Characterize your PROTAC hook effect with flexible and in-solution affinity measurements for results you can trust

Once the screening for warheads and ligase ligands is over and you finally have fully assembled PROTAC candidates, you need to characterize the ternary complex hook effect. In order to select candidates that enhance cooperativity — a proven approach that mitigates the extent of the hook effect by favoring ternary complex stability — it's important to determine the biophysical parameters of the ternary complex.

Dianthus with Spectral Shift technology — a plate-based affinity screening platform that handles the throughput needed for hit identification and lead validation — also excels in the characterization of ternary complexes and the hook effect with these advantages:

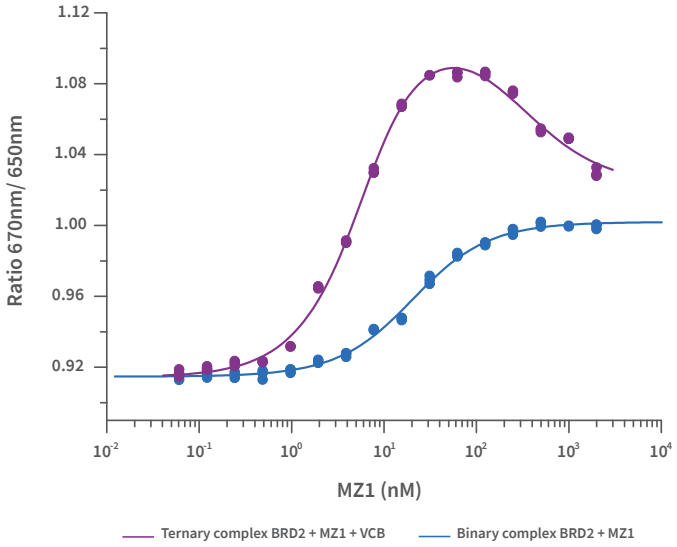
1. Get a flexible assay setup — you have the option to label the POI or E3 ligase when you measure binding affinities
2. Measure affinities in solution — so you don't stress out about having to immobilize ternary complex components
3. Generate data that agree with a mathematical model for three-component binding equilibria, so you feel confident about your results

Hook effect characterization of a highly cooperative ternary complex

The binary and ternary complex formed by target BRD2, PROTAC MZ1, and ligase VCB were characterized with measurements performed in solution with labeled BRD2 using Dianthus with Spectral Shift. To calculate the affinity constants the data obtained were fit using a model for three-component systems[1].

The affinity of the binary complex BRD2 + MZ1 was determined first (K_d = 18.8 nM), followed by the characterization of the ternary complex BRD2 + MZ1 + VCB under conditions that revealed the hook effect. The concentration of MZ1 that results in maximum ternary complex formation was determined to be 57 nM. Additionally, the formation of the ternary complex confirmed previously reported positive cooperativity[2] (α = 9.3) which is known to correlate with efficient ubiquitination and target degradation.

Dianthus with Spectral Shift offers in-solution measurements and options to label the POI or ligase, plus generates data in good agreement with a model for three-component binding equilibria for results you feel confident about.



[1]Douglass et al *J Am Chem Soc* (2013), 135, 6092-6099
[2]Zengerle et al *ACS Chem Biol* (2015), 10, 1770-1777

Choose the Dianthus that fits your PROTAC characterization needs

Get a plate-based and microfluidics-free affinity screening platform. No fluidics means no maintenance, so your projects don't get delayed due to downtime. Dianthus is ready whenever you need it — non-stop, 24/7. All you have to do is choose a model that matches your requirement for affinity sensitivity.

Dianthus Pico

Your best option if the success of your PROTAC projects requires screening for binders with millimolar to picomolar affinities

Dianthus

Your solution if you know you'll only need to characterize interactions with millimolar to nanomolar affinities

NanoTemper's mission is to create biophysical tools
for scientists to tackle their challenging characterizations

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