Detecting endogenous protein interactions and RIPTACs with no-wash assays: The new frontier for AlphaLISA™ *SureFire*® assay technology

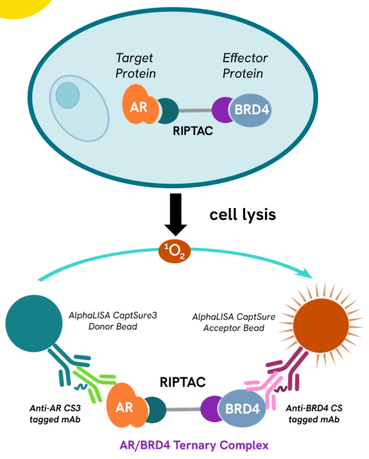
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1 Overview

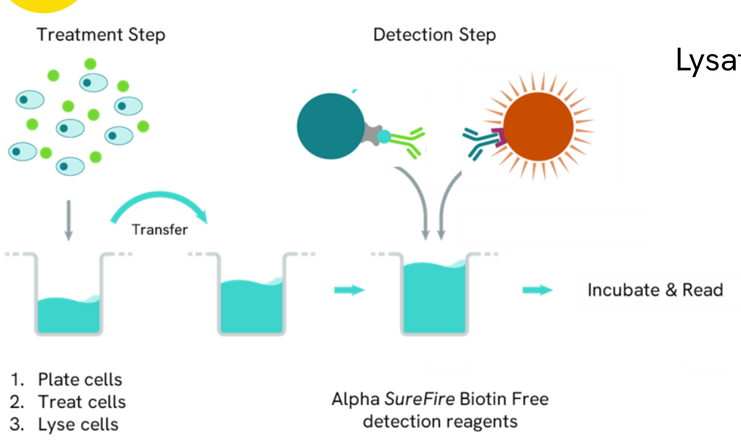
- Protein-protein interactions (PPIs) are fundamental drivers of cellular function. When dysregulated, they can contribute to the development of diseases, including cancer, neurodegenerative disorders, and autoimmune conditions, making PPIs compelling therapeutic targets.
- Among emerging therapeutic strategies, induced proximity is rapidly gaining traction as a novel method for modulating disease biology. Regulation Induced Proximity Targeting Chimeras (RIPTACs) leverage this concept through a "hold-and-kill" mechanism, driving the formation of a stable complex between a disease-associated target protein and an essential effector protein. By disrupting the effector protein's function, this induced interaction can ultimately alter the disease state under investigation.
- Measuring endogenous PPIs and induced ternary complexes within native cellular environments has long posed technical challenges. The AlphaLISA™ *SureFire*® platform solves this by providing direct detection of protein interactions in endogenous cell systems.

2 RIPTAC Technology: 'Hold & Kill'



- HLD-0915, a first-in-class AR-BRD4 RIPTAC for metastatic castration-resistant prostate cancer.
- Recruits Androgen Receptor (target protein) and BRD4 (effector protein) into a stable ternary complex thereby abrogating BRD4 function and leading to cell death selectively in AR positive cells.

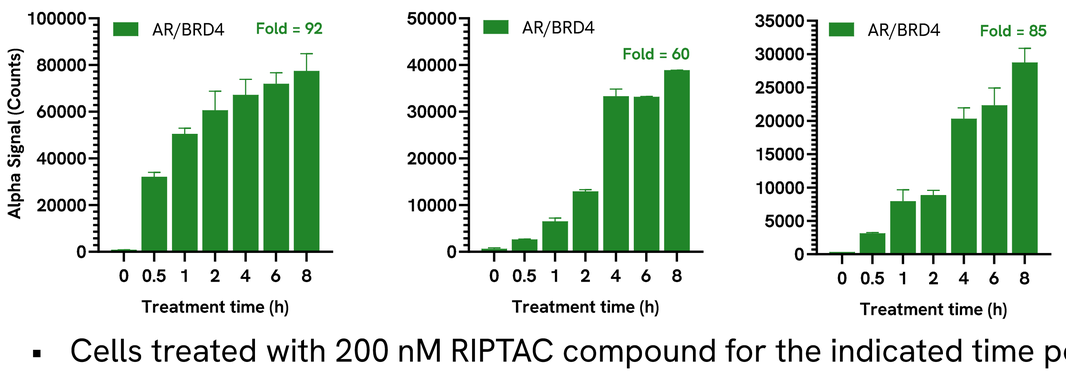
3 General experimental procedure



Lysate split multiple ways to screen for:

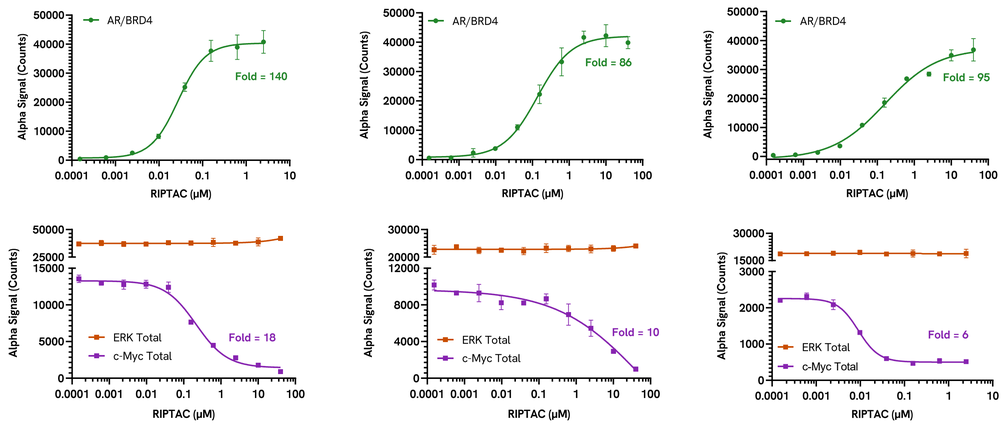
- AR/BRD4 Ternary Complex
- Transcription factors
- Apoptotic markers
- Stress markers
- All measured using AlphaLISA™ *SureFire*® assay technology

4 RIPTAC-mediated induction of AR/BRD4 in prostate cancer cells

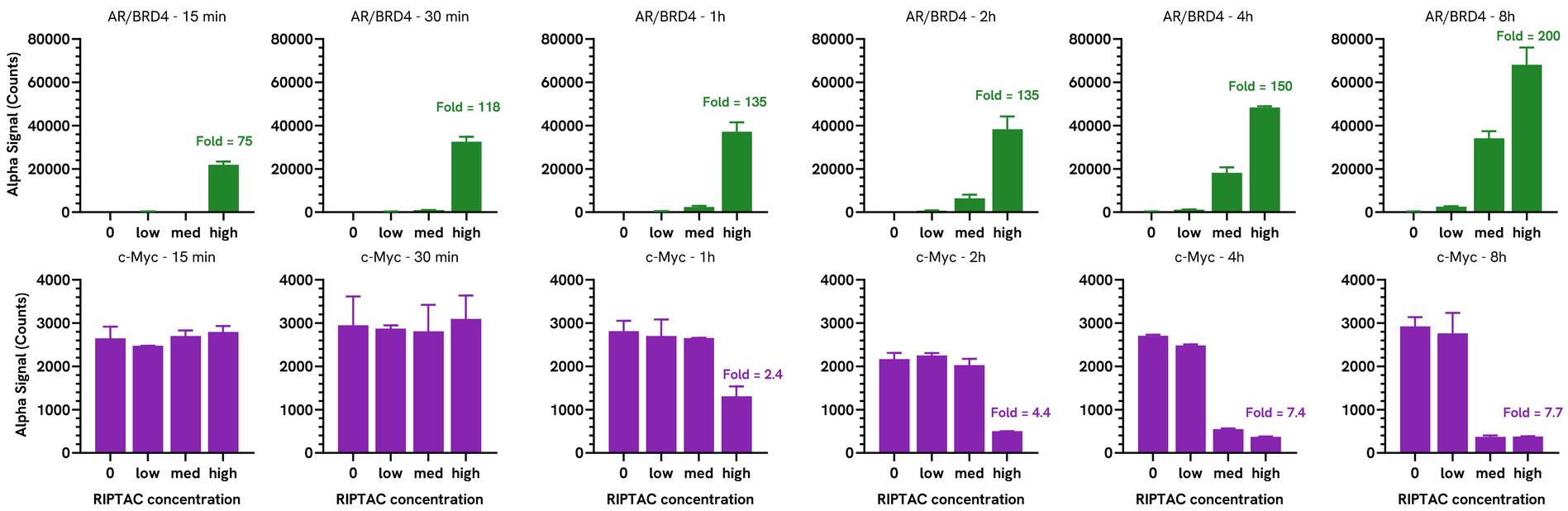


- Cells treated with 200 nM RIPTAC compound for the indicated time points.

