Biochemical Assay Kits for High-Throughput PROTAC Optimization

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Abstract

Proteolysis targeting chimeras, or PROTACs, are heterobifunctional molecules that bind both a target Protein-of-Interest (POI) and a specific E3 ligase. This binding targets the POI for degradation through the ubiquitin-proteasome system in the cell.

Therapeutic approaches using PROTAC have advantages over classical drug therapy including targeting otherwise undruggable proteins and requiring relatively low amounts of compound. PROTAC-based drugs are already in clinical trials, and there is a high demand for the development of new PROTAC molecules. PROTAC development requires multiple rounds of High-Throughput Screening (HTS) for complete optimization. These include identifying an optimized binder for both the POI and the E3 ligase while also finding a suitable linker between the two parts of the PROTAC molecule. Given the promise of PROTAC technology, there is a great need for biochemical tools to simplify and advance the optimization process.

Introduction

Bromodomain (BRD) containing proteins are promising candidates for PROTAC targeting. These proteins are highly significant for cancer and other human diseases; however, traditional inhibitors provide only modest clinical effect. We developed biochemical assays that allow efficient HTS of PROTAC molecules directed towards BRD proteins.

In our system, GST-BRD3 serves as the BRD protein, while FLAG-Cereblon or VHL (Von-Hippel Lindau) serve as the E3 ligases. An Alpha-screen approach provides easy detection of binding to both the POI and the E3 ligase. These assays allow HTS of BRD-aimed PROTACs, as well as competitive inhibition testing toward the PROTACs. We are currently developing additional HTS approaches to screen kinase-aimed PROTACs, as well as to determine the PROTAC effects at the cellular level.

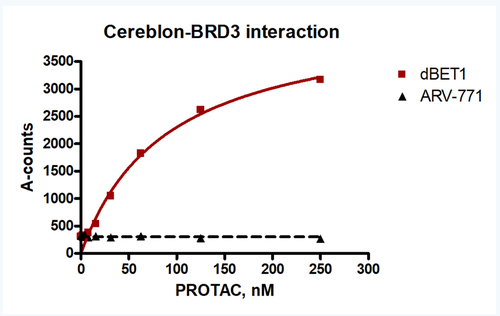
Advantages

- Direct highly specific assays
- Biochemical assays simplify design of proper PROTAC molecules
- Allows PROTAC optimization
- Allows HTS screening
- Allows quantitation of interaction
- Applicable to different formats
- Requires only a standard microplate reader

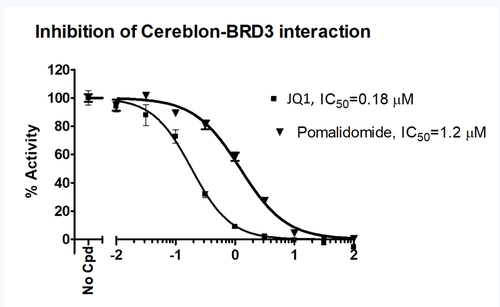
Summary

BPS Bioscience, Inc. offers a portfolio of simple and effective tools for high-throughput PROTAC optimization. These tools include biochemical assays for primary screen of test molecules as well as assessment of PROTAC efficiency.

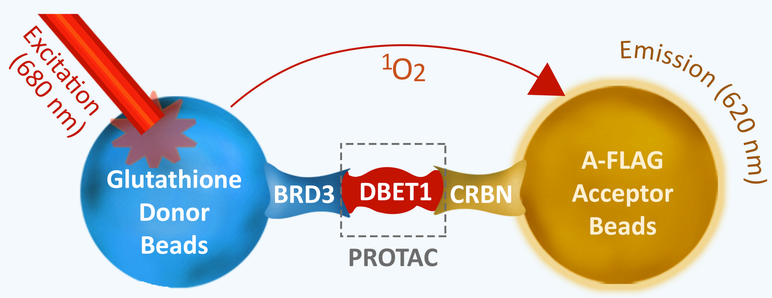
Biochemical PROTAC Assay A: BET Bromodomain-CRBN Binding (BPS Bioscience #79770)



Titration of PROTACs at fixed concentration of BRD3 and Cereblon. PROTAC-mediated interaction of Cereblon with BRD3, measured using the PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding, BPS Bioscience #79770.

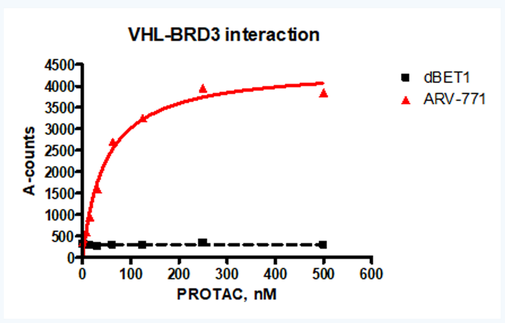


Effect of BET bromodomain or CRBN inhibitors. Inhibition by (+)-JQ1 (BPS Bioscience #27401) or Pomalidomide of dBET1-mediated interaction of Cereblon with BRD3, measured using the PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding, BPS Bioscience #79770.

