

Fast-Tracking PROTAC® Discovery using Direct-to-Biology: Utilising Biophysical and Cellular Assays to Target Aurora Kinase A

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Background

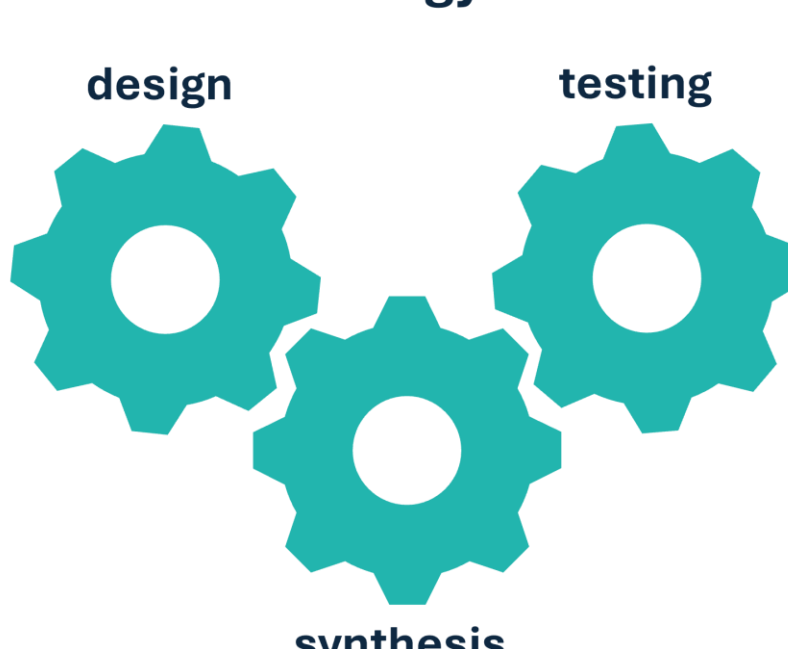
Direct-to-Biology (D2B)

- Combines plate-based chemical synthesis with rapid high-throughput biological screening
- Removes lengthy purification steps by directly screening crude reaction mixtures for desirable activity
- Increases throughput and reduces cost compared to traditional synthesis
- Reduces environmental impact of hit synthesis by limiting the need for solvent-heavy purification steps

Conventional Chemistry:



