

# Lumit™ protein interaction immunoassays using protein tags and biotinylated small molecules

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## 1. Introduction

Protein interactions, including protein-protein interactions (PPI) and protein-small molecule interactions, play critical roles in numerous cellular processes such as trafficking, signaling, apoptosis, and proliferation. As major drivers of signaling cascades, deregulated protein interactions are implicated in disease states including autoimmune diseases and cancers. Thus, modulating protein interactions is a major focus in drug discovery research.

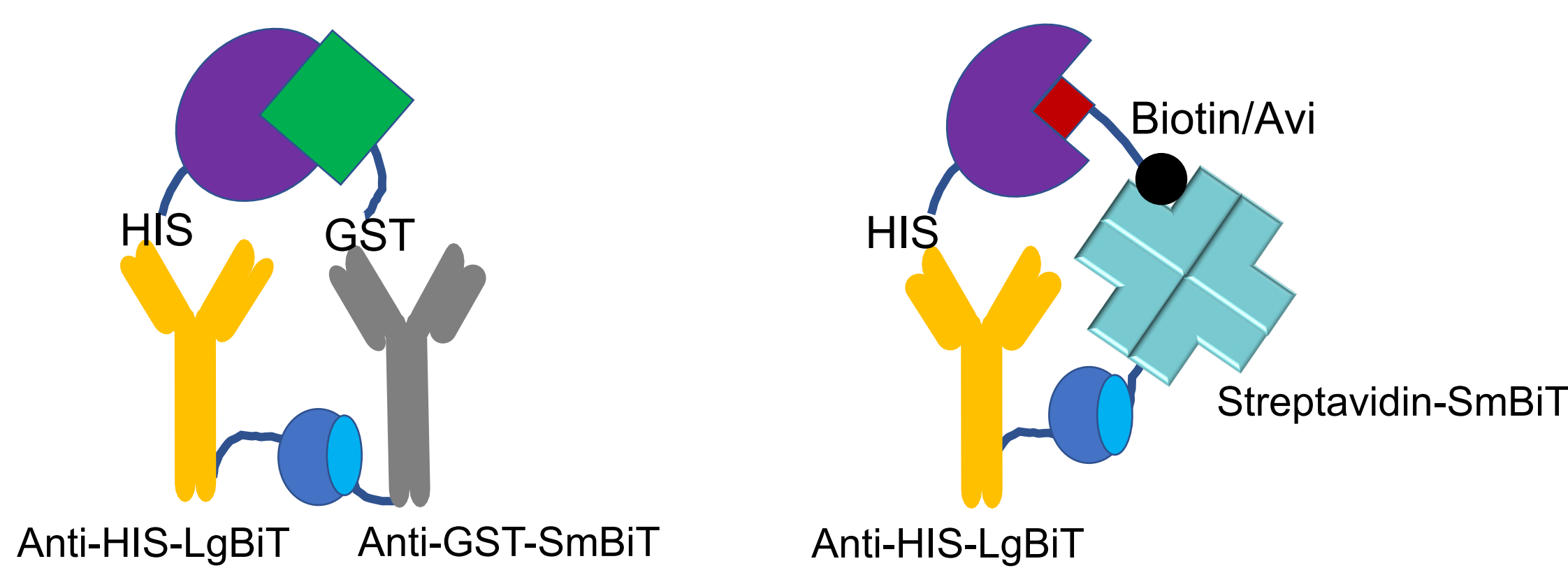
We present Lumit™ Protein Interaction Immunoassay using protein tags, a homogenous (no-wash) bioluminescent assay to measure protein-protein or protein-small molecule interactions in solution. These assays have a simple, add and read protocol and enable fast, highly sensitive assays suitable for multiplexing and high throughput screens.

Here, we demonstrate the utility of Lumit™ Protein Interaction Immunoassays for monitoring:

- Interactions between KRAS mutants and RBD-cRAF.
- Small molecule inhibitors of tyrosine kinases.
- PROTAC-mediated ternary complex formation.
- Autoubiquitination of E3 ligase Cbl-b.

