CRBN COOPERATIVITY ASSAY

Celtarys' **CRBN cooperativity assay** is a homogeneous **fluorescence polarization** (FP) assay designed to assess how a **protein of interest (POI)** influences the apparent binding affinity of a CRBN-recruiting PROTAC for the **CUL4A/RBX1/DDB1/CRBN complex**. The assay uses **CELT-077** as a fluorescent CRBN tracer and compares matched PROTAC displacement curves in the absence (binary) and presence (ternary) of the POI.

A POI-dependent shift in the displacement curve provides a measure of apparent cooperativity under the assay conditions. This comparison helps characterize how the POI influences CRBN engagement by a PROTAC.

Why this assay?

This assay provides the following advantages:

- **Direct comparison** of binary and POI-containing conditions for the same **PROTAC.**
- Quantitative concentration–response curves and apparent **cooperativity (α)** assessment.
- **Homogeneous fluorescence polarization** readout with no washing or separation steps.
- **High-affinity fluorescent tracer**: CELT-077 has a low nanomolar affinity for the CRBN complex.
- **Expert-run fluorescence polarisation** screening service for fast and reliable data generation
- As an FP assay, it involves a **limited number of reagents and steps** simplifying implementation.
- **384-well format** suitable for comparative profiling of multiple PROTAC candidates.

How does FP (fluorescence polarization) work?

The principle of the assay is based on the **change** in **rotational mobility** that occurs when a **fluorescent ligand binds to a larger target protein.** Free CELT-077 rotates rapidly in solution and, when excited by plane-polarised light, produces a low fluorescence polarisation signal. When CELT-077 binds to the target protein CRBN, its effective molecular volume increases, rotational relaxation slows down and the **emitted light** retains a **higher degree of polarisation**, resulting in a higher FP signal (Figure 1). CRBN-binding compounds compete with CELT-077 for the same binding site, displacing the tracer in a concentration-dependent manner and reducing the FP signal.

To evaluate cooperativity, the **same PROTAC is tested under two experimental conditions:**

- **Binary condition**: CRBN + PROTAC + CELT-077.
- **Ternary condition**: CRBN + PROTAC + POI + CELT-077.

The displacement curves obtained under both conditions are compared to determine whether the presence of the POI modifies the apparent affinity of the PROTAC for CRBN. The **cooperativity factor (α)** is calculated as the ratio between the binary and ternary apparent binding affinities:

- **α > 1: Positive cooperativity**. The presence of the POI enhances the apparent affinity of the PROTAC for CRBN.
- **α ≈ 1: No detectable cooperativity**.
- **α < 1: Negative cooperativity**. The presence of the POI reduces the apparent affinity of the PROTAC for CRBN.

Positive cooperativity may favor ternary complex formation, whereas negative cooperativity indicates less favorable interactions under the tested conditions.

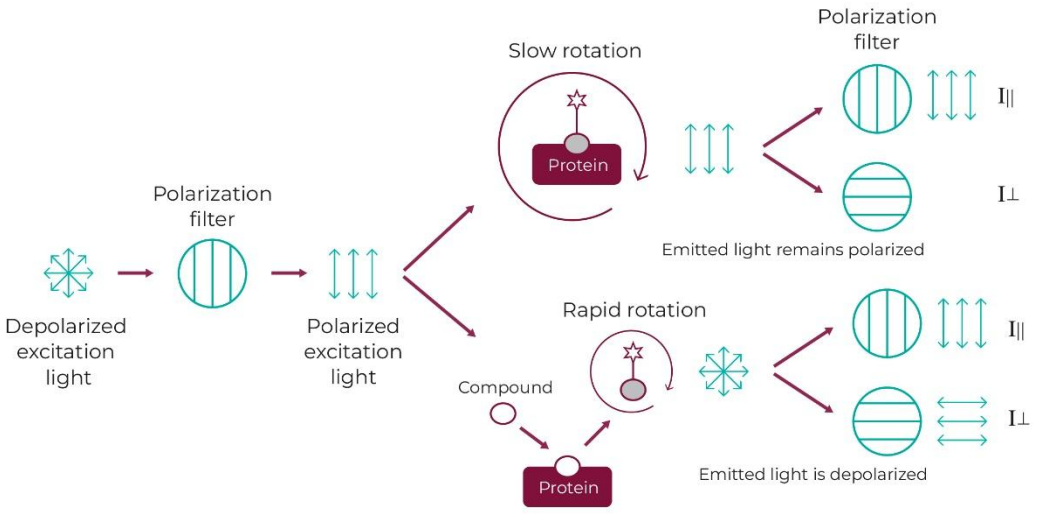

Figure 1. Depiction of the basis of FP assays.

What you receive

PDF report containing cooperativity assessment results, including **IC50**, **apparent Ki** values and **cooperativity factors (α).**

Workflow & timelines

- **Scope & quote:** Define the experimental requirements, select the Protein of Interest (POI) and receive a tailored quotation.
- **POI sourcing:** Customers may provide their own purified POI or request Celtarys to source and purchase the appropriate recombinant protein on their behalf. Any associated protein acquisition costs will be included in the quotation.
- **Sample shipment:** Ship PROTAC samples and the POI (if customer-supplied), following Celtarys' sample submission guidelines.
- **Assay execution:** Conduct the cooperativity assay under optimized experimental conditions.
- **Data analysis & reporting:** Evaluate binary and ternary binding affinities, calculate cooperativity factors (α) and deliver a comprehensive results report.

Note: Experimental timelines will be confirmed based on assay requirements, sample availability and any necessary protein procurement or optimization.

Sample requirements (summary)

- **PROTAC samples**: Preferably supplied as 10 mM stock solutions in DMSO or as lyophilized solids, with sufficient material for the selected concentration-response assays.
- **Protein of Interest (POI):** Customers can either supply their own purified recombinant protein or simply specify the desired target protein. For customer-supplied proteins, please provide protein identity, purity, concentration, formulation buffer and recommended storage conditions. For proteins sourced by Celtarys, our team will assist in selecting a suitable commercially available option.
- **Sample identification**: All samples must be clearly labelled with unique identifiers matching the corresponding documentation.
- **Stability & handling**: Please indicate specific storage, transport or formulation requirements to ensure optimal sample integrity.

Note: Protein procurement is subject to commercial availability and suitability for the assay. Any additional costs will be communicated and agreed upon before purchase. Celtarys Research will handle samples strictly in accordance with the instructions provided on the sample submission form. Celtarys is not responsible for any deterioration, loss of activity or delays resulting from inadequate packaging, non-compliant transport or failure to specify optimal storage/handling conditions. The customer must clearly indicate all special requirements (e.g., light protection: shipping in amber vials and/or wrapped in aluminum foil; temperature control with a validated carrier and recorder; inert atmosphere; humidity/pH limits; required diluent/solvent; maximum freeze-thaw cycles) and ensure that the product is packaged in accordance with them. Samples arriving outside the specified conditions or without complete documentation may be rejected or require a new shipment at the customer's expense and within the timeframe determined by the customer.