CASSS Bioassays 2023

Novel Fluorescence-Linked Immunosorbent Assay to Evaluate Bispecific Antibody Potency

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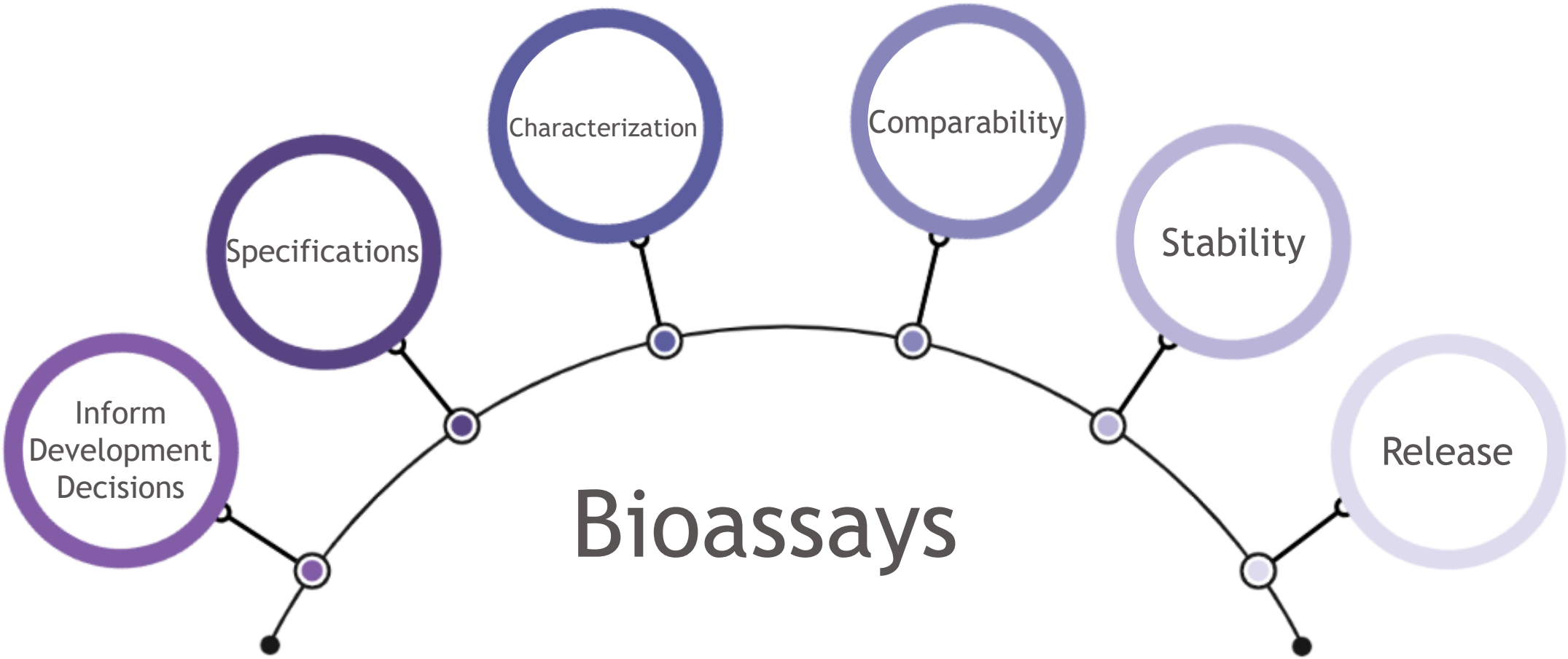
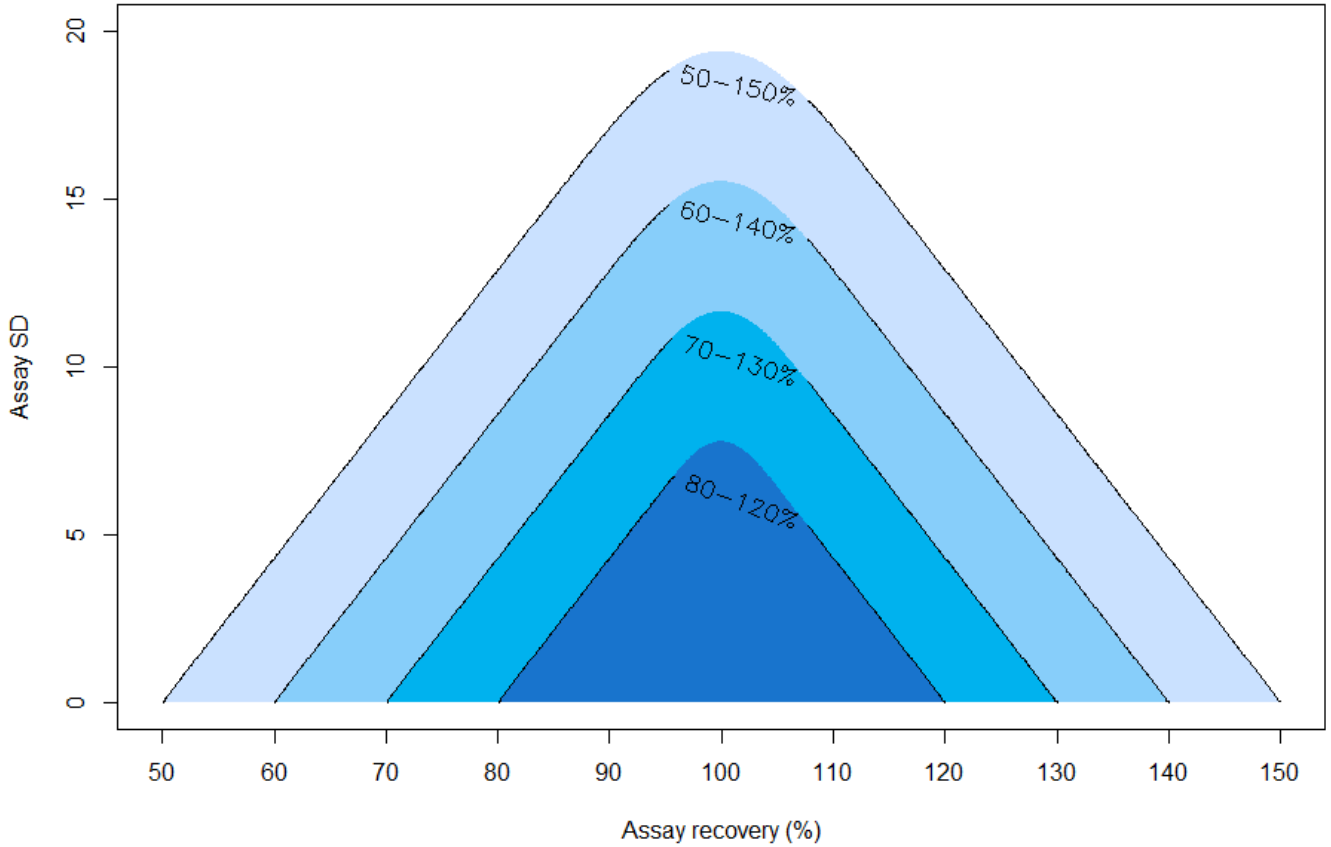
Bristol Myers Squibb™