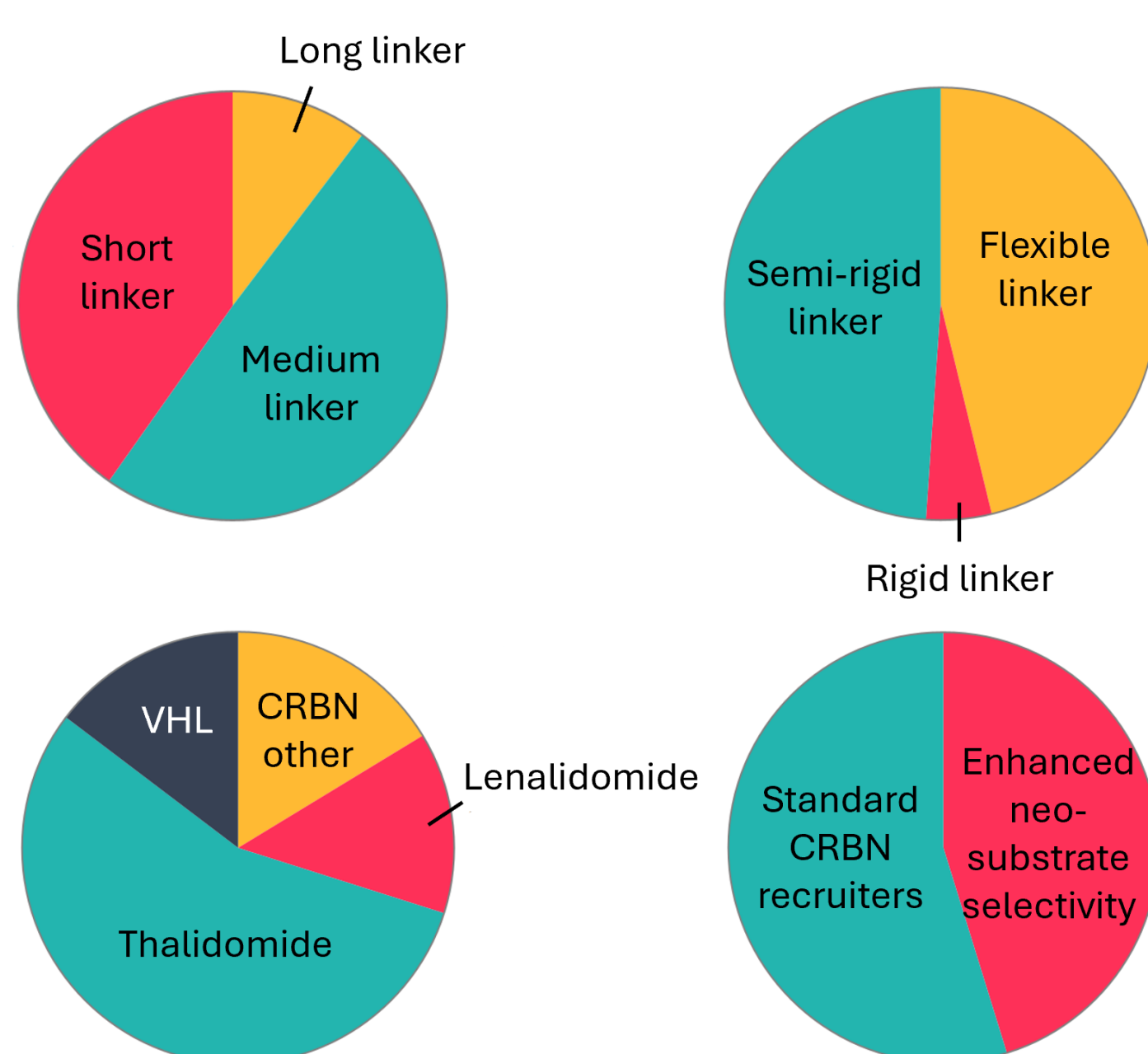
Direct-to-Biology:



D2B removes the purification bottleneck from conventional chemistry, allowing for a flexible and efficient approach to hit finding

Domainex's PROTAC toolbox

- PROTACs are heterobifunctional molecules that dual-bind an E3 ligase and a protein of interest (POI) to induce POI degradation
- Domainex has generated a proprietary PROTAC toolbox consisting of ~160 partial PROTACs (linker + E3 ligase binder)
- Toolbox includes Cereblon (CRBN) and VHL ligands, and a wide variety of linker lengths and types
- Plate-based reactions of partial PROTACs with POI binders rapidly produces D2B plates with novel PROTAC candidates for screening



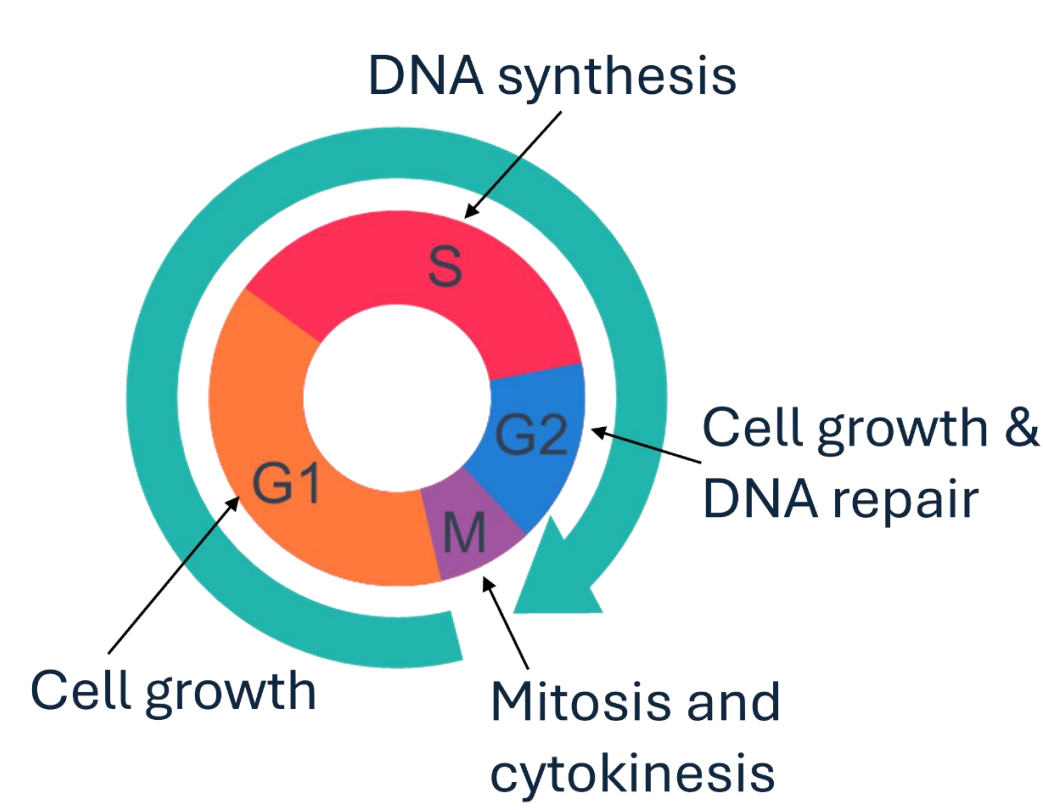
E3 ligase recruiter and linker compositions in Domainex's PROTAC toolbox

