Leveraging Bivalent Molecules and Biophysical/Biochemical Techniques for Enhanced Therapeutic Potential

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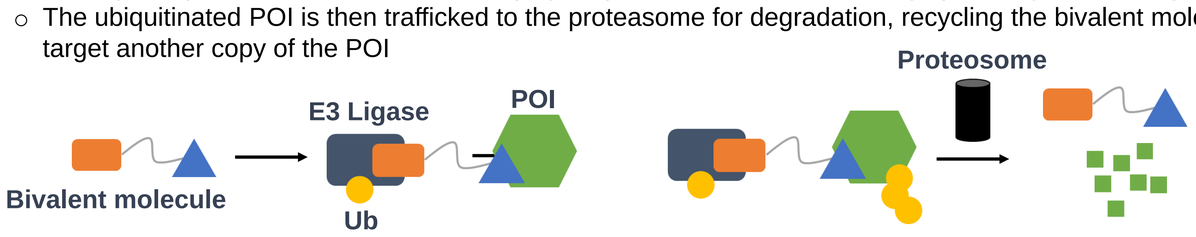
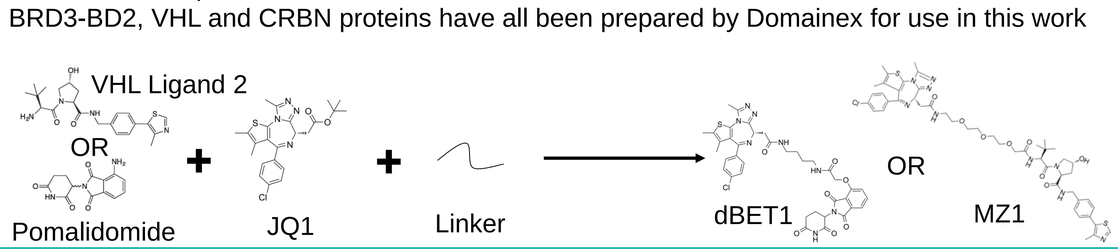
Introduction

- Bivalent molecules are heterobifunctional molecules with three components: a protein-of-interest (POI) binding moiety, a linker and an E3 ubiquitin ligase warhead
- Bivalent molecules can hijack the ubiquitin-protease system (UPS) which results in degradation of the POI
- Mechanism of action:
  - Recruits POI and E3 ligase to form a ternary complex
  - Ternary complex formation facilitates the polyubiquitination of the POI through proximity to an E3 ligase
  - The ubiquitinated POI is then trafficked to the proteasome for degradation, recycling the bivalent molecule to target another copy of the POI

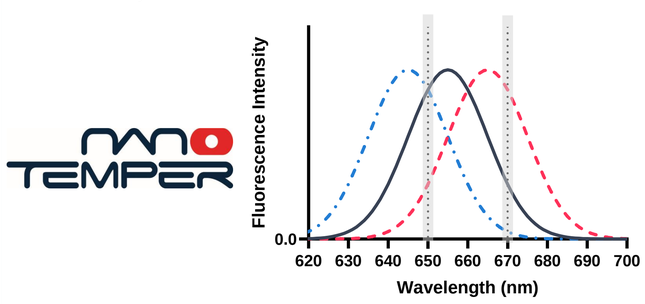


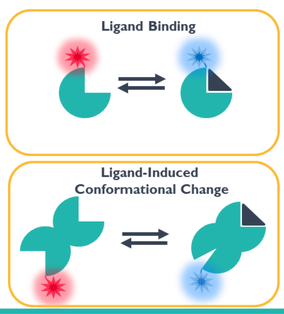
- This case study uses MZ1 and dBET1; bivalent molecules that connect a ligand for BRD3-BD2 (or other BRD proteins) and either VHL or CRBN warheads
- BRD3 is a member of the bromodomain and extra-terminal motif (BET) protein family and a therapeutic target for various diseases including cancer
- Cereblon (CRBN) and von Hippel-Lindau (VHL) are commonly utilized E3 ligases in bivalent molecule development
- BRD3-BD2, VHL and CRBN proteins have all been prepared by Domainex for use in this work