Bioassays play an integral role in the development of biologics products



Performance target is critical for bioassay choice

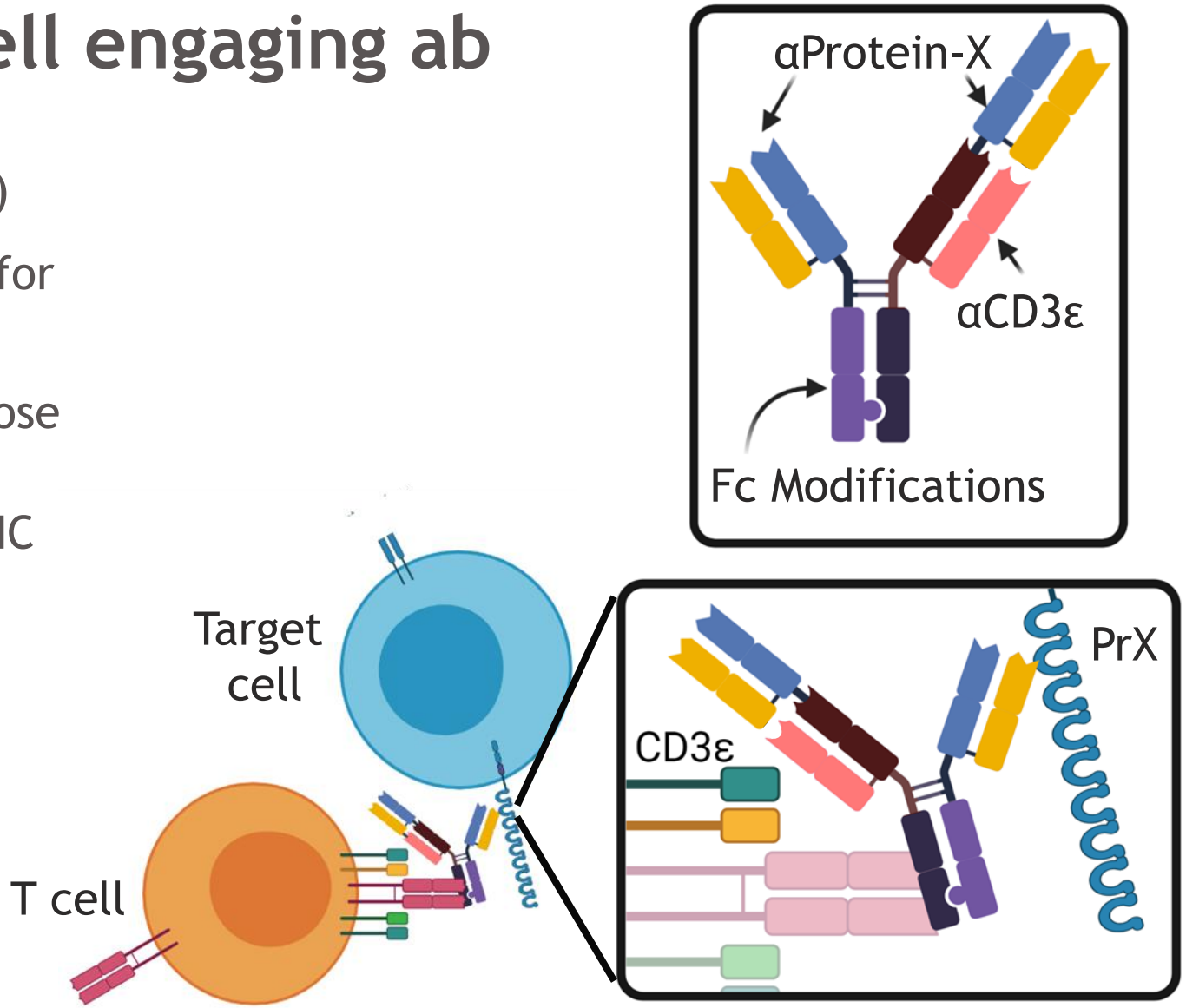


Area below each contour line represents
required assay performance to achieve ≤1% OOS (Out-of-Specification) rate for given specification limits

Bioassays require highly accurate and precise methods to reduce risk of false OOS and to generate sufficient confidence in reportable results

Mechanism for BMS-X T-cell engaging ab

- Targets unique epitope on Protein-X (PrX)
- T-cell engager (TCE) utilizes 2+1 format for optimized T cell activation
- PrX *x* CD3 bsAb brings tumor cells into close proximity with T cells:
  - CD3 crosslinking, independent from MHC restriction
  - Cell killing based on PrX expression
- Null Fc effector functions



Establishing a comprehensive, phase-appropriate potency strategy

Lot Release and Stability:

- Direct-binding ELISA assays (plate-bound CD3 or PrX)

-or-

- Fluorescent ELISA (FLISA) with immobilized TCE

Biological Characterization:

- Verification of null Fc effector function
- FcRn binding kinetic evaluation

Early objectives

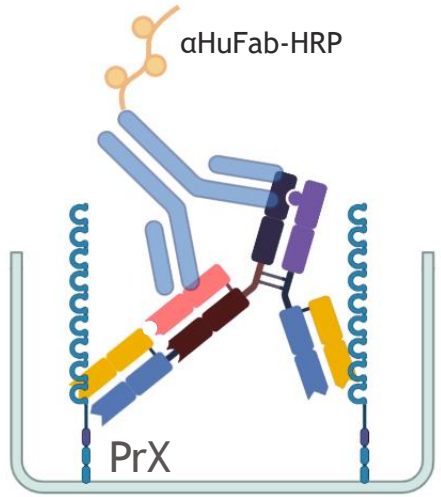
•Cell-based assay reflective of cell killing

Late objectives

- Cell-based reporter assay

Strategy for BMS-X potency assay and IND readiness

- Two ELISAs run in tandem to detect individual arms of bispecific
  - Immobilize rHu-CD3ε and rHu-PrX on separate plates
  - Incubate with BMS-X
  - Detect with anti-HuFab-HRP and TMB substrate