Aurora Kinase A (AurA)

- Key cell cycle regulator (overexpressed in cancer)
- Literature precedence for CRBN-mediated AurA degrader
- Used as a tool target to demonstrate D2B potential for development of CRBN-mediated novel PROTACs



AurA PDB ID: 4C3R



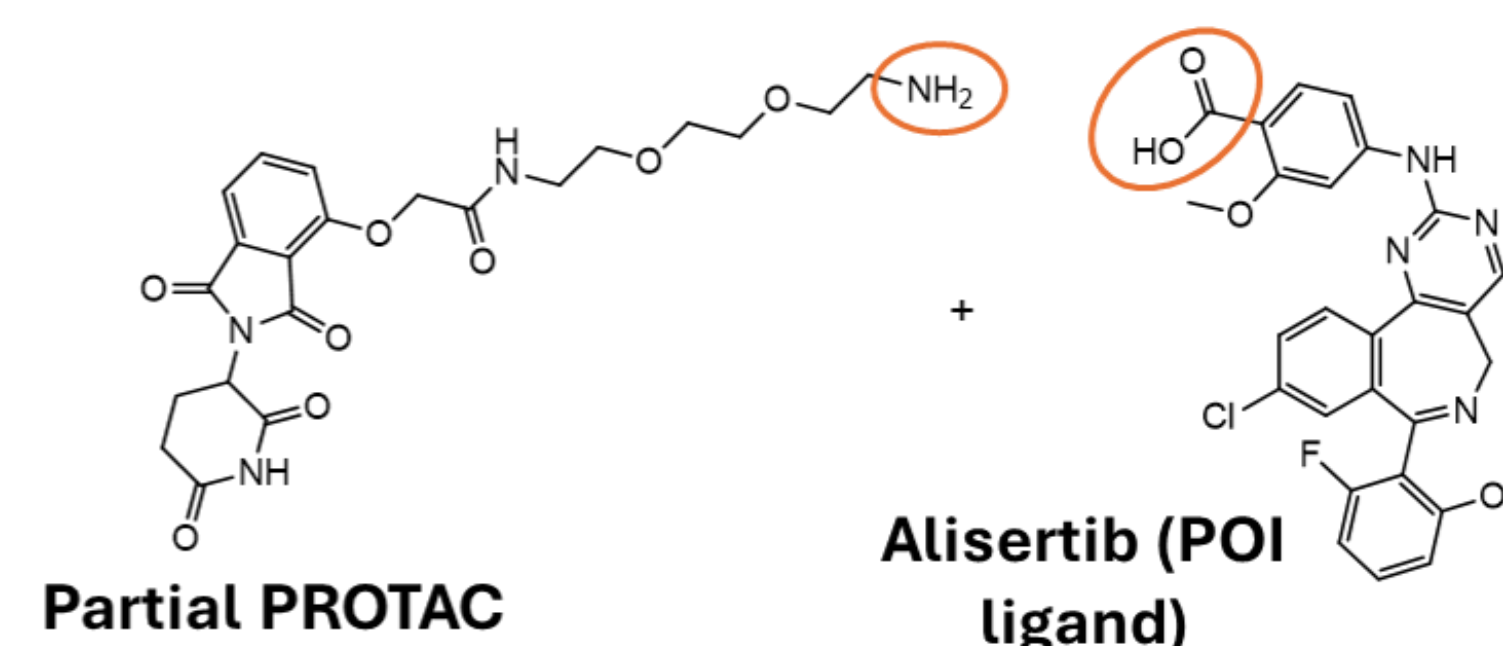
The cell cycle

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D2B Driven Discovery of Aurora Kinase A Degraders

PROTAC synthesis

- Linking Alisertib (AurA ligand) to Thalidomide (CRBN ligand) forms a potent and highly specific PROTAC-mediated AurA degrader (JB170) for use as a positive control
