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PROTAC Pool Synthesis And Direct-to-Biology Platform

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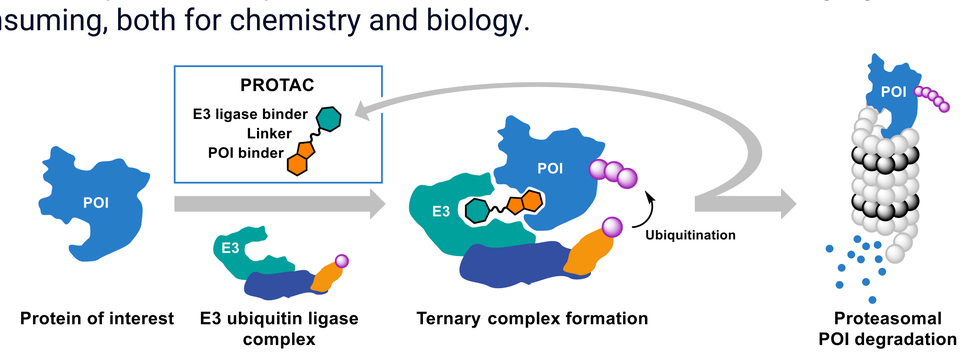
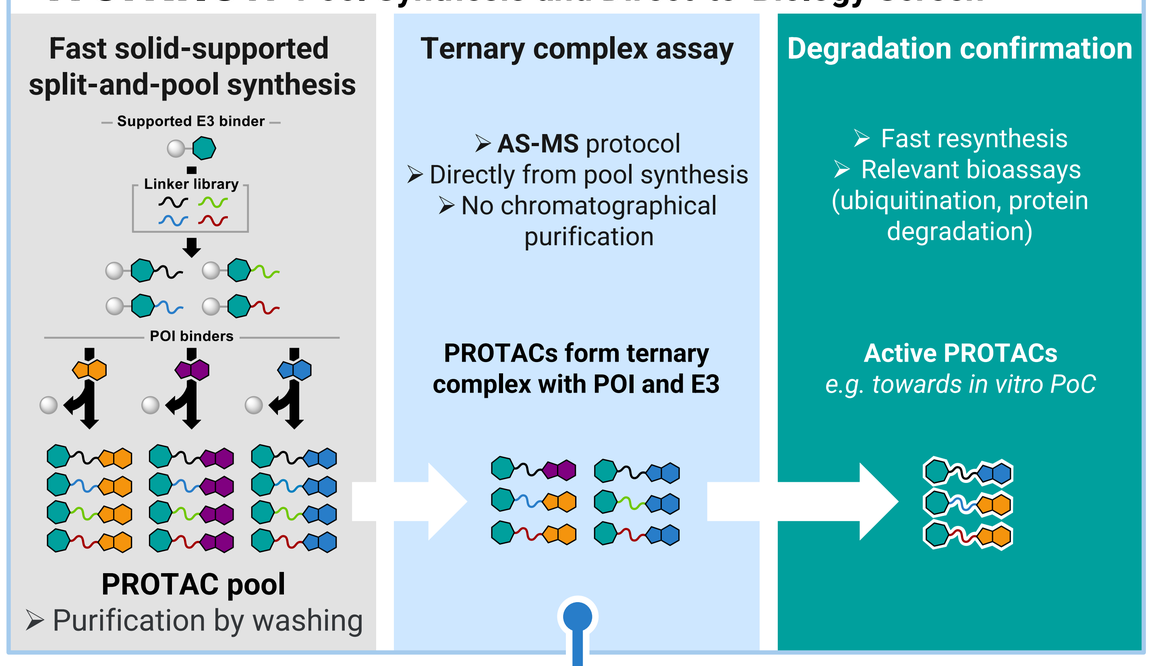
Introduction

Proteolysis targeting chimeras (PROTACs) are heterobifunctional molecules and 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, both for chemistry and biology.

