A Novel Approach to Target Engagement for Drug Discovery

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Abstract

Target engagement is a key step in early-stage drug discovery. Measuring the binding of a drug to its intended therapeutic target assists in understanding its efficacy and mechanism of action. Characterizing this in a cellular and disease relevant background that includes the relevant cytoplasmic mediators and proteins adds further biological context. It is an advantage to measure compound engagement with a validated target early in the screening cascade, as it helps accelerate the process of identifying and prioritizing drug candidates. Here we describe a proprietary chemical protein stability assay (CPSA), comparable to market alternatives, that can be employed simply, economically and at scale to directly measure drug: target interactions in cellular lysates.

Introduction

CPSA is a distinctive technology licenced to Selvita from Medicines Discovery Catapult (MDC). After exposing cells or lysates to compounds of interest, the protein target is treated with a chemical denaturing agent, and the proportion of the protein in its folded and denatured states are assessed. If the compound has bound to the target, the protein will become more stable with higher tolerability of a chemical denaturant. This leads to a shift in the denaturant concentration response curve compared to a suitable control and indicates that the protein and the compound are bound to one another.

Experimental Workflow

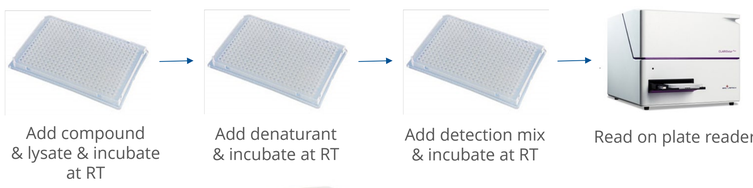


Figure 1: CPSA is a plate-based mix and read assay, compatible with automation and scalable to both 384 and 1536-well formats. Few steps make it simple to run, the use of lysates reduces variability and removes any need for protein generation, and there is no requirement for specialist equipment or high temperature incubations. It is also inexpensive to use, making it fully amenable to HTS

(MTS data is not shown but is available here: https://md.catapult.org.uk/publications/mdc-chemical-protein-stability-assay-cpsa-a-novel-target-engagement-approach/)

Comparison to an Alternative Assay

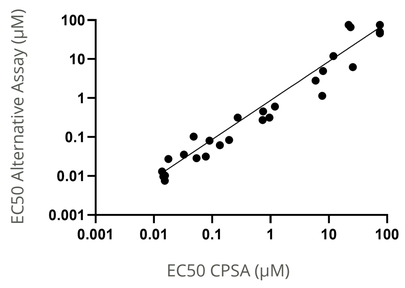


Figure 2: EC_{50} comparison between CPSA and an alternative thermal denaturation assay. Target engagement to p38 was assessed for the same compound set in both chemical (CPSA) and thermal (commercially available alternative) denaturation assay formats. Cellular lysates were screened in a 384-well format. There was significant correlation between the two technologies (r = 0.79 p=,0.0001). Data courtesy of Medicines Discovery Catapult.