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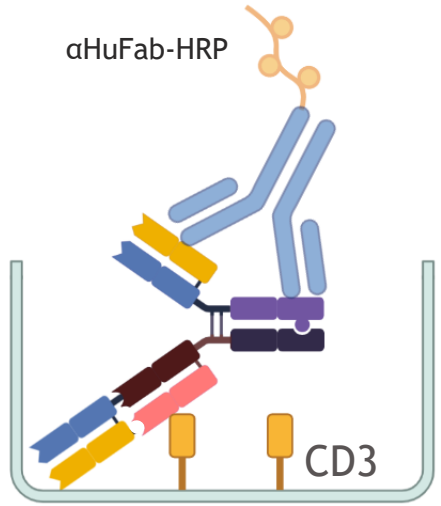

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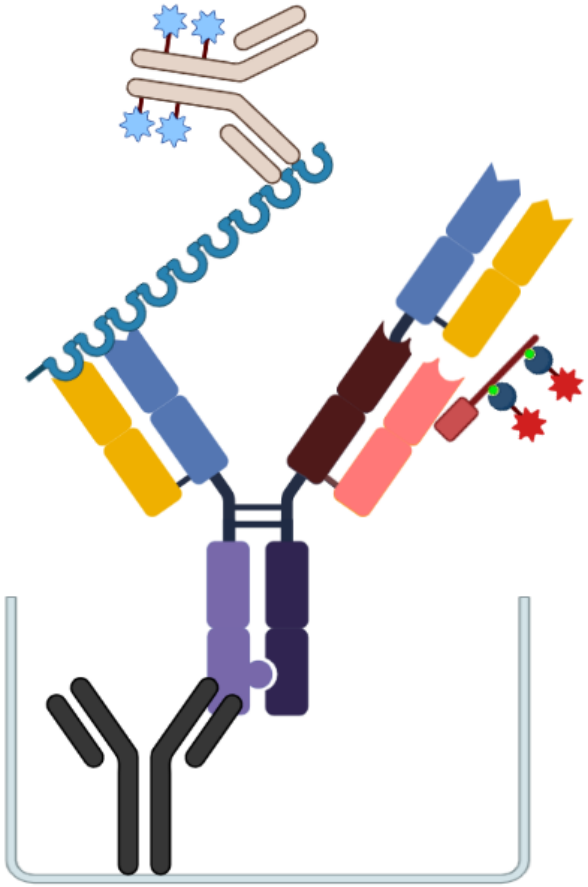
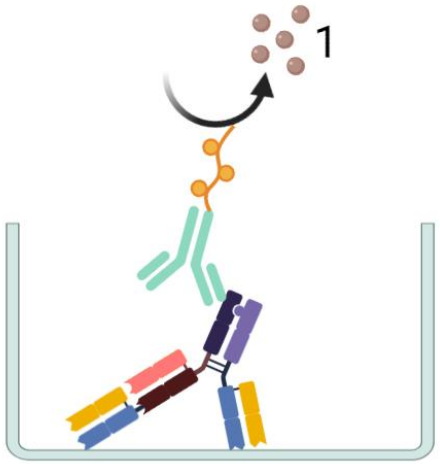
  - Detect with anti-HuFab-HRP and TMB substrate

- Multiplexed immunoassay with dual fluorescence detection
  - Immobilize BMS-X via anti-Hu Fc
  - Incubate with both rHu-PrX and rHu-CD3ε-biotin
  - Develop with anti-PrX Fluor1 and Streptavidin (SA) Fluor2



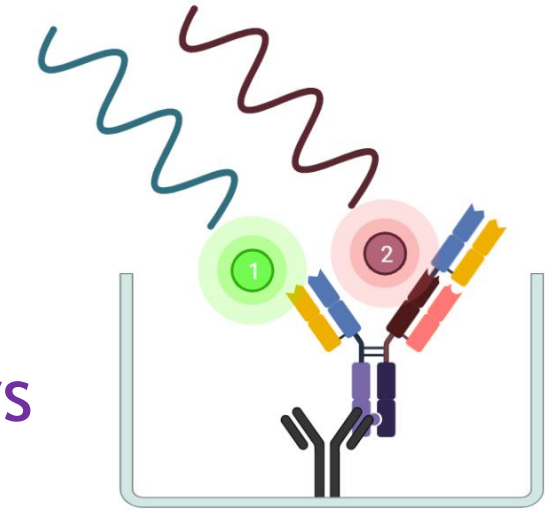
FLISA presents multiple advantages as a potency assay

- Multiplexed immunoassay with dual fluorescence detection
  - Simultaneous detection of PrX and CD3ε binding
  - Single assay format to reduce on-hands time
  - Stability-indicating for all binding domains

