Direct-to-Biology Platform for Accelerated PROTAC Discovery

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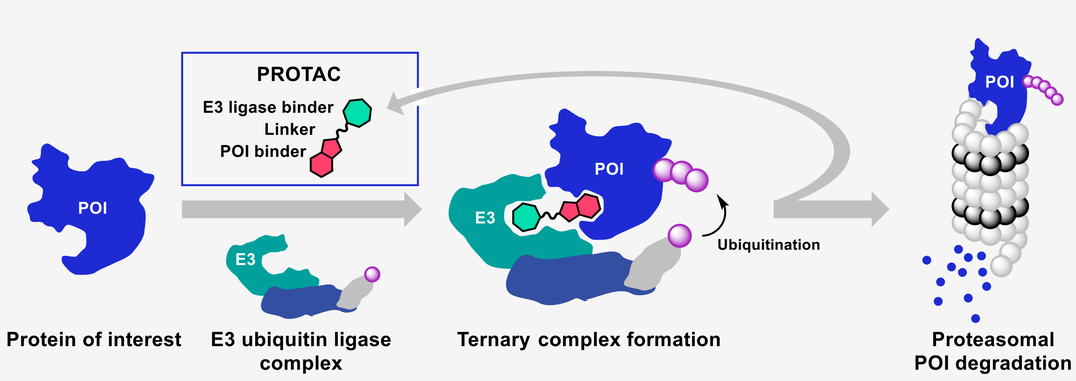
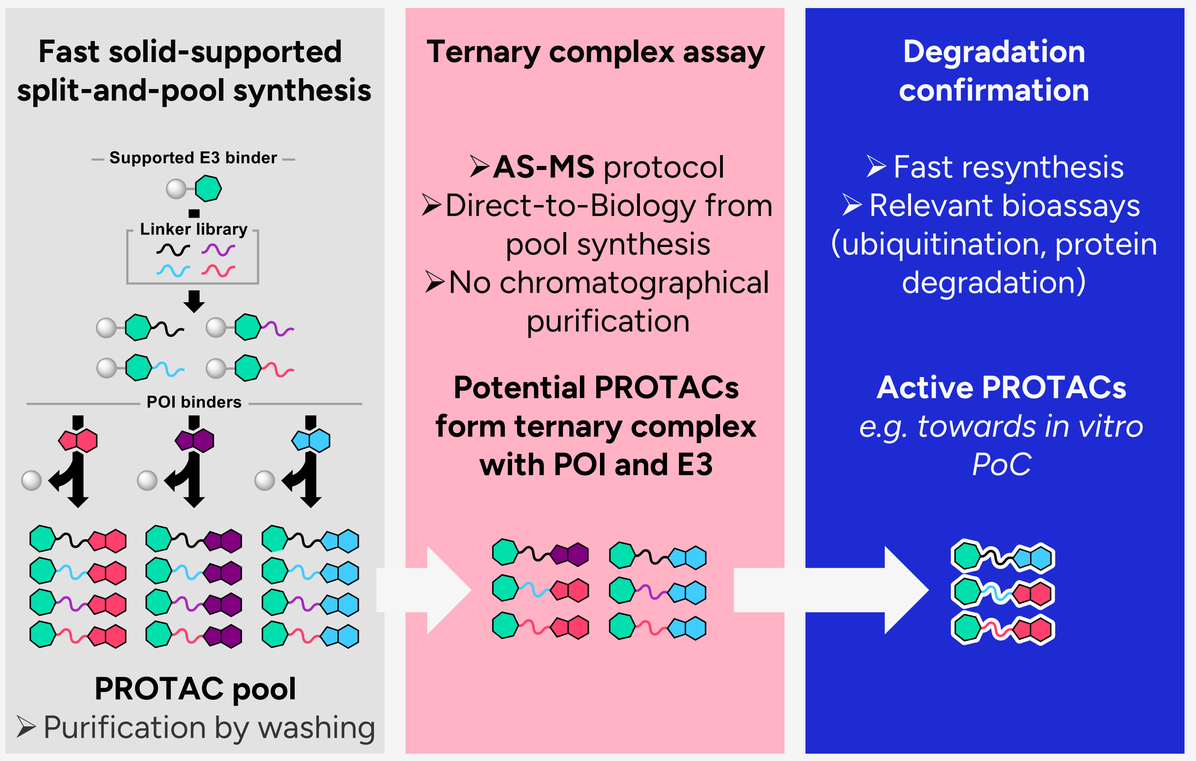
Introduction