Target Engagement Can be Measured with Different Detection Methods

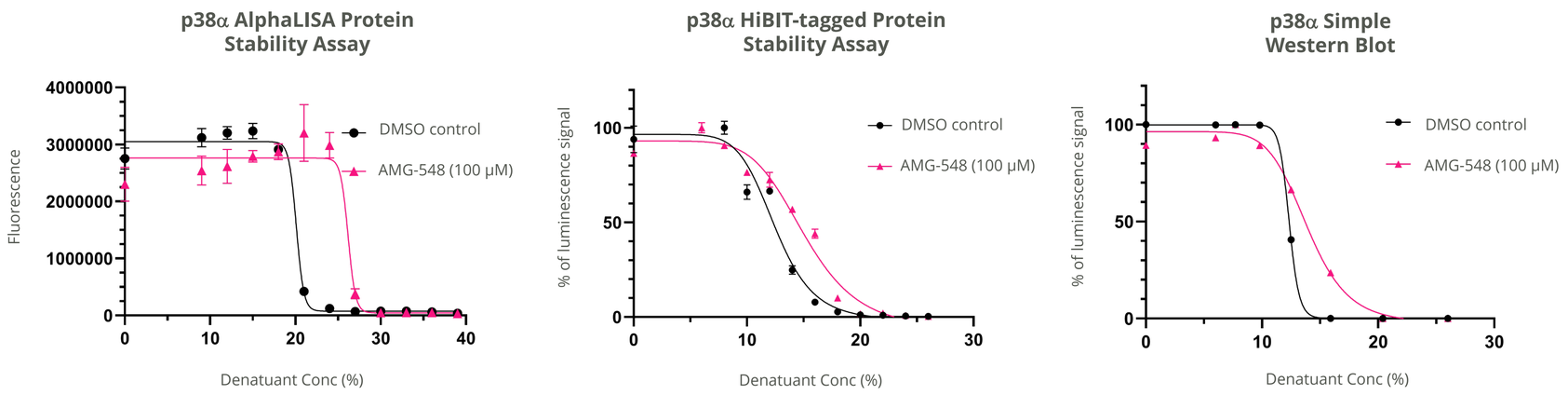
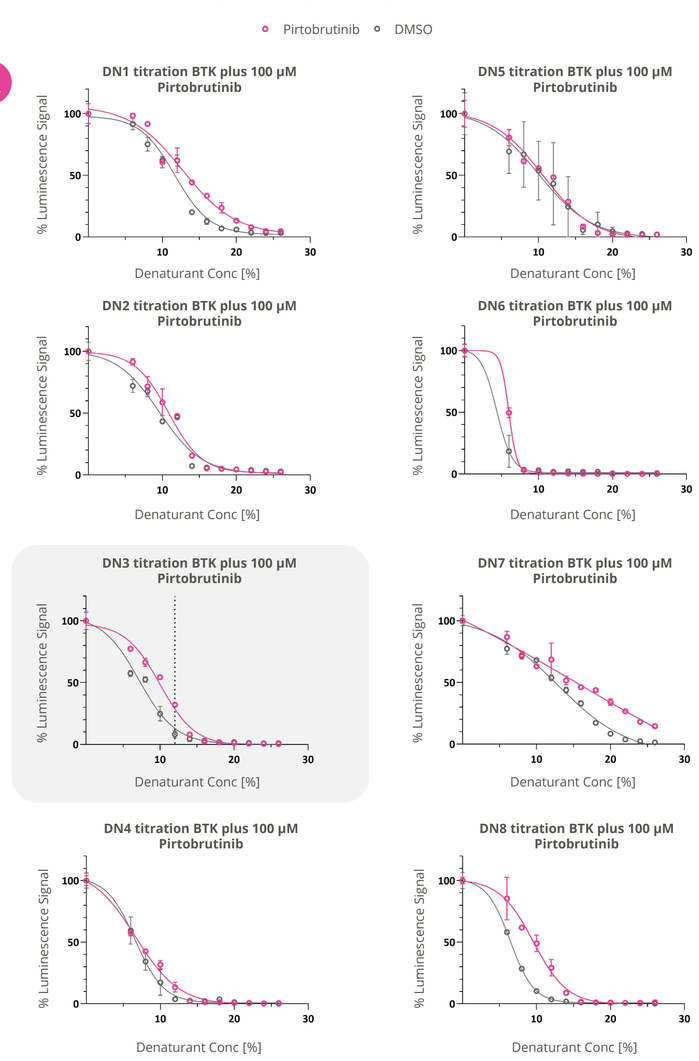
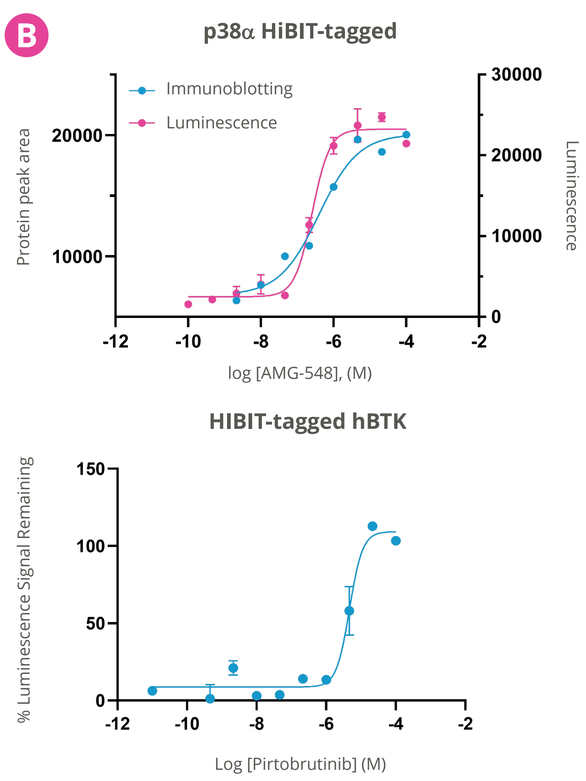