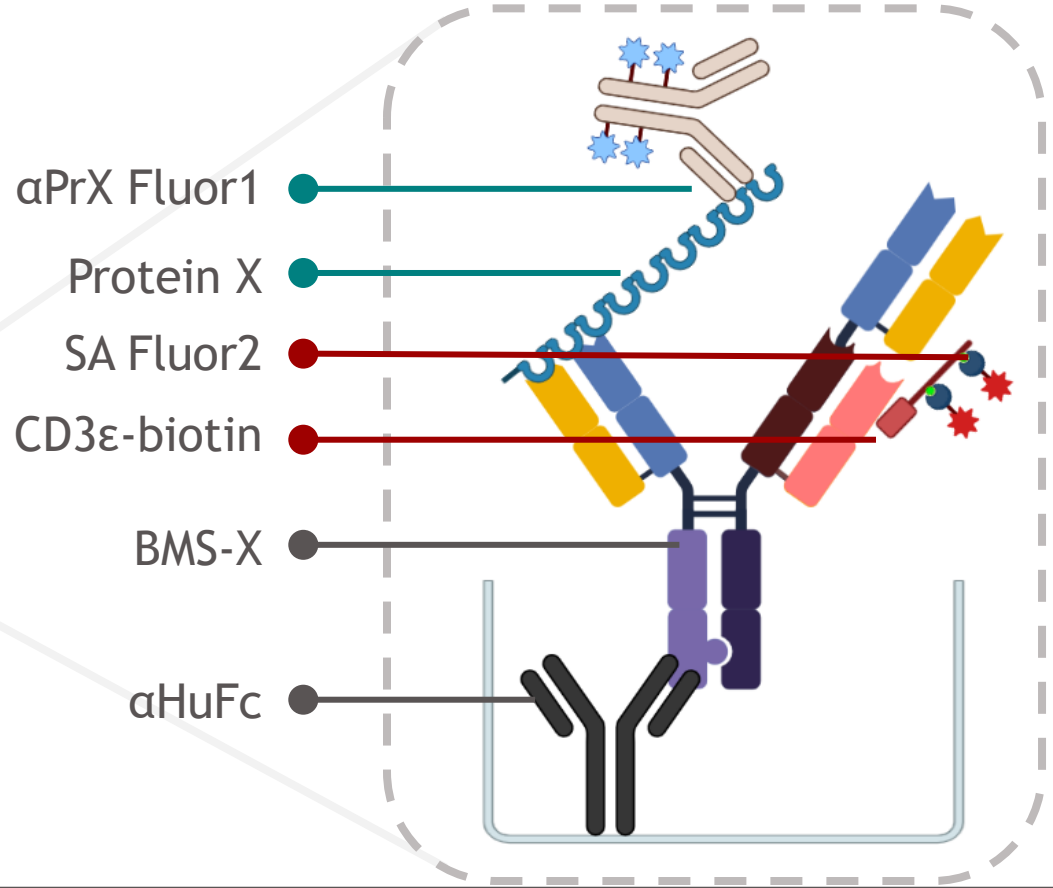


ELISA
1 well, 1 signal
2 plates

VS

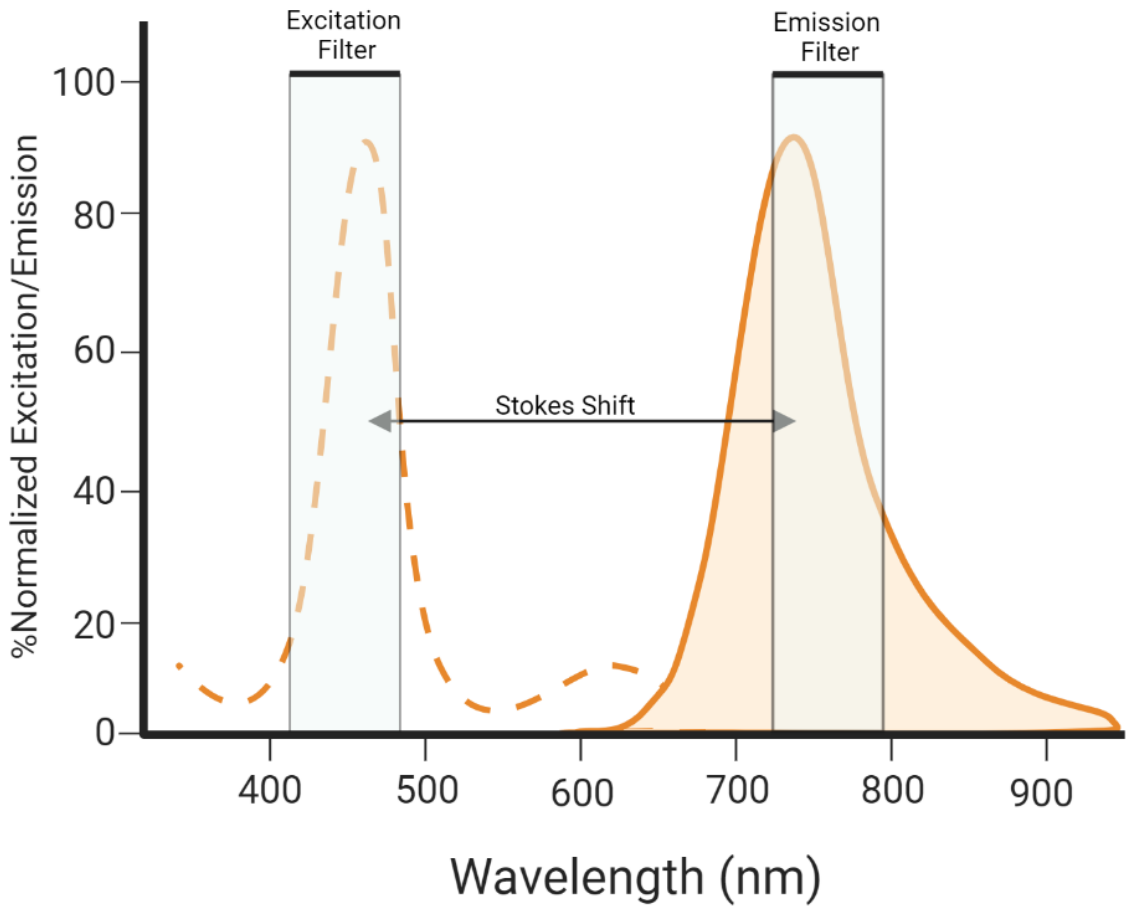


FLISA
1 well, 2 signals
1 plate



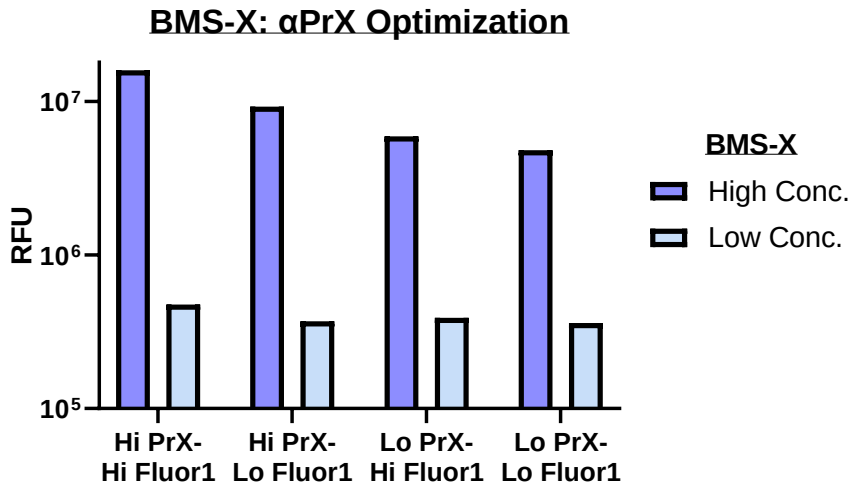
Can fluorescence substitute for chromogenic signal?

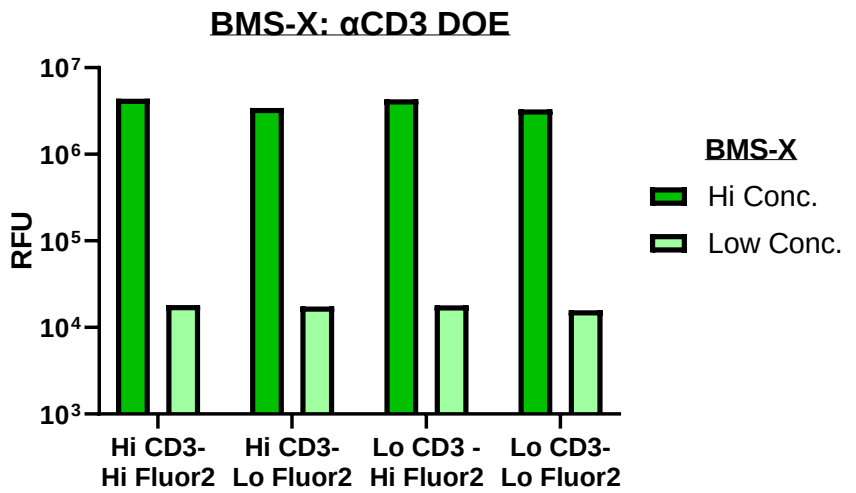
- Large stokes shift with distinct excitation and emission spectra
  - Minimize overlap between fluorophores' spectra and spillover from excitation laser
- Sufficient brightness
  - Maximize signal-to-noise ratio
- Photostability
  - Robust stability for handling on the bench
- Compatible fluorophore pairs; QC friendly