Proteolysis targeting chimeras (PROTACs) are heterobifunctional molecules that induce target protein degradation. As an emerging therapeutic modality, it holds great promise for undruggable targets. Known to be beyond rule-of-five chemical space, the optimization of PROTACs can be challenging and time-consuming, for both chemistry and biology.

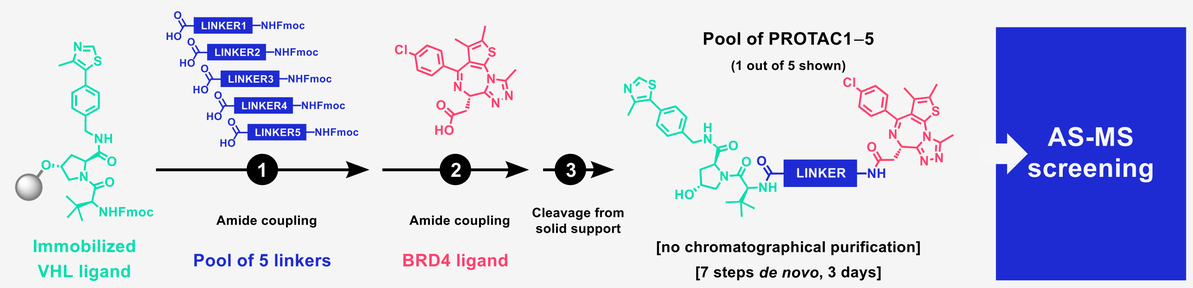


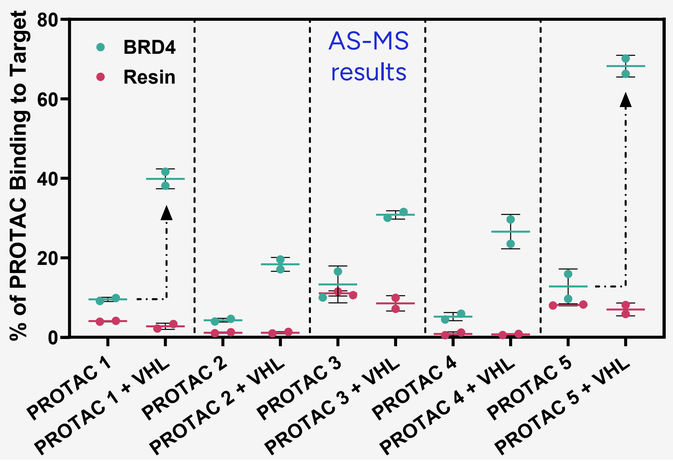
The goal of this study was to develop a novel platform to accelerate PROTAC discovery. It combines an intrinsically fast and undemanding multi-step chemical synthesis with a direct-to-biology[1] AS-MS[2] ternary complex assay.

Workflow Pool Synthesis and Direct-to-Biology Screening



Case Study Discovery of VHL-Recruiting BRD4 PROTACs



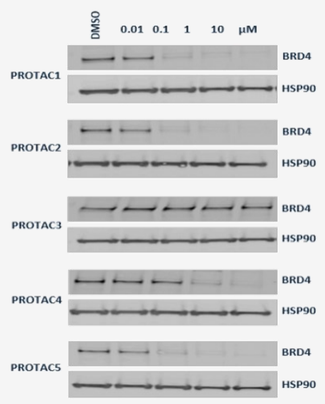


1. The pool synthesis: Multi-step amide chemistry on solid support (only main steps shown). Then, the molecule pool is directly analyzed in an AS-MS assay regarding the formation of a VHL:PROTAC:BRD4 ternary complex (dotted arrows: potential cooperativity) for each PROTAC from the unseparated crude mixture. This allows pre-selecting candidates.