## 2. Lumit™ Protein Interaction Immunoassay using protein tags

Lumit: A bioluminescent and homogeneous immunoassay powered by NanoBiT technology



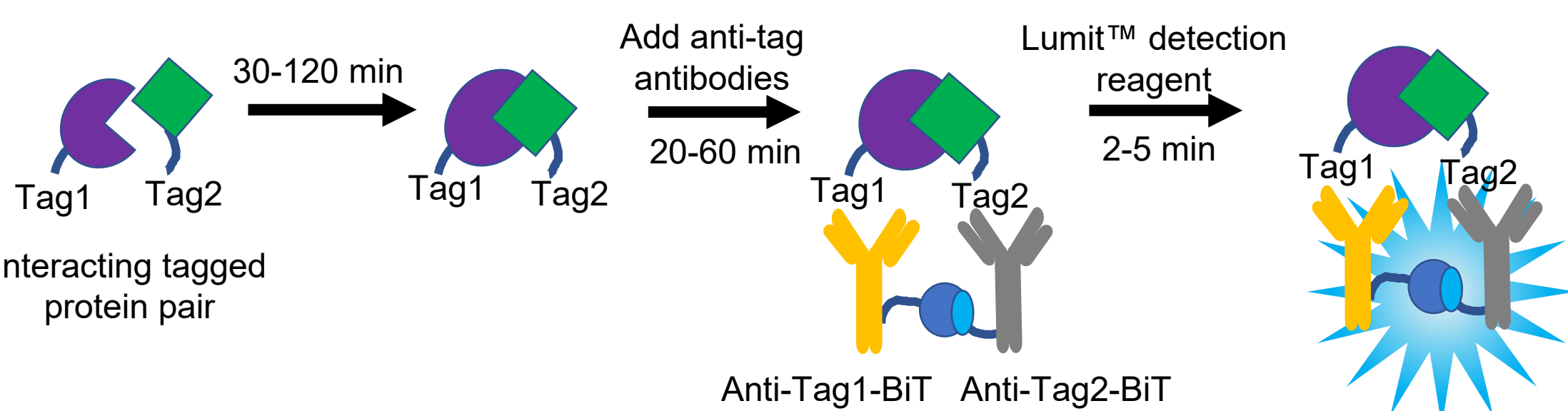
### Protein-Protein Interaction

Lumit™ Protein Interaction Immunoassays are based on SmBiT- and LgBiT- labeled anti Tag antibodies and Streptavidin.

### Protein-Small Molecule Interaction

When two tagged proteins or a protein and small molecule interact in the presence of corresponding Lumit™ reagents, the two anti Tag antibodies come into proximity and SmBiT and LgBiT complement to form an active enzyme that emits a bright luminescence signal in the presence of substrate.

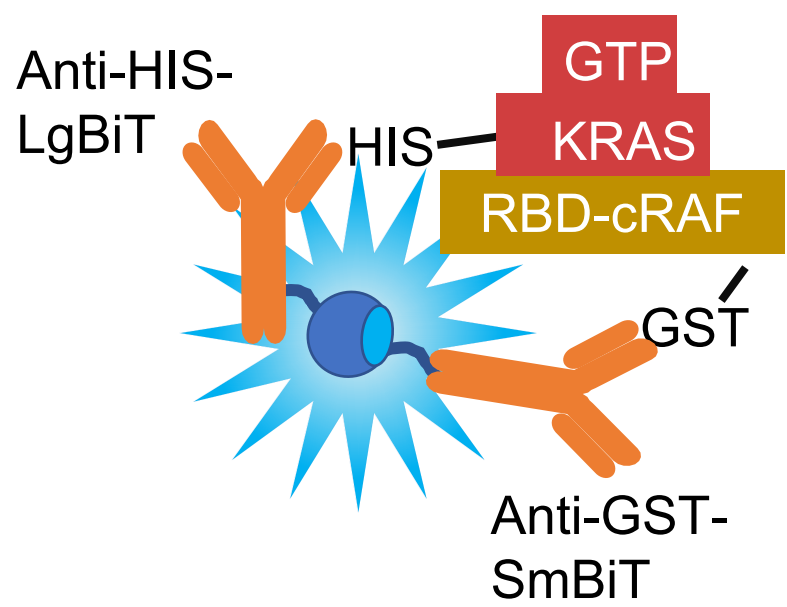
## 3. Simple workflow of Lumit™ Protein Interaction Immunoassay



Reagents are available for the following tags:

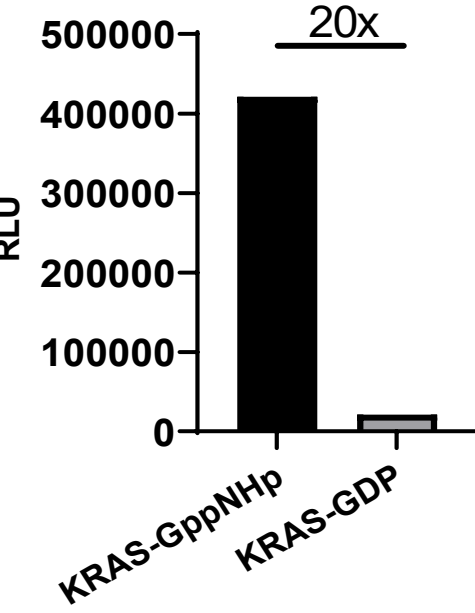
- 6HIS
- GST
- Human IgG
- FLAG®
- Biotin/AVI

## 4. Lumit™ KRAS-RAF Assay monitors KRAS-GTP-dependent interaction with cRAF

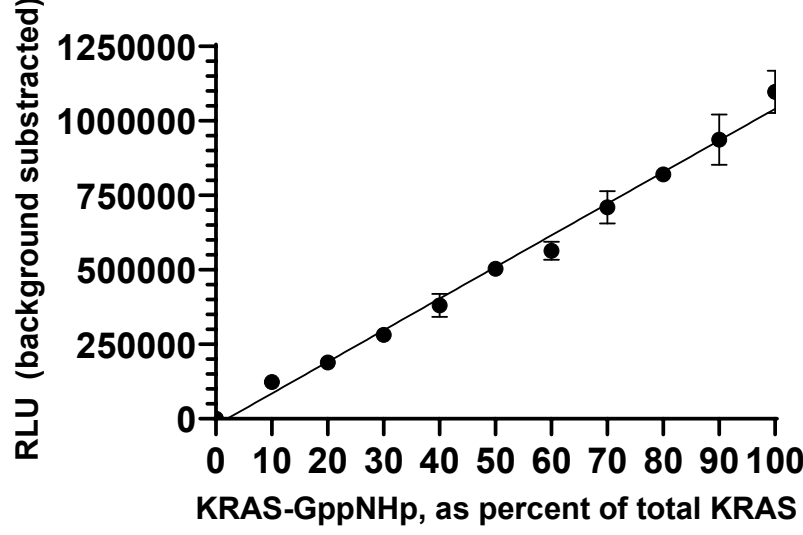


For Lumit™ KRAS/c-RAF interaction assays we used HIS-tagged KRAS(G12C) and GST-tagged RBD-cRAF, along with LgBiT-labeled anti-HIS and SmBiT-labeled anti-GST.