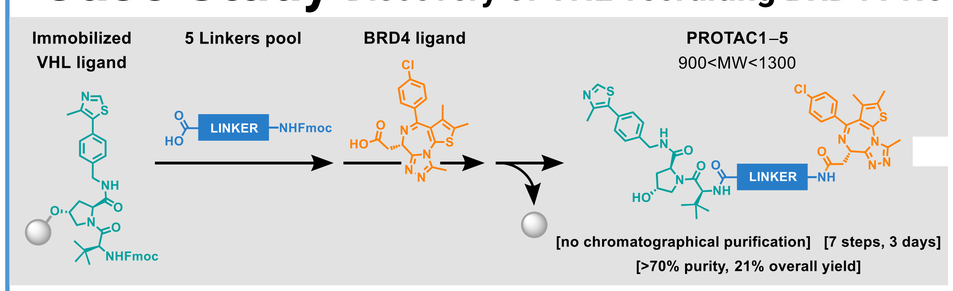


The aim of this study was to develop a novel platform to accelerate PROTAC discovery. It combines an intrinsically fast and undemanding chemical synthesis with a direct-to-biology(ref 1) AS-MS(ref 2) ternary complex assay.

Workflow Pool Synthesis and Direct-to-Biology Screen

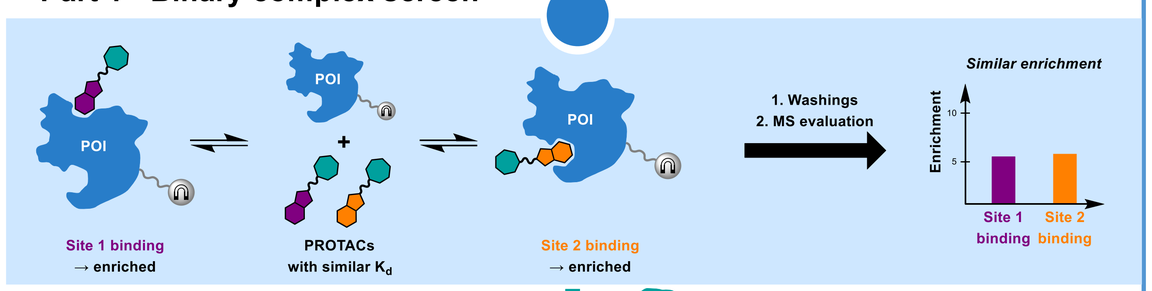


Case Study Discovery of VHL-recruiting BRD4 PROTACs

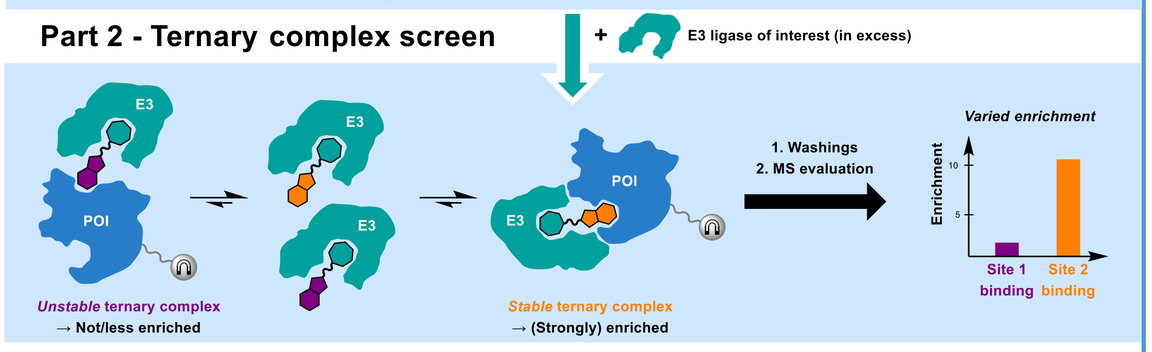


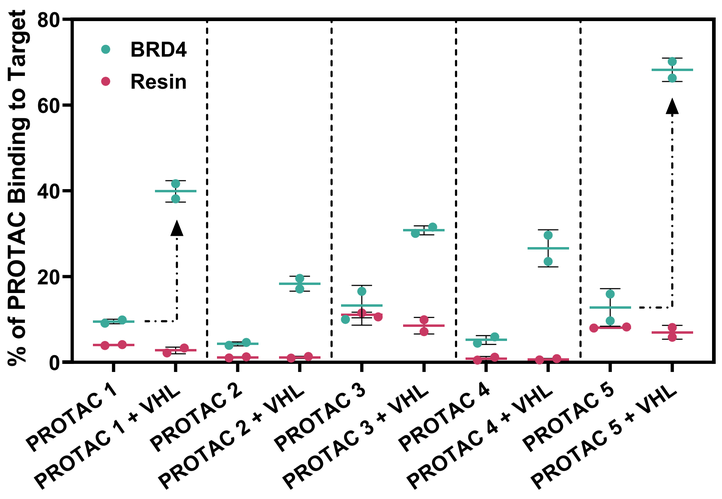
AS-MS screening

Part 1 - Binary complex screen



Part 2 - Ternary complex screen





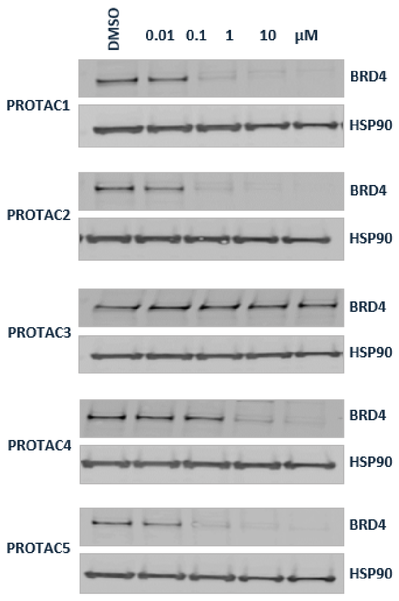
- Pool synthesis: 7 steps, 3 days, 70% purity, 21 % overall yields, no purification.
- AS-MS assay: provides information on formation of VHL:PROTAC:BRD4 ternary complex and co-operativity (dotted arrows) directly from a crude mixture.
- 5 PROTACs formed stable ternary complex with BRD4 and VHL (PROTAC1 = Mz1)

AS-MS assay for PROTACs principle

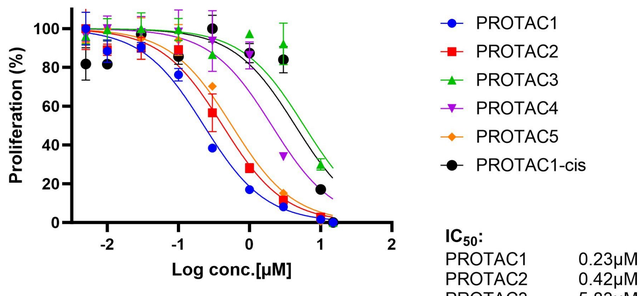
Affinity selection-mass spectrometry (AS-MS) screening with immobilized proteins of interest (POI) allows for the identification of POI ligands and, when combined with an E3 ligase, ternary complex-forming PROTACs.(ref 2)

Case Study Confirmation of ASMS results by cell assays

Western Blot
K562 cells, after 48h treatment



Proliferation assay
K562 cells, after 48h treatment



