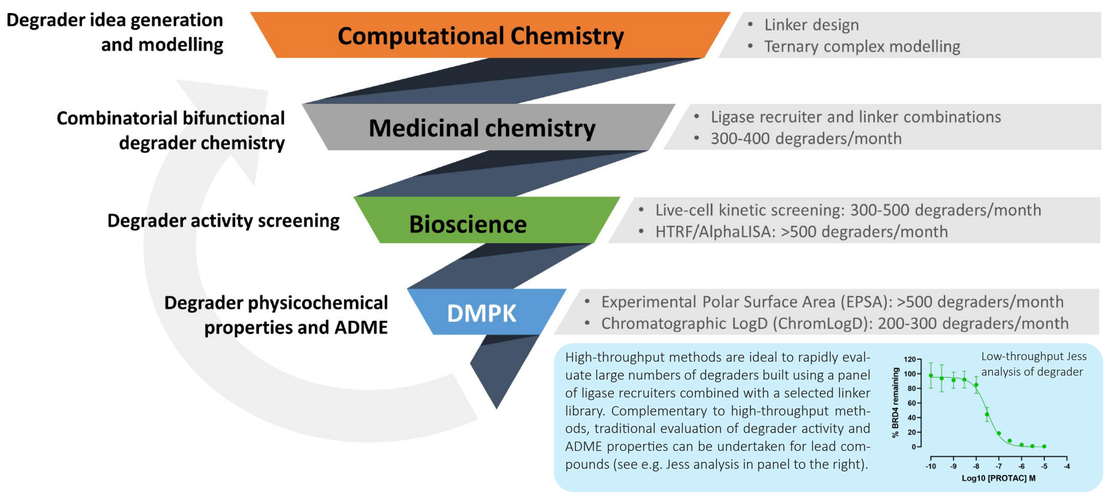


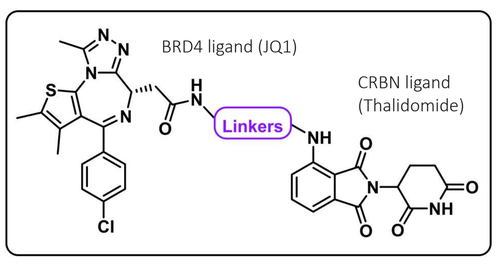
Figure 1: Overview of the general workflow of the degrader development platform. Computational approaches to generate linker ideas feed into high-throughput medicinal chemistry to rapidly generate hundreds of bifunctional compounds. High-throughput compatible activity assays are used to screen degraders for activity against the target, alongside of experimental determination of physicochemical properties. Iterative cycles of triage and refinement drives development of lead compound degraders for detailed analysis.

High Throughput Bifunctional Degrader Synthesis

Optimization of bifunctional molecules or degraders presents a number of challenges. Over the past few years, since the emergence of the TPD field, degrader optimization has been an empirical process often using single compound synthesis. This iterative approach to linker optimization is time and resource consuming. To accelerate this process, Sygnature has been using its expertise in high-throughput synthesis and applied it to degrader molecules.

Overview of synthetic steps for high throughput degrader chemistry capability assessment using CRBN (ligase) and BRD4 (model target) ligands. For initial testing, 24 compounds were synthesized in parallel.

Capability compounds were generated using various linkers combined with ligands for BRD4 and CRBN. Amide coupling was utilized in this initial method assessment.



Yield
- Range: 7-58%
- 80% of compounds > 25%
- Average purity > 85%

Process
- Straightforward
- Short cycle time

Ongoing optimizations
- Other coupling chemistries
- Use of scavenger resins

General combinatorial approach under development

Ligase recruiters
- CRBN
- VHL
- IAP
- DCAF15
- DCAF16
- FEM1B
- AHR
- KEAP1
- Novel ligases
- Etc.

Linkers
- Alkyl
- PEG
- Alkyne
- Rigidified
- Etc.