KRAS/RBD-cRAF binding

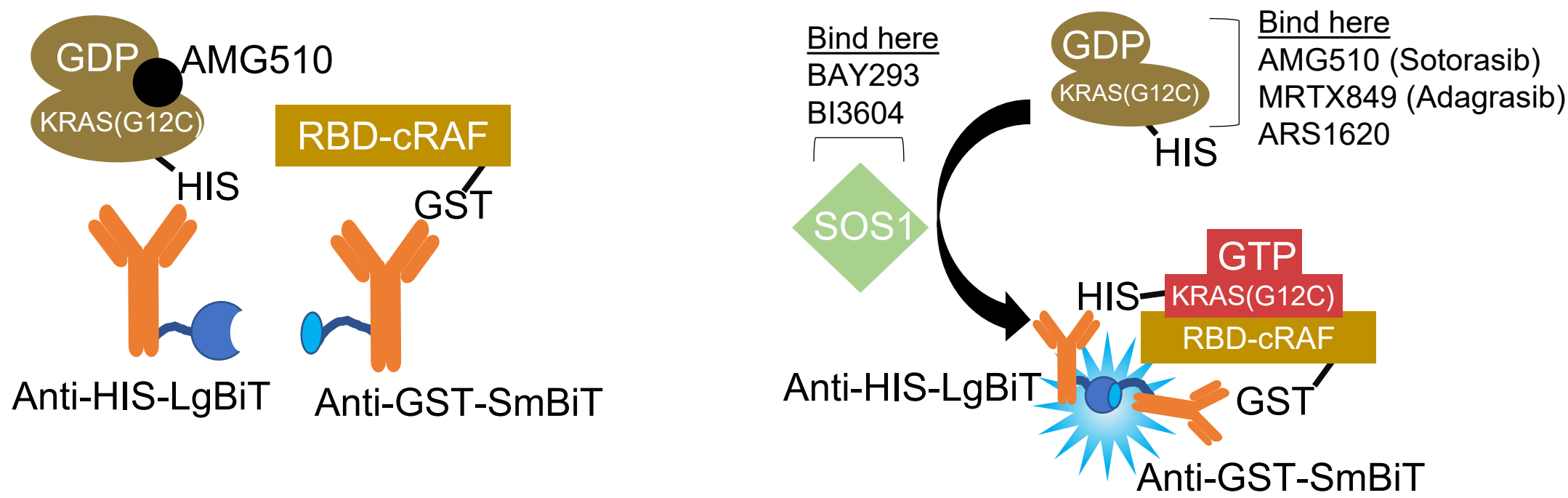


KRAS/RBD-cRAF binding

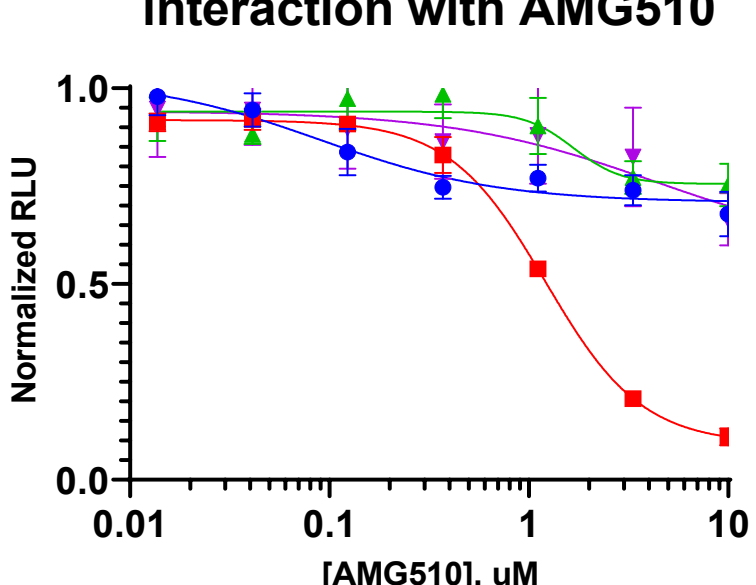


Lumit™ KRAS-cRAF assay has a wide assay window (~20 fold, left) and excellent resolution (right), which allows small changes in KRAS/c-RAF binding to be monitored accurately and reproducibly.