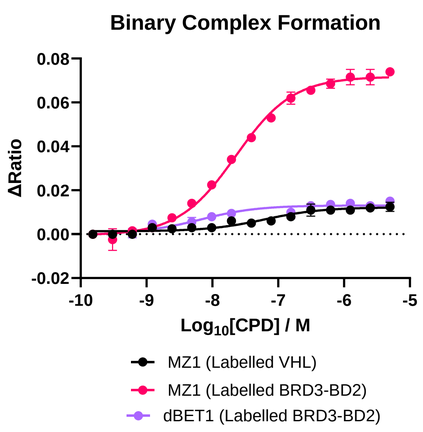


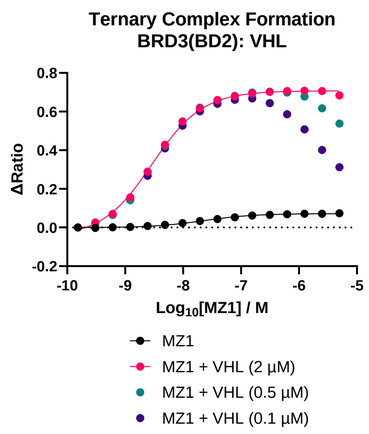
Spectral Shift

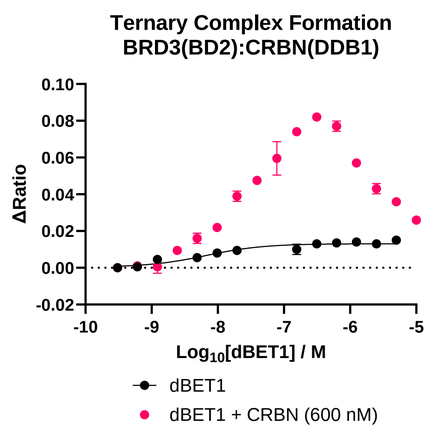
- Fluorescence-based biophysical technique used to determine ligand binding
- Requires modification of a target protein with NanoTemper's 2nd generation RED dye
- The dye's chemical environment can be affected directly when the ligand binds in close proximity or through confirmational changes induced by ligand binding, which cause a shift in the emission wavelength
- K_D derived by plotting the ratiometric measurement (FI 670 nm / 650 nm) against ligand concentration
- Generally, a better S/N ratio than MST and TRIC with less sensitivity to aggregates









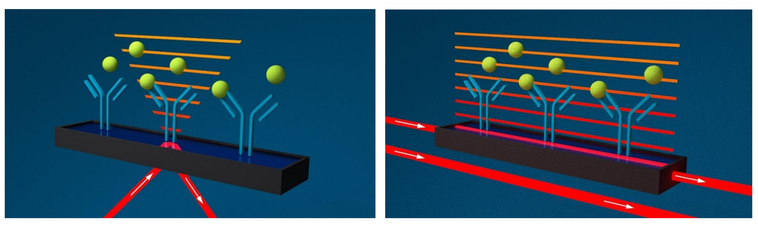


	MZ1	dBET1
Binary K_D (nM)	23	7
Ternary K_D (nM)	3	24
α value	7.6	0.3

Grating-Coupled Interferometry (GCI)

- Label-free rapid kinetic assessment for binding characterisation
- Creoptix WAVEdelta system uses (GCI) – analogous to Surface Plasmon Resonance (SPR)
  - Light travels over the entire length of the chip rather than a single point
  - More binding events contribute to the signal making the system more sensitive
- Novel microfluidics that are part of the disposable biosensor cartridge
  - Enables measurements of very fast off-rates up to 10 s^{-1}
  - Amenable to crude samples, harsh chemicals, and particles up to 1000 nm
- Proprietary waveRAPID (Repeated Analyte Pulses of Increasing Duration) method

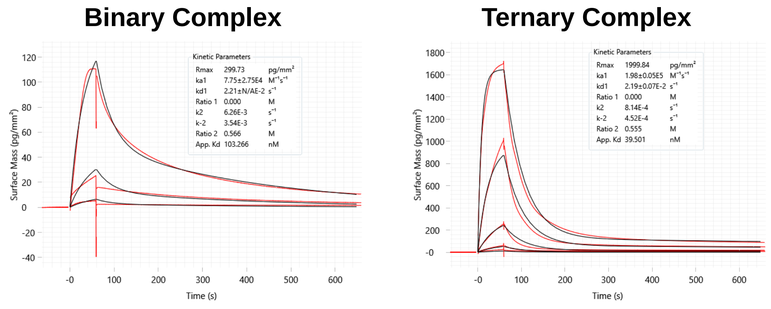
SPR **GCI**



BRD3 Immobilization and MZ1 binding

1000 ~ pg/mm² for binary binding
785 ~ pg/mm² for ternary binding
Data fitted to conformational change