Target ligand

Figure 2: Our approach to high throughput synthesis of bifunctional degraders. Top panel shows our current workflow, exemplified and tested with the ligase ligand thalidomide combined with a small linker library for attachment to the BRD4 ligand JQ1. Whereas a single ligase ligand was used in this example, the approach is suitable for parallel synthesis using multiple E3 recruiters, and this capability is currently being evaluated at Sygnature. Further development will include assessment of chemistries beyond amide coupling and use of scavenger resins in place of reverse-phase HPLC.

Computational Approaches for Bifunctional Degrader Design

Ternary complex models are used alongside known ligand SAR to design and prioritise new degraders for synthesis.

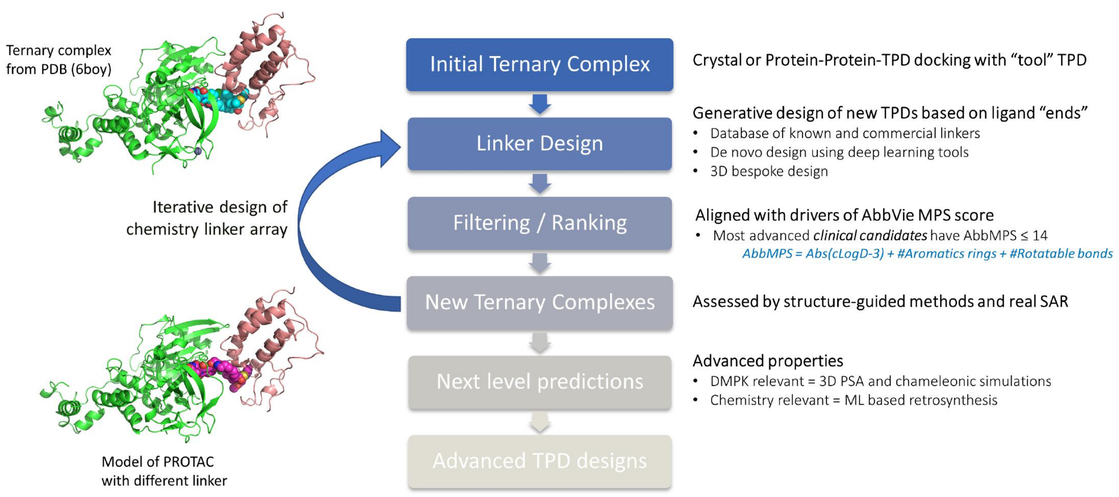


Figure 3: A virtual library of novel degraders is built from a "toolbox" of pre-curated warheads and linkers, and prioritised for synthesis based on fit to the ternary complex model(s) and "beyond rule of 5" predicted properties. Alongside this, bespoke *de novo* designs are generated in the 3D models using a range of methods. Emerging experimental results are used to refine the ternary complex models and produce next generation designs. In the early stages of design, emphasis is on complementarity to the ternary complexes but as the degraders advance and become more synthetically challenging, lower throughput models for DMPK drivers become more critical for prioritisation.