- Plate-based chemistry was used to amide couple forty representative partial PROTACs to Alisertib for the generation of novel AurA degraders for screening



Amide coupling of Alisertib with partial PROTACs to form novel degraders

Spectral Shift: Driving a D2B Campaign via Biophysics

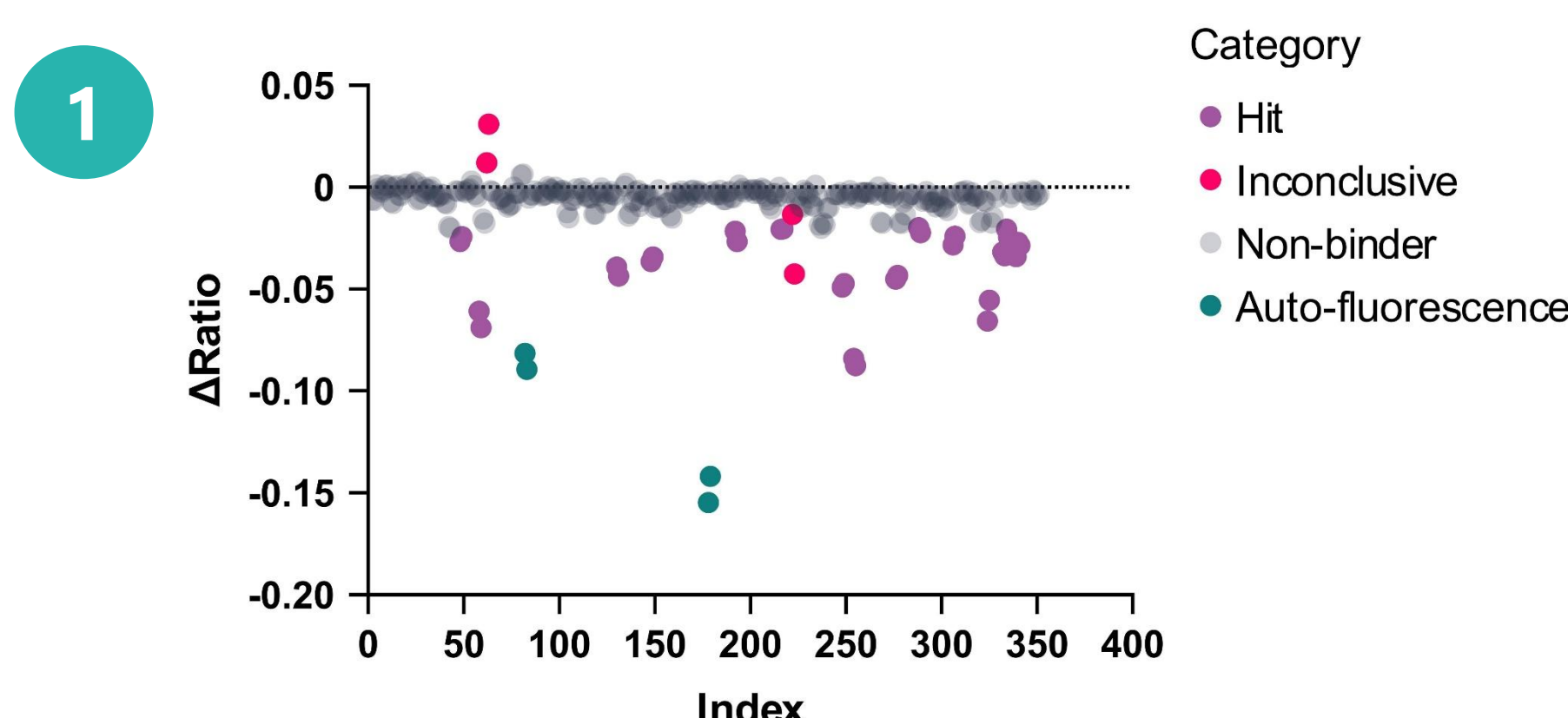
Spectral shift (NanoTemper) works on the principal that ligand binding will change the chemical environment of a fluorophore resulting in measurable changes in emission spectra