IC_{50}:

PROTAC1	0.23µM
PROTAC2	0.42µM
PROTAC3	5.83µM
PROTAC4	2.06µM
PROTAC5	0.56µM
PROTAC1-cis	4.40µM

Dose-dependent BRD4 degradation observed for PROTAC 1, 2,4 and 5 in K562 cells

K562 cell viability was decreased by PROTACs in dose-dependent manner

Conclusions and Outlook

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

1. **A solid-supported split-and-pool synthesis approach to generate VHL–BRD4 PROTACs has been developed**
   - **No purification other than washings is needed in between and after the synthesis**
   - **The synthesis is robust, efficient, and adoptable for automated synthesis, upscaling, and pool expansion**
   - **The applicability beyond VHL ligands (*e.g.*, CRBN ligands, ref 6) has been demonstrated and is currently under further investigation**
2. **An AS-MS ternary complex assay enables the identification of PROTACs which form stable ternary complexes with the POI and the studied E3 ligase**
   - **The high-throughput protocol allows evaluation of hundreds of PROTACs within days, by increasing the pool size and by combining various pools**
   - **PROTACs that formed ternary complex in ASMS assay also demonstrated BRD4 degradation and anti-proliferative activity in K562 cell line in dose-dependent manner**

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(ref 7)

References

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