High Throughput Live-Cell Kinetic Degrader Screening

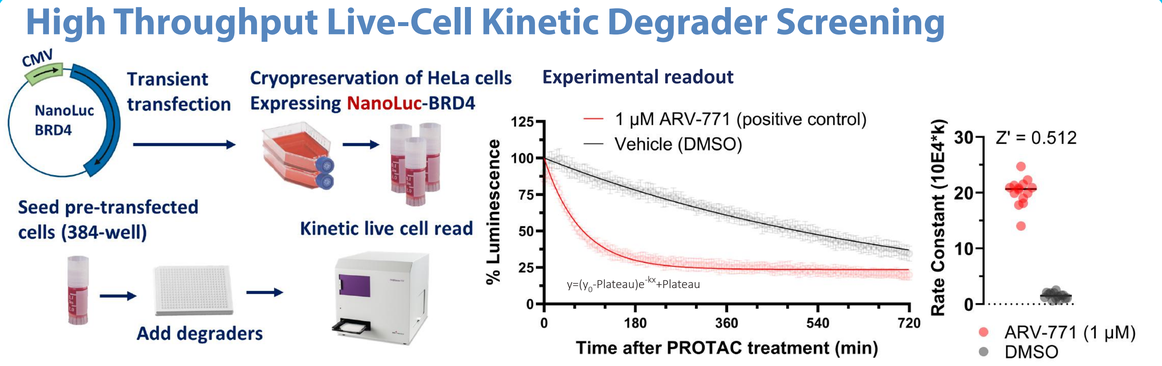


Figure 4: The live-cell (HeLa) method was set up using transient transfection, with Nano-Luciferase tagged BRD4 as a model. The ARV-771 PROTAC was used as a positive control for BRD4 degradation. The exponential decay rate-constants *(Riching et al.)* derived from curve-fits generated a Z' compatible with compound screening.

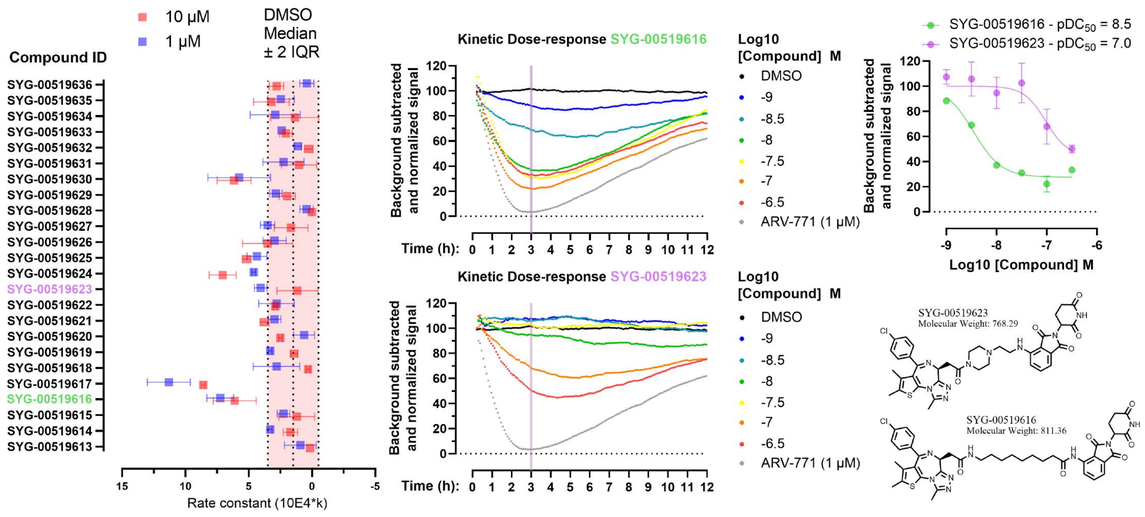


Figure 5: Using the live-cell kinetic set-up, 24 compounds made using high-throughput chemistry were screened at two concentrations to lower the possibility of false negatives due to the hook effect. A cut-off of two interquartile ranges (IQR) around the DMSO median was set for assigning hits. Compounds showing activity were taken forward for dose-response analysis (two compounds shown here) in kinetic mode. DC_{50} values were calculated at three hours post treatment. Clear discrimination of activity could be observed for the tested compounds. Transient transfection was used for the method development here, however for screening purposes, endogenously tagging the target with Nano-Luciferase or HiBiT is desirable due to endogenous control of protein expression levels.

In vitro DMPK for Bifunctional Degraders

Chromatographic LogD and experimental polar surface area (EPSA)
- High throughput
- Cost-effective
- Low sample consumption
- Highly reproducible
- Informative for bioavailability

ChromLogD and EPSA method assessment
- ChromLogD and EPSA methods set-up at Sygnature
- Test on 24 bifunctional degraders made by HT chemistry
- Successful and rapid generation of data on lipophilicity and polarity

Extended characterization of established degraders
- ChromLogD and EPSA vs. TPSA
- Metabolic stability
- HeLa cell membrane permeability

Extended characterization of established bifunctional degraders

Parameter		dBET1	dBET6	ARV-825	ARV-110
ChromLogD		2.43	2.86	3.33	3.72
EPSA (Å²)		104	105	98	99
TPSA (Å²)		194	194	205	181
Hepatocyte Cl_{int} (µL/min/10^6 cells)	Mouse	267	>277	>277	6.7
	Rat	153	269	209	18.2
	Dog	46.9	101	>277	6.3
	Human	36.1	62.2	66.3	8.5