1. Single shot binding screen

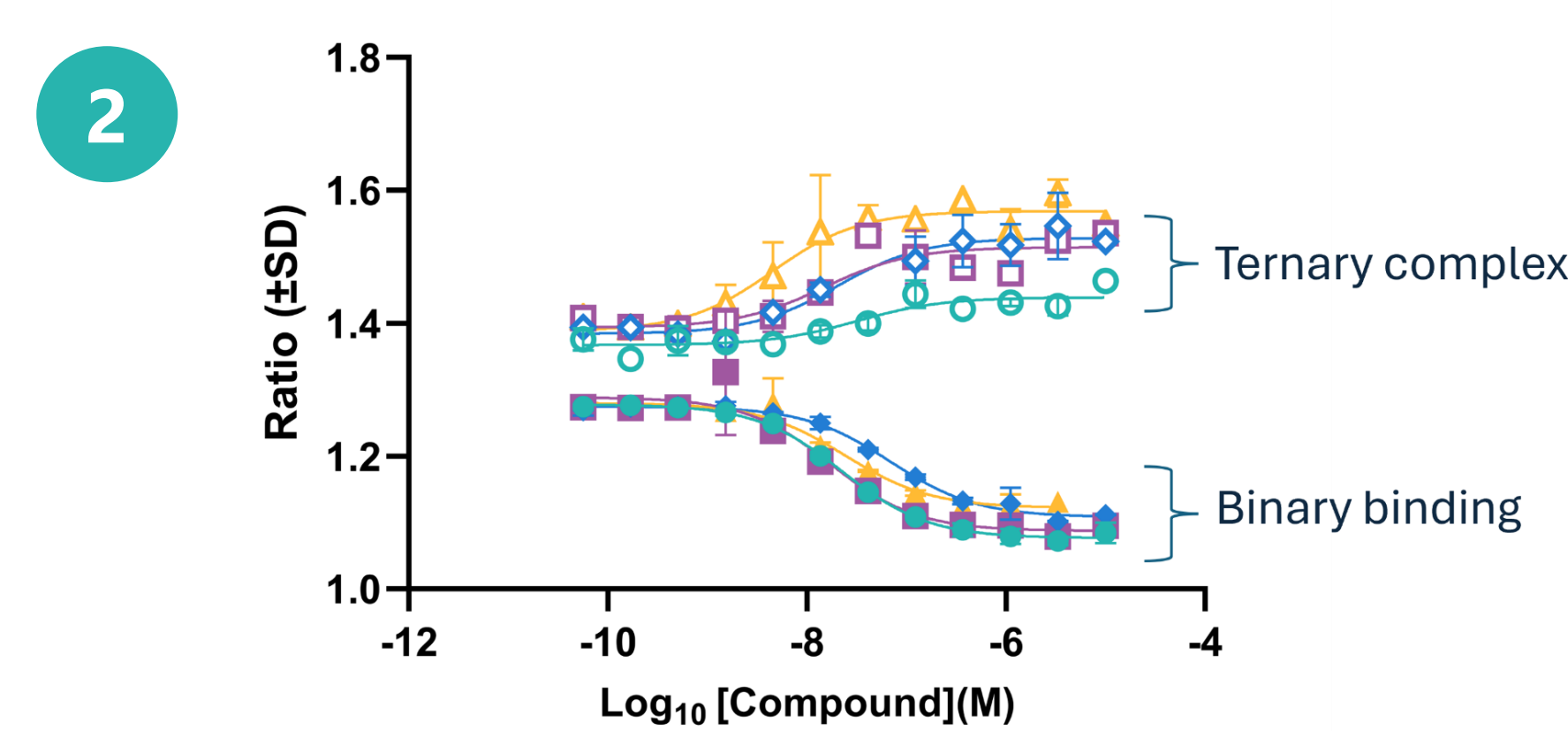
- Crude D2B mixtures were screened at a single concentration against fluorescently-labelled AurA to identify degraders capable of binding the target