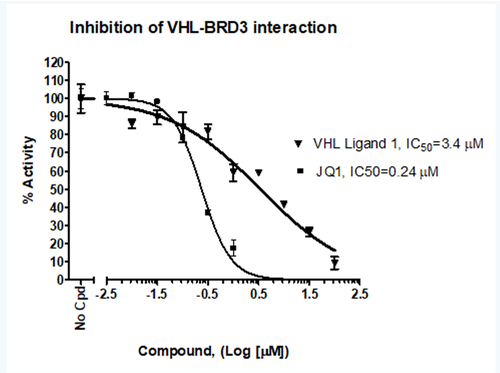


Cereblon (CRBN) (BPS Bioscience #100329)
BRD3(BD2) (BPS Bioscience #31033)

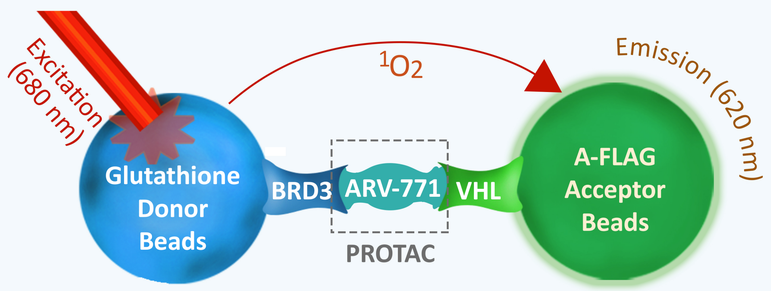
Biochemical PROTAC Assay B: BET Bromodomain-VHL Binding (BPS Bioscience #79790)



Titration of PROTACs at fixed concentration of BRD3 and VHL. PROTAC-mediated interaction of VHL with BRD3, measured using the PROTAC Optimization Kit for BET Bromodomain-VHL Binding, BPS Bioscience #79790.

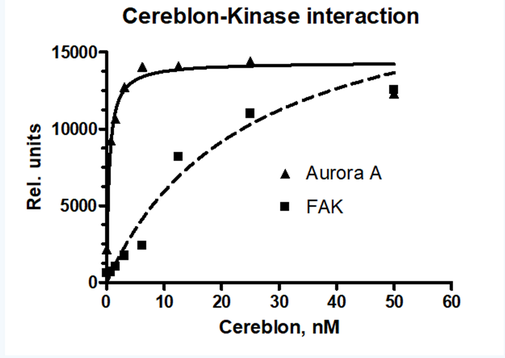


Effect of BET bromodomain or VHL inhibitors. Inhibition by (+)-JQ1 (BPS Bioscience #27401) or VHL Ligand 1 of the ARV-771-mediated interaction of VHL with BRD3, measured using the PROTAC Optimization Kit for BET Bromodomain-VHL Binding, BPS Bioscience #79790.

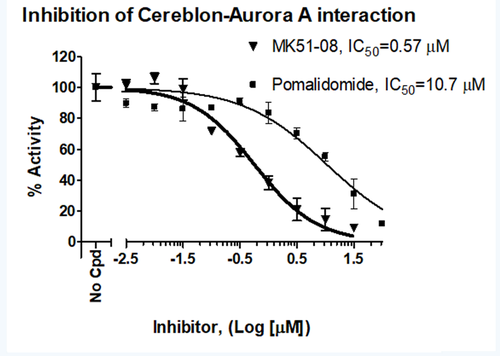


VHL – Von Hippel Lindau (BPS Bioscience #100373)
BRD3(BD2) (BPS Bioscience #31033)

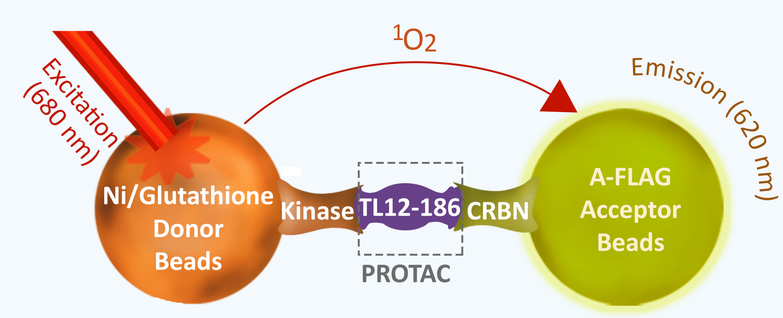
Biochemical PROTAC Assay C: Kinase-CRBN Binding (BPS Bioscience #79822)



Titration of CRBN at fixed concentration of kinases and TL12-186. PROTAC-mediated interaction of CRBN with Aurora A and FAK kinases, measured using the PROTAC Optimization Kit for Kinase-CRBN Binding.



Effect of kinase or CRBN inhibitors. Inhibition by MK51-08 or Pomalidomide of the TL12-186-mediated interaction of CRBN with Aurora A kinase, measured using the PROTAC Optimization Kit for Kinase-CRBN Binding.



CRBN – Cereblon (BPS Bioscience #100329)
His-Aurora A (BPS Bioscience #100112)
GST-FAK kinase (BPS Bioscience #40420)

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