Chromatographic LogD was determined by RP-HPLC method for the determination of the octanol–water distribution coefficients at pH 7.4 *(Lombardo et al.)* using a C18 column. EPSA values were measured using supercritical fluid chromatography using a Chirex (S)-Val and (R)-NEA, 4.6 x 50 mm column. Both assays were calibrated using a set of 7 marker compounds. Metabolic stability studies were run by incubating compounds with hepatocytes followed by LC-MSMS analysis.

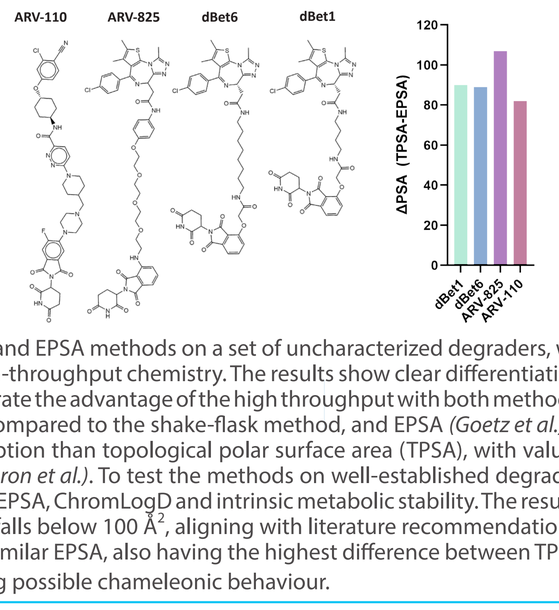


Figure 6: To assess the high-throughput ChromLogD and EPSA methods on a set of uncharacterized degraders, we used the 24 compounds made at Sygnature using high-throughput chemistry. The results show clear differentiation of measured physicochemical properties and demonstrate the advantage of the high throughput with both methods. In addition, ChromLogD has a wider dynamic range compared to the shake-flask method, and EPSA *(Goetz et al.)* is believed to be a more reliable predictor of oral absorption than topological polar surface area (TPSA), with values <100 Å² being targeted for intestinal permeability *(Caron et al.)*. To test the methods on well-established degrader compounds, we assessed a small set of compounds for EPSA, ChromLogD and intrinsic metabolic stability. The results show that the EPSA for the clinical ARV-110 degrader falls below 100 Å², aligning with literature recommendations. Interestingly, the ARV-825 tool compound showed a similar EPSA, also having the highest difference between TPSA and EPSA (ΔPSA) of the tested compounds, suggesting possible chameleonic behaviour.

Summary

We show here the basis for a platform to rapidly develop novel bifunctional degraders. The platform rests on high-throughput combinatorial chemistry to efficiently generate a high number of linker and ligase recruiter combinations for a given target. This, coupled with our high-throughput assays for assessment of degrader activity and experimental physicochemical properties (EPSA, ChromLogD) quickly generates data to support compound progression. Although not covered here, we also support identification and development of novel E3 ligands which can directly feed into the existing platform workflow to quickly generate and evaluate degraders working via novel ubiquitin ligases.

References

- Riching KM, et al., Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action. ACS Chem Biol. 2018 Sep 21;13(9):2758-2770.
- Goetz GH, et al., EPSA: A Novel Supercritical Fluid Chromatography Technique Enabling the Design of Permeable Cyclic Peptides. ACS Med Chem Lett. 2014 Aug 4;5(10):1167-72.
- Caron G, et al., Steering New Drug Discovery Campaigns: Permeability, Solubility, and Physicochemical Properties in the bRo5 Chemical Space. ACS Med Chem Lett. 2021 Jan 5;12(1):13-23.
- Lombardo F, et al., ElogD(oct): a tool for lipophilicity determination in drug discovery. 2. Basic and neutral compounds. J Med Chem. 2001 Jul 19;44(15):2490-7.

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