2. Ternary complex formation assay

- Demonstrating ternary complex formation is an essential step in any induced proximity modality, in this case used to confirm PROTAC-mediated recruitment of CRBN to AurA
- Concentration-dependent binding profiles dramatically differ upon inclusion of CRBN with AurA, indicating effective E3 ligase recruitment by our PROTACs



Single concentration binding assessment of D2B compounds to labelled Aurora Kinase A

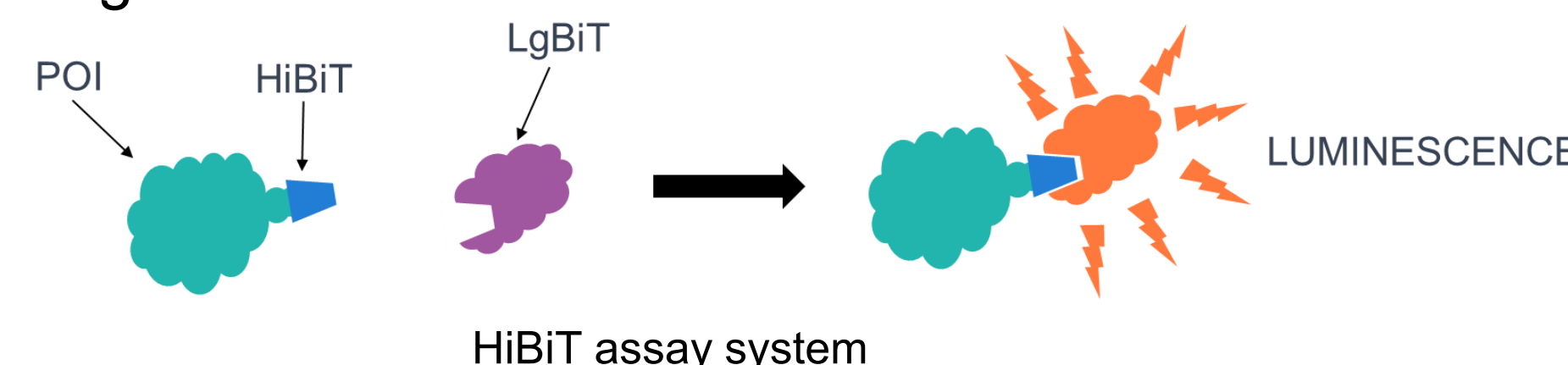


Concentration-dependent binary binding and ternary complex formation of select hits from single shot screen