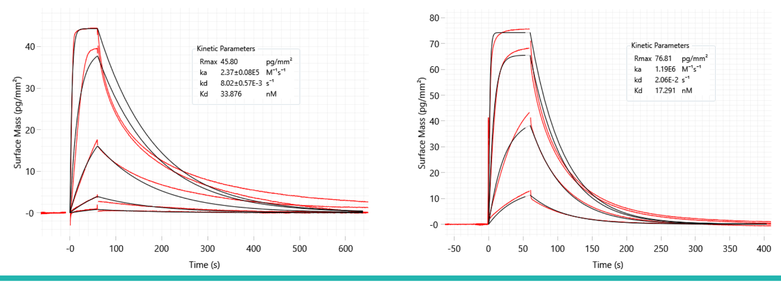
Binary Complex **Ternary Complex**



	K_D (nM)
Binary (MZ1)	103
Ternary (MZ1:VHL)	40

VHL Immobilization and MZ1 binding

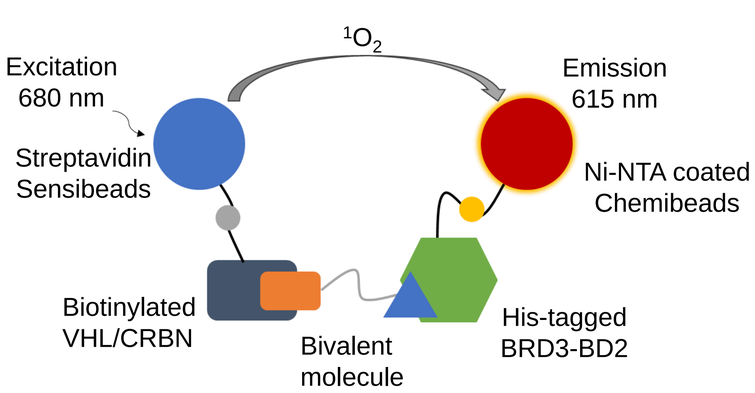
~ 2400 pg/mm² for binary binding
~ 400 pg/mm² for ternary binding
Data fitted to 1:1 kinetics



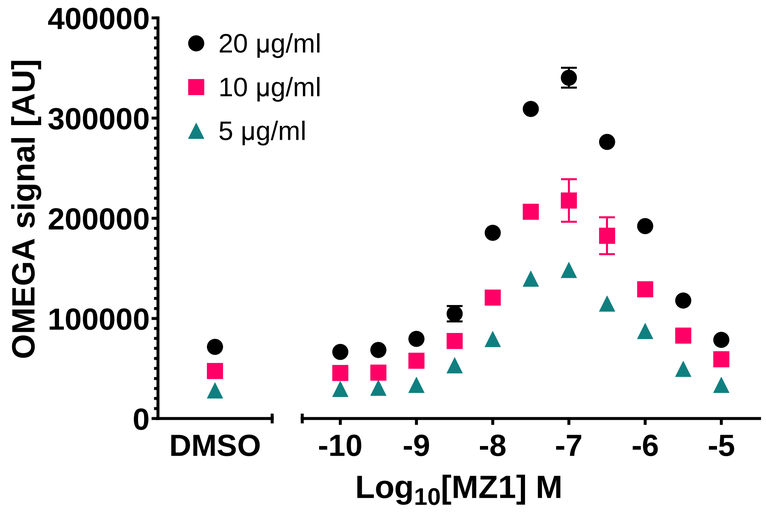
	K_D (nM)
Binary (MZ1)	33
Ternary (MZ1:BRD3)	17

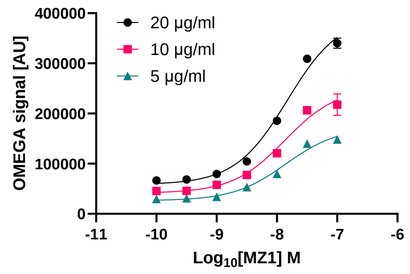
Proximity-based Biochemical Assays

- Homogenous, no-wash immunoassays with high sensitivity and wide dynamic ranges.
- Simple to adapt to high throughput and automated processes.
- Excitation of the donor bead at 680 nm generates singlet oxygen molecules.
- If the donor is in proximity of the acceptor, the generated singlet oxygen can cause a cascade of reactions leading to chemiluminescence at 615 nm.
- Can be used for sandwich or competition assays.



BRD3(BD2):VHL





Bead Concentration (µg/mL)	EC_{50} (nM)	Literature K_D (nM)
5	14.0	7 (ITC) 8 (SPR)
10	13.6	
20	15.0	

Summary

- Targeted Protein Degradation (TPD) represents a transformative approach in drug discovery, offering an innovative alternative to traditional small-molecule inhibitors.
- Bivalent molecules in TPD has enhanced the efficiency and durability of this therapeutic method
- At Domainex, advanced biophysical and biochemical techniques have been successfully employed to characterize molecular interactions:
  - Spectral Shift
  - Grating-Coupled Interferometry (GCI)
  - Proximity-based FRET (Alpha/OMEGA)
- These techniques accurately quantify complex affinities, advancing understanding of TPD and its therapeutic potential.
- Domainex can these techniques to support a diverse array of drug discovery initiatives and therapeutic modalities.

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about our drug-discovery platform, please contact: **enquiries@domainex.co.uk**

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