## 5. Lumit™ KRAS-RAF Assay can be used to investigate small molecule inhibitors

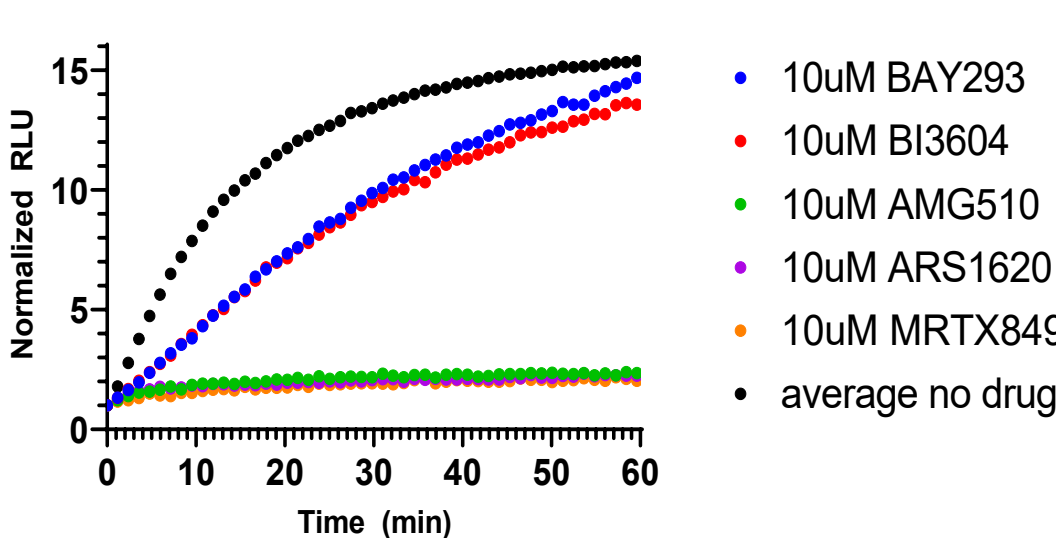


KRAS(G12C)/RBD-cRAF interaction with AMG510



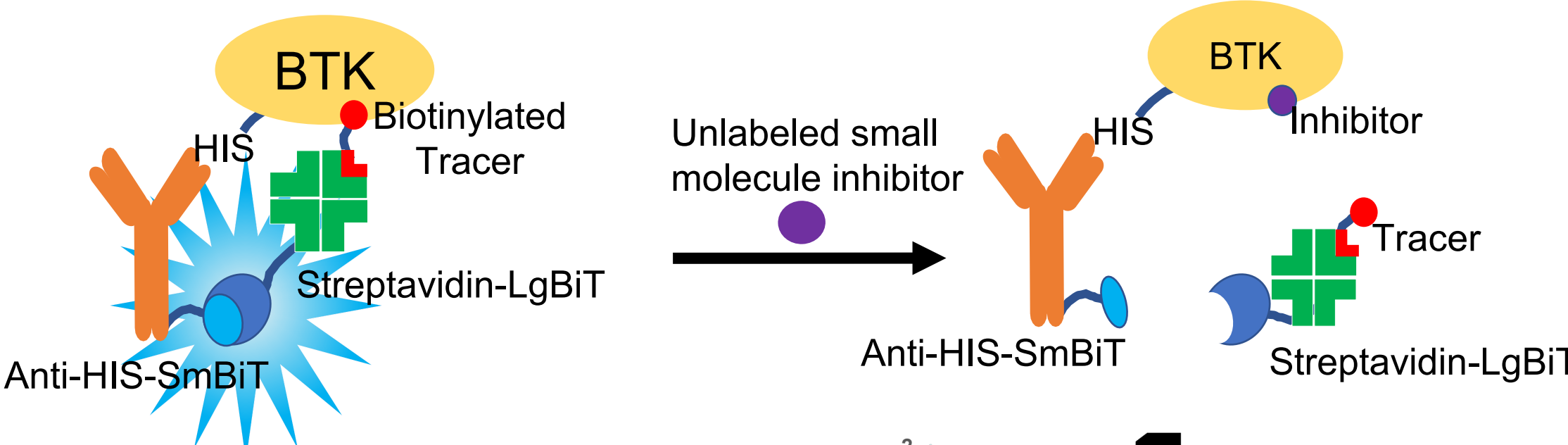
Lumit™ KRAS-cRAF assay can be used to test the specificity of small molecule inhibitors for panels of KRAS mutants.

Kinetics of KRAS(G12C)/RBD-cRAF interaction

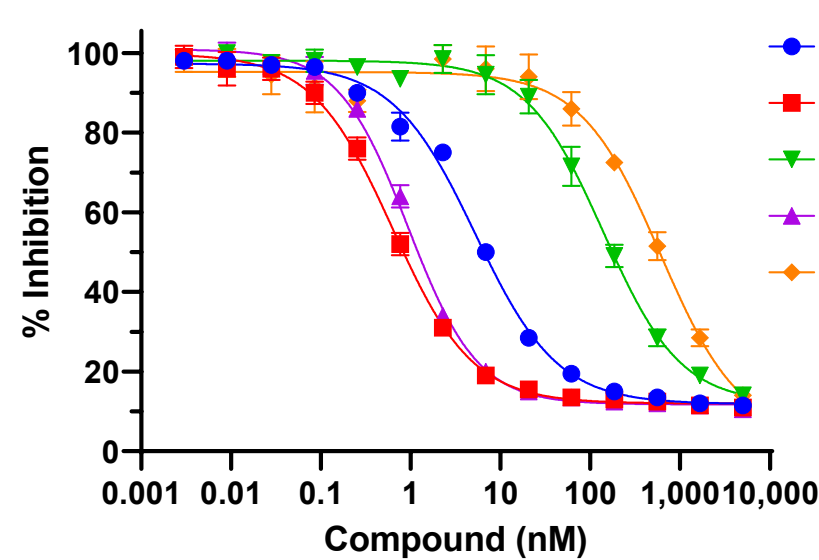


The KRAS activation cycle can be reconstituted *in vitro* and the kinetics of KRAS/cRAF binding can be monitored in the presence of small molecule inhibitors as a reflection of SOS1-mediated GDP/GTP exchange.