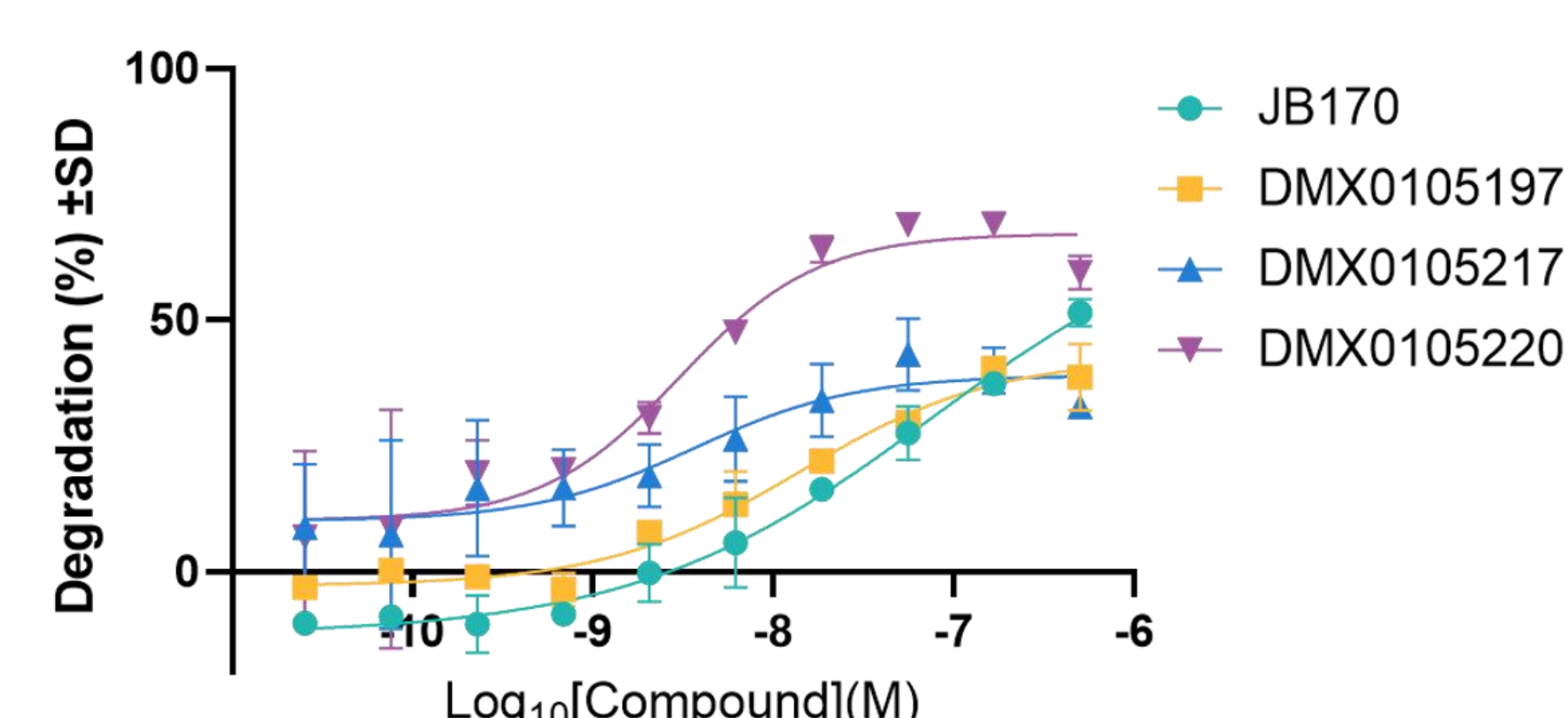
From Spectrum to System: Cellular Validation of D2B Hits

HiBiT reporter assay

- HiBiT is an 11 amino acid tag that complements with LgBiT protein to form a functional luciferase, with resultant luminescent signal proportional to cellular HiBiT-tagged protein content
- MOLT4 cells expressing HiBiT-tagged AurA were treated with JB170 and candidate novel PROTACs, resulting in a reduction in luminescent signal consistent with AurA degradation