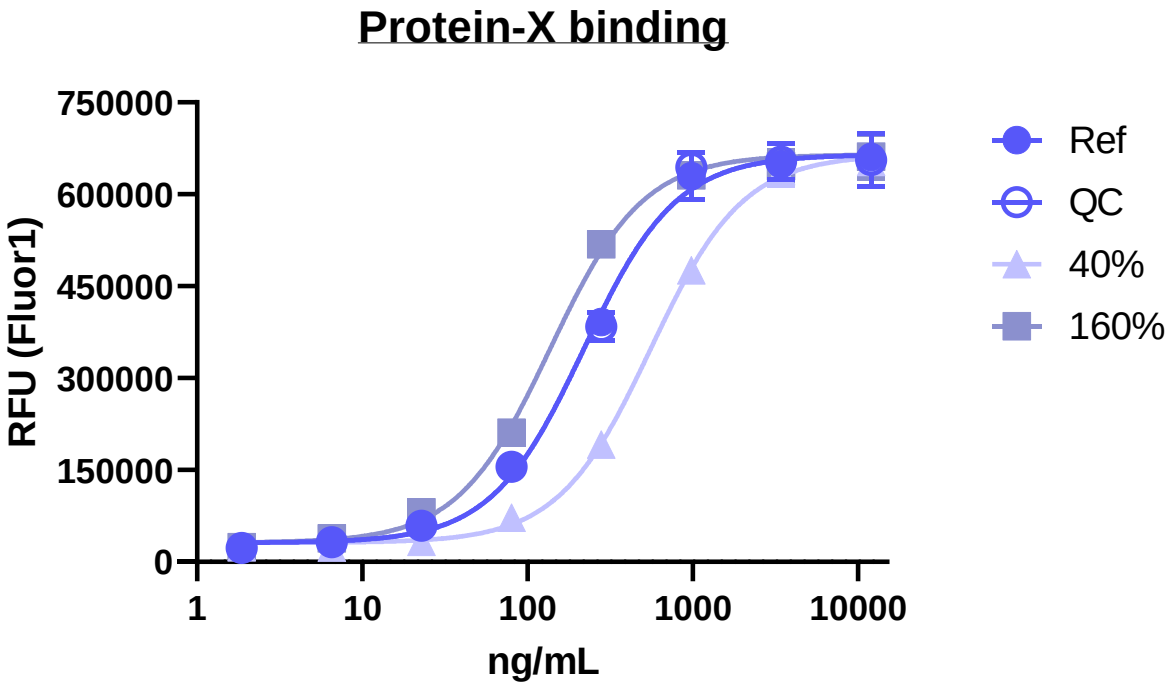
FLISA optimization considerations

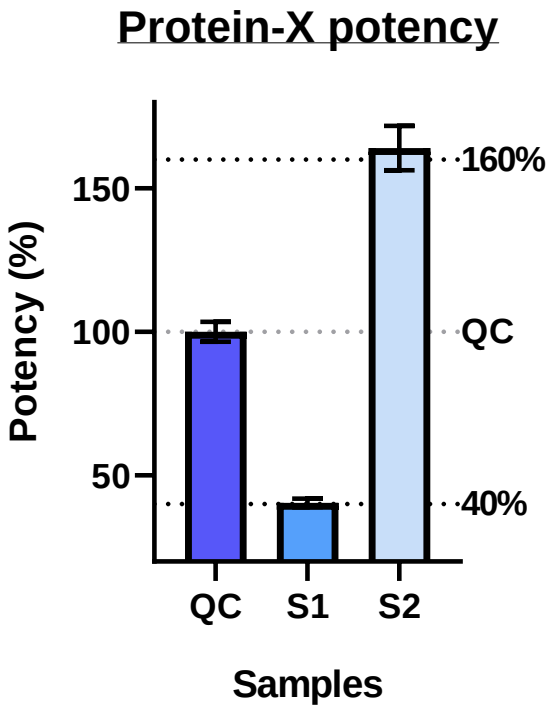
- Initial assay conditions optimized for:
  - Fluorophore compatibility
  - Coating antibody concentration
  - Antibody targets and fluorophores concentrations
- Optimal 8-point curve dilutions with a single dilution scheme to satisfy both targets
- Plate map to evaluate positional effects
- Additional, contributing factors (ie. buffer composition)
- Robustness evaluations of varying conditions (OFAT and DOE)