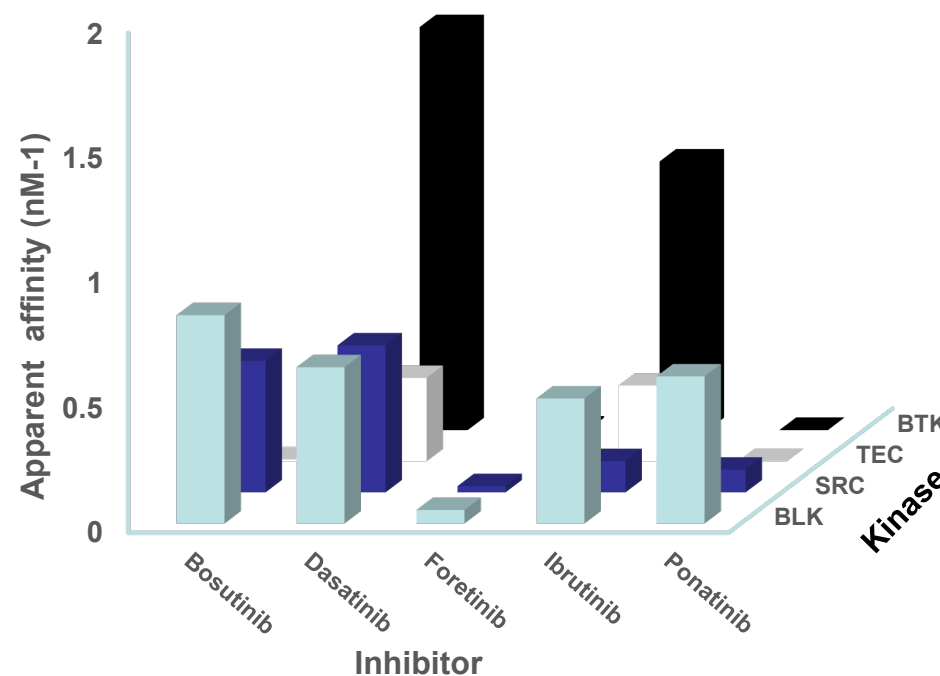
## 6. Lumit™ Protein Interaction Immunoassay for monitoring small molecule interactions



BTK with small molecule inhibitors

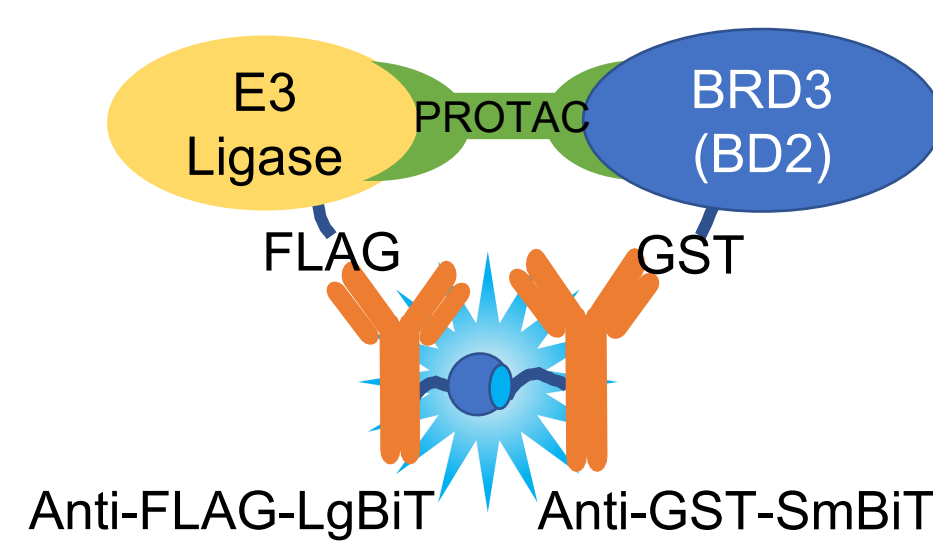


Lumit™ Kinase Assay enable sensitive and rapid screening of small molecule inhibitors.