5 AR/BRD4 induction in a dose-dependent manner – 4h treatment



6 Kinetics of AR/BRD4 induction and c-Myc downregulation in VCaP cells

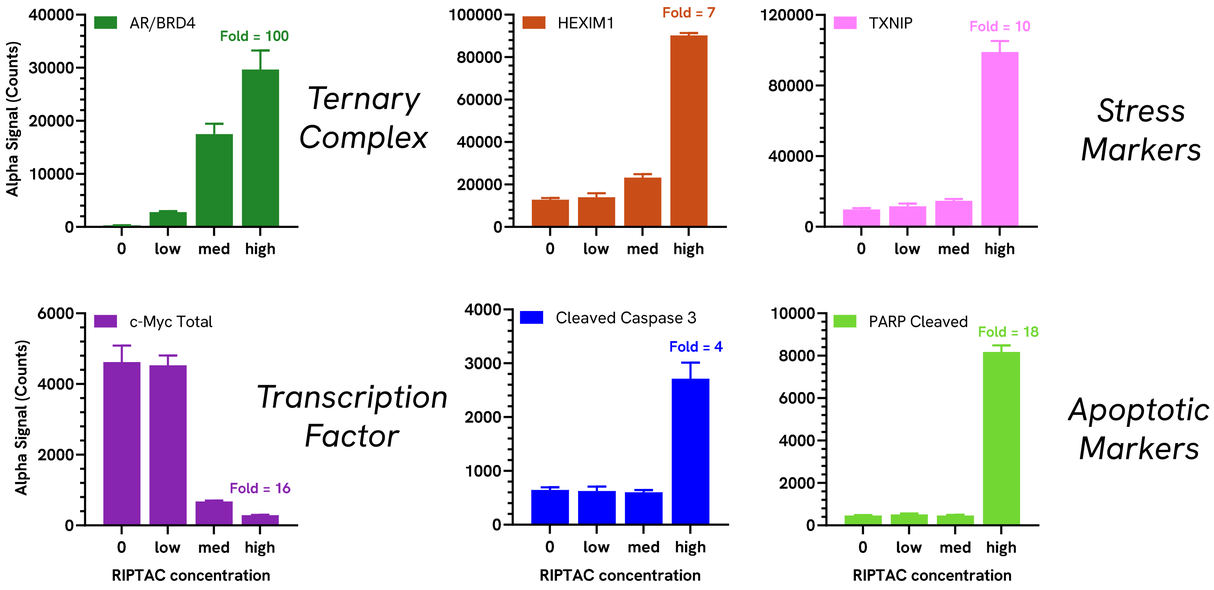


- Cells treated with RIPTAC compound.
- High (50 μM), medium (3.2 nM) & low (128 pM).
- Cells lysed and screen for AR/BRD4 and c-Myc.

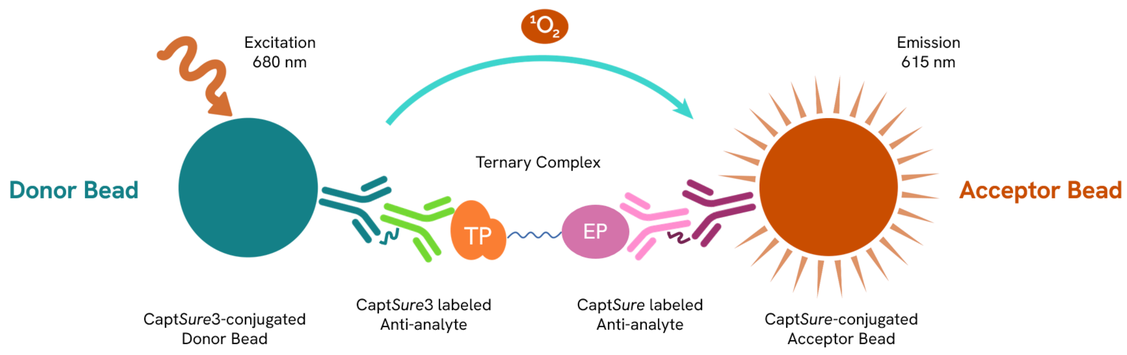
- Ternary Complex significantly increased from 15 mins.
- Significant downregulation of c-Myc total levels.

7 Detection of AR/BRD4 & cellular markers in VCaP cells

24h treatment with various concentrations of RIPTAC compound



8 The modular & flexible platform design of AlphaLISA™ *SureFire*® for Ternary Complex assay construction



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With a catalogue of > 700 ***Alpha SureFire*** cell-based assays, an expansive number of permutations exist for combining '**Target**' and '**Effector**' protein antibodies from our existing portfolio to build new cellular **Ternary Complex** assays.