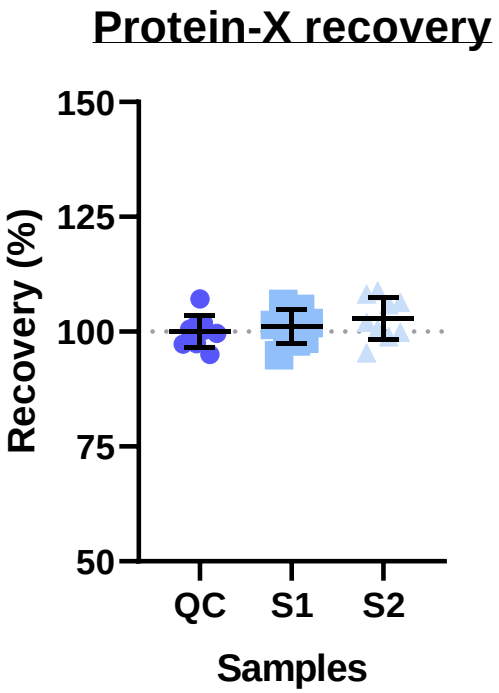




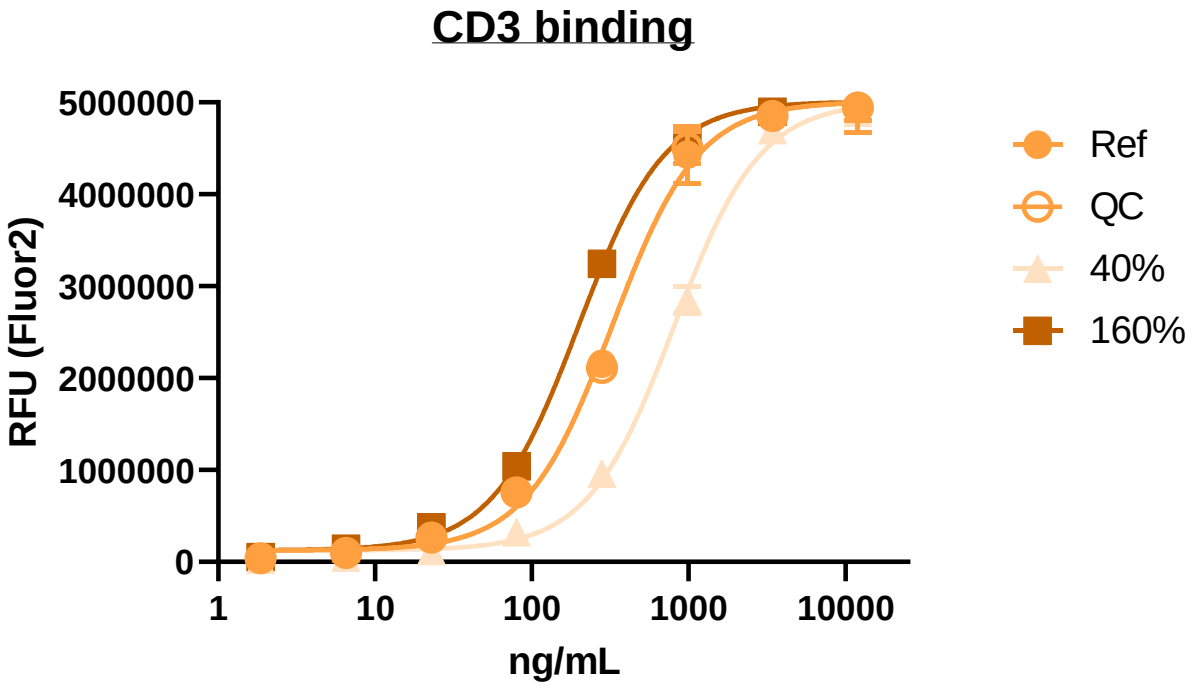
Performance of FLISA: Protein-X binding