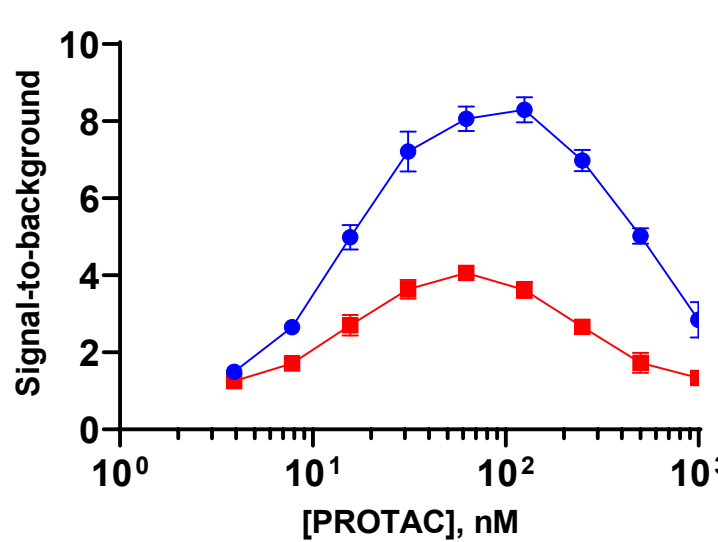
Lumit™ Kinase Assay can be adapted for high throughput formats, enabling comparisons of apparent affinities across multiple inhibitors and targets.

## 7. Lumit™ PROTAC Assay monitors ternary complex formation

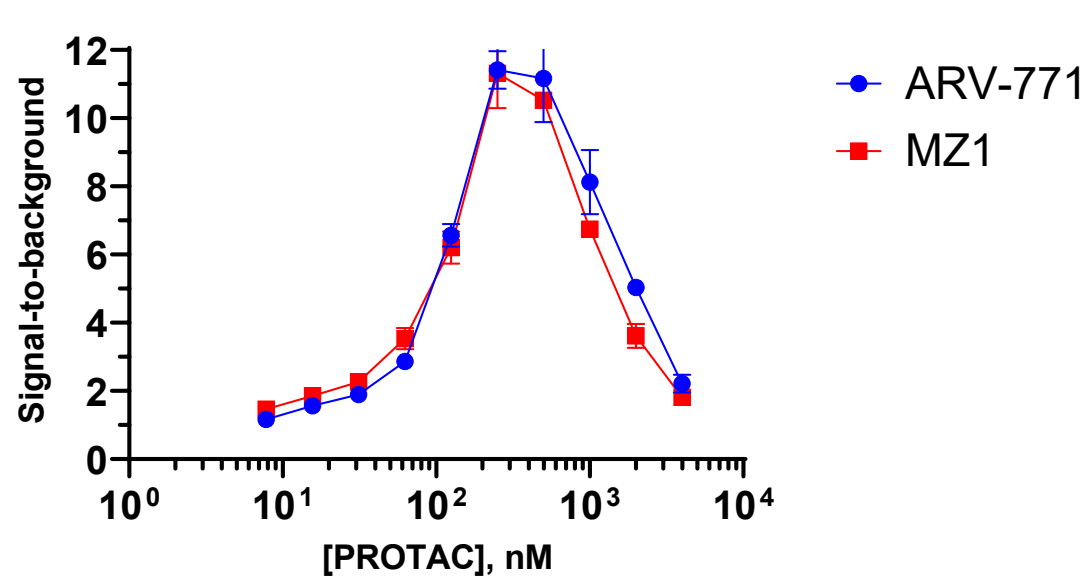


With BRD3(BD2) as our target protein, we monitored PROTAC-mediated ternary complex formation with cereblon complex and VHL complex using anti-FLAG-LgBiT and anti-GST-SmBiT Lumit™ reagents.