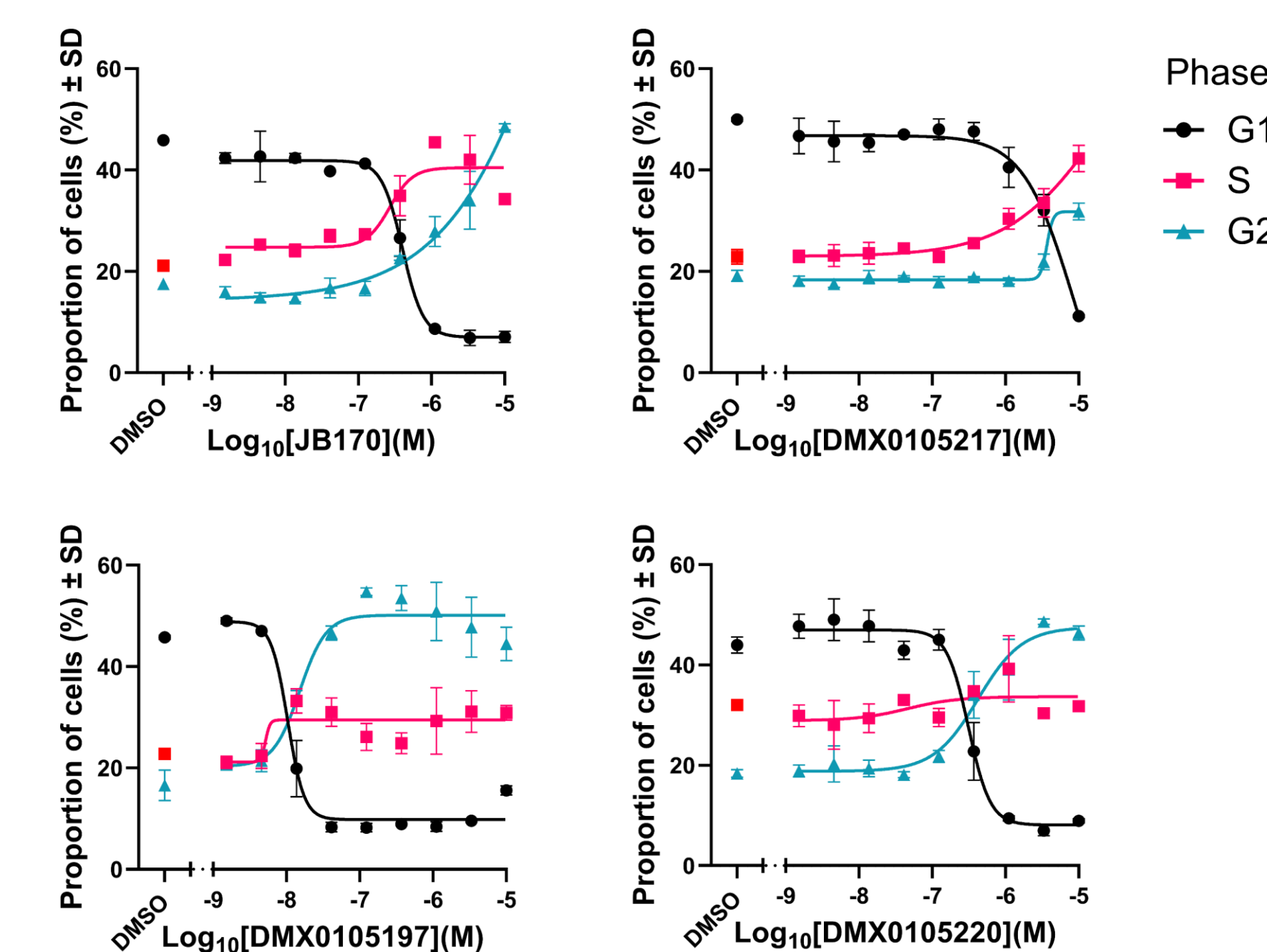
HiBiT assay system



PROTAC-mediated concentration-dependent degradation of Aurora Kinase A by JB170 and novel PROTACs

Using flow cytometry to measure cell cycle arrest

- THP1 cells were treated with concentration response curves of JB170 and select D2B compounds
- Following fixation, cells were stained with propidium iodide (fluorescent DNA intercalator) to measure DNA content
- A cell cycle analysis model was applied to determine the proportion of cells in each phase of the cell cycle
- Data shows JB170 and our novel PROTACs trigger cell cycle arrest in G2 phase in a concentration-dependent manner
- This functional effect is consistent with AurA's proposed role in regulating the transition from G2 to M phase of the cell cycle



Compounds that induce degradation of Aurora Kinase A trigger concentration-dependent cell cycle arrest in G2 phase

Summary

- Aurora Kinase A served as a model target for the integration of Domainex's PROTAC and D2B platforms, with both cellular and biophysical screening approaches used to rapidly identify novel degraders
- This approach could be applied to targeted protein degradation programmes and beyond, accelerating drug discovery to bring benefits to patients with unparalleled speed

