Production of Active Cereblon and Analysis by SPR

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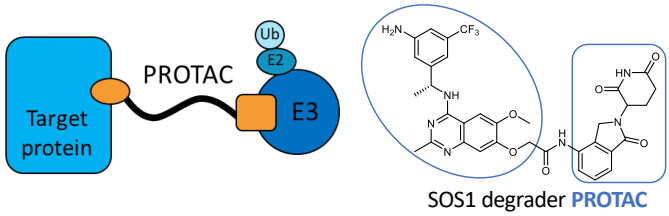
PEAK PROTEINS
A SYGNATURE DISCOVERY BUSINESS

1 PROTACs (Proteolysis Targeting Chimeras)

PROTACS bring together a target protein and a E3 ubiquitin ligase - triggering **targeted protein degradation** and selectively reducing the level of target protein within the cell



SOS1 degrader **PROTAC**

Advantages over traditional inhibitors:

- ✓ Degradation of multiple copies of protein allows use of low therapeutic doses
- ✓ New routes to undruggable targets by binding outside of catalytic pockets
- ✓ Increase the value of tight binding but inactive lead compounds
- ✓ Overcome drug resistance caused by mutations in catalytic pockets

2 Production of Cereblon at Peak Proteins

Cereblon is an E3 ligase which has been demonstrated to efficiently degrade substrates targeted by PROTACs

Peak Proteins expressed biotinylated **Cereblon** as a complex with full-length DDB1 in HEK cells

- Efficient purification strategy resulted in **high purity protein**
- Cell culture optimisation **enhanced yield 10-fold**
- **Selective biotinylation** using the Avi-tag enables **efficient**, **directional immobilisation**

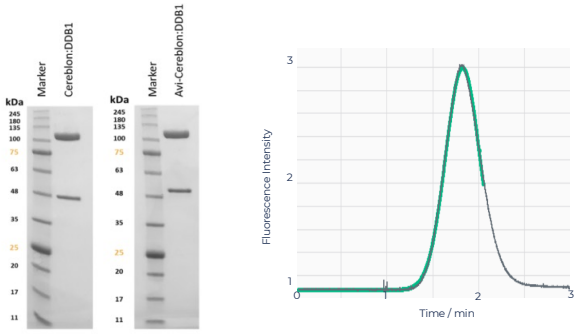


Figure 1 Left: SDS-PAGE of purified Cereblon:DDB1
Right: Fida analysis of purified Cereblon showing raw (grey) and fitted data (green)

Fida (Flow-Induced Dispersion Analysis) measured a **complex size of 4.5 nm** in good agreement with the crystal structure of the **complex**

The **PDI of < 0.0001** and absence of spikes in the raw data indicates high homogeneity with no aggregates

3 Highly active Cereblon demonstrated using SPR

Cereblon was used in SPR to demonstrate the formation of binary and ternary complexes using an **SOS1 degrader PROTAC** and **SOS1** (also produced in-house at Peak Proteins)

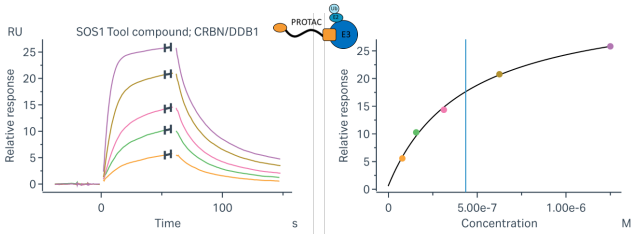


Figure 2: Binary complex formation analysis by SPR

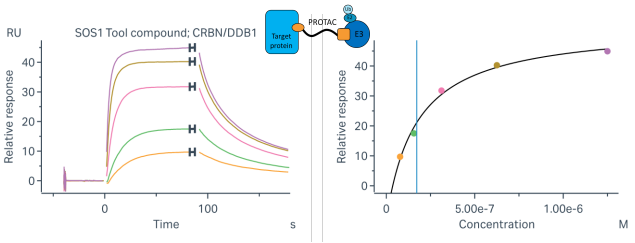


Figure 3: Ternary complex formation analysis by SPR

Cereblon was shown to be **highly active (>85%)** - much higher than material from alternative suppliers

This indicates that the immobilized **Cereblon**/DDB1 complex on the SPR chip surface is well-folded and accessible for PROTAC binding

Peak Proteins and Sygnature Discovery can help your degrader projects

- Peak Proteins can deliver highly active, purified Cereblon:DDB1 and have experience producing other degrader E3-ligases, including VHL / VCB and multiple DCAF proteins
- Sygnature Discovery can offer a full drug discovery package and has experience of designing, testing, and evaluating targeted protein degraders in customers' drug discovery projects
- The CHARMED platform available at Sygnature Discovery allows rapid discovery and assessment degraders for your protein of interest