Figure 3: Measurement of protein stability in multiple detection technologies. Target engagement to p38α was assessed in cellular lysates in a multi-well format. Binding of AMG-548 to p38α shifted the proportion of the folded to denatured protein compared to the DMSO control. This potency shift was detected by AlphaLISA, HiBiT and Western blot technologies. Denatured proteins disrupt the mechanism of action of detection technologies, resulting in disruption to protein proximity measurements or antibody binding. Here a different chemical denaturant was used for each technology and a final denaturant concentration determined for subsequent experiments.

Determine Target Appropriate Denaturant



Measure Target Engagement



CPSA Technology Can Identify Inhibitors Specific to WT or Mutated Target Proteins

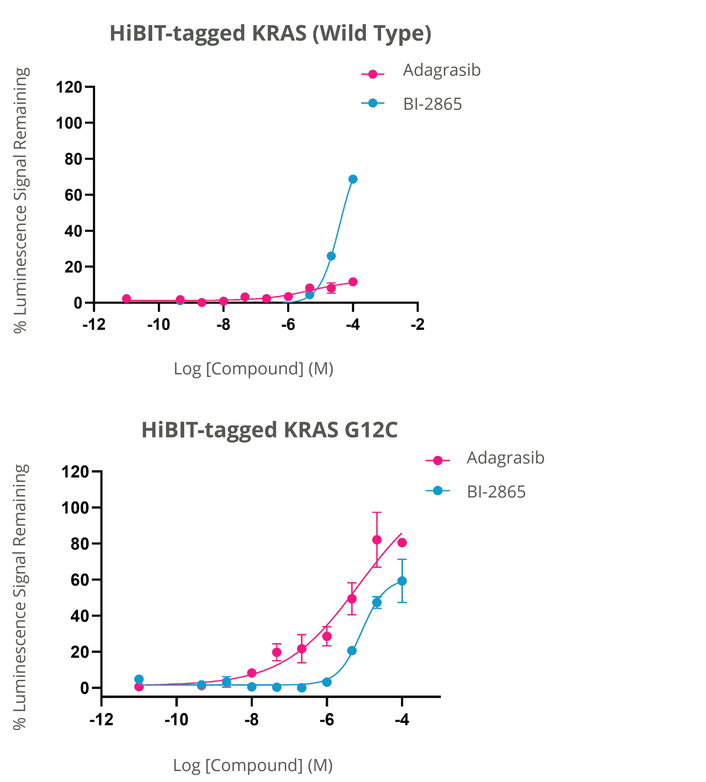


Figure 4: Application of CPSA to multiple cellular targets. Lysates were generated from HeLa (p38) or HEK cells (BTK & KRAS) overexpressing HiBiT-tagged target proteins. For each target, the optimal chemical denaturant type and concentration was determined. [A] shows an example of this for p38. Assays were run in 96-well (p38) or 384-well (BTK & KRAS) microtiter plates with the appropriate denaturant. Protein binding was measured via the Nano-Glo® HiBiT Lytic Detection System. [B] Target appropriate control compounds were screened in concentration response to determine pXC50s. There was parity between XC50s independent of the detection technology used. [C] Two inhibitors of KRAS were screened against HiBiT tagged WT protein or the G12C mutation. Adagrasib is specific for the KRAS G12C mutation and BI-2856 is a pan-RAS inhibitor. CPSA differentiated between the two, with target engagement absent for Adagrasib in the WT cell lysates.

Summary

CPSA is a target engagement technology which has been shown to give comparable pharmacology and data quality to commercial temperature driven protein stability assays (for those targets tested). Cost savings, a simplified HTS-compatible workflow and the scalability of CPSA make it an ideal screening tool for determining cellular target engagement for drug discovery targets. Selvita has demonstrated CPSA target engagement for multiple protein targets, and we continue to collaborate with Medicines Discovery Catapult to further expand CPSA applications and ultimately support researchers to make confident, data-driven decisions as they advance new therapeutic programs.