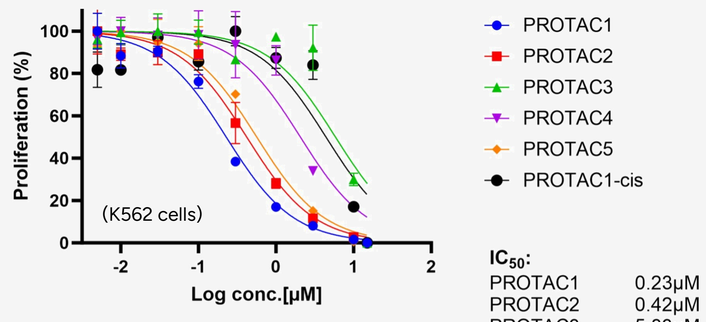
2. AS-MS assay for ternary complex formation
Affinity selection-mass spectrometry (AS-MS) screening for potential PROTACs, which are enriched in this binding assay by forming a ternary complex with an immobilized protein of interest (POI) and a soluble E3 ubiquitin ligase.[2] This degradation prerequisite is used to prioritize candidates for downstream assays.

3. Confirmation of protein degradation by orthogonal assays
Candidates were evaluated individually by their degradative effect on BRD4 (Western blot) and in a functional cellular assay, identifying true degraders and proving that the ternary complex they induce is productive for degradation.

Degradation assay (BRD4): Western blot



Functional assay: Anti-proliferative activity



IC_{50}:	
PROTAC1	0.23µM
PROTAC2	0.42µM
PROTAC3	5.83µM
PROTAC4	2.06µM
PROTAC5	0.56µM
PROTAC1-cis	4.40µM

Results and Discussion

Part 1 – Synthetic chemistry

- 5 VHL–BRD4 PROTACS (**PROTAC1–5**, 900<M_W<1300) were synthesized on solid support using a Fmoc strategy, building on validated ligands for the VHL E3 ubiquitin ligase and the POI BRD4 (**VH032**[4], and (+)-**JQ1**[5])
- The 7-step *de novo* synthesis was completed in 3 days without the need for chromatographic purification, an inherent feature of the solid support
- The PROTAC pool was obtained with >70% purity and 21% overall yield
- No residual unreacted E3 ligase binder, linker, or POI binder was observed

Part 2 and 3 – AS-MS ternary complex assay and degradation confirmation

- Among the 5 compounds, **PROTAC1** (= **Mz1**[3]) and **PROTAC5** showed the most significant enhancement of binding to BRD4 in the AS-MS assay
- A negative control (*cis*-**Mz1**, data not shown) showed no enhancement
- Productive POI degradation induced by the AS-MS hits was confirmed by Western blot, showing dose-dependent degradation for **PROTAC1**, **2**, **4**, and **5**, (not **3**), demonstrating the utility of this direct-to-biology workflow

Conclusions and Outlook

A direct-to-biology PROTAC discovery platform has been established:

1. **Solid-supported split-and-pool synthesis** to generate potential PROTACs

   Multi-step synthesis: No purification steps other than washings

   The synthesis is **robust**, **efficient**, and **adoptable to automation**, **upscaling,** and **pool expansion**

   The applicability beyond VHL E3 ligase ligands (*e.g.,* **cereblon ligands**[6]) has been developed as well (not shown)

2. **AS-MS ternary complex assay** allows for the **identification** of potential **PROTACs** by their ability to stabilize ternary complexes with the POI and the E3 ligase under study. The protocol is **ready for medium to high throughput,** allowing the evaluation of up to **hundreds of potential degraders within days**, by increasing the pool size and by combining various pools

We believe **this platform can speed up PROTAC discovery cycles** and can be extended to other **heterobifunctional chemical entities** in the induced proximity-based drug discovery field.[7]

References

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