Cereblon complex + BRD3(BD2) ternary complex formation

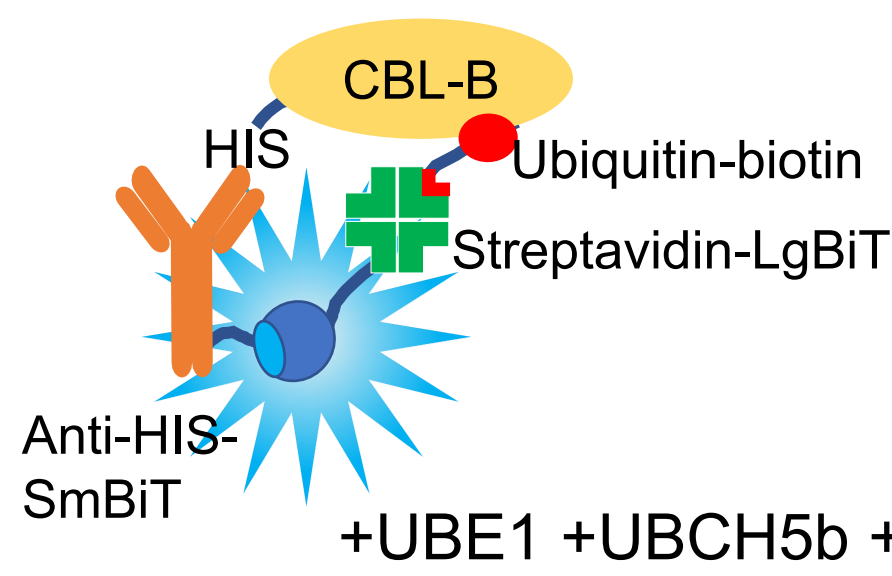


VHL complex + BRD3(BD2) ternary complex formation

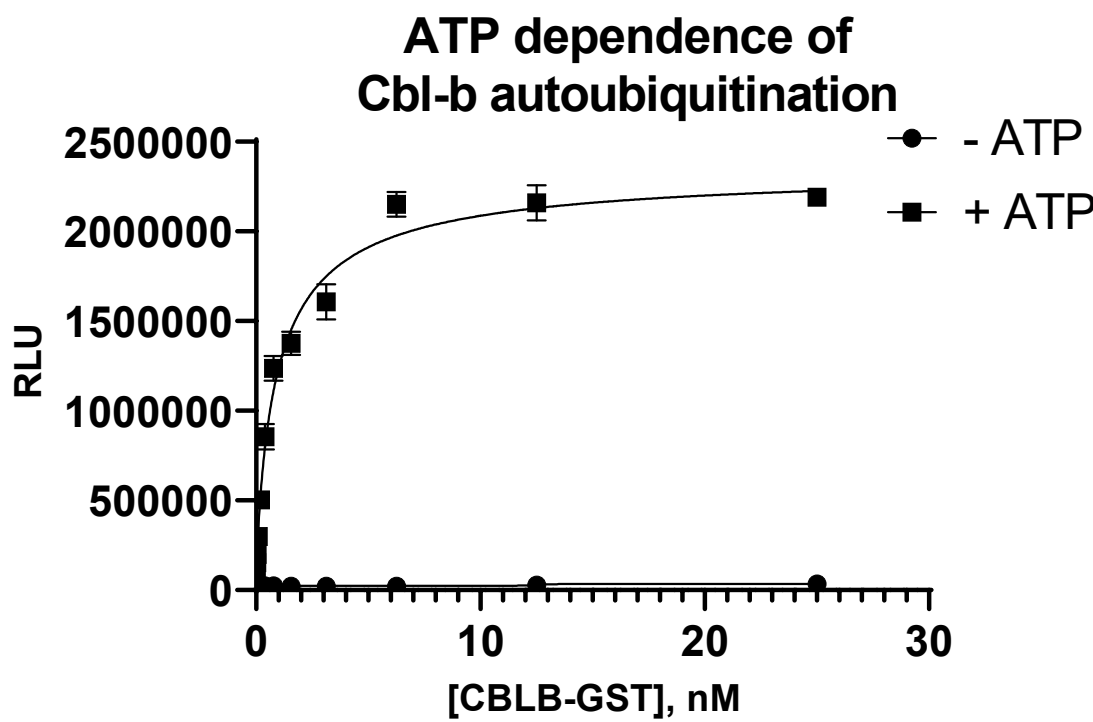


Lumit™ PROTAC Assays can monitor ternary complex formation across different E3s, target proteins, and PROTACs

## 8. Lumit™ Ubiquitination Assay monitors autoubiquitination of E3 ligase



To monitor autoubiquitination of E3 ligase Cbl-b, we incubated Cbl-b with ubiquitin activating protein (UBE1) and ubiquitin conjugating protein (UBCH5b) in the presence of ATP. Ubiquitination was monitored with streptavidin-LgBiT and anti-HIS-SmBiT.



Lumit™ can be used to monitor autoubiquitination of E3 ligase Cbl-b in the presence of E1, E2, and ATP with a ~70-fold assay window compared to control (no ATP) conditions.

## 9. Conclusions

We have developed a luminescent signal-based homogeneous immunoassay platform (Lumit™) to study protein-protein and protein-small molecule interactions.

- No wash steps or surface immobilization required.
- Assays are rapid (30-60min) and simple.
- Simple luminometer is needed for detection.
- Assays can be scaled down for 384-well plates.
- Luminescence detection provides a wide assay window.

Lumit™ Protein Interaction Immunoassays complement luminescent cell-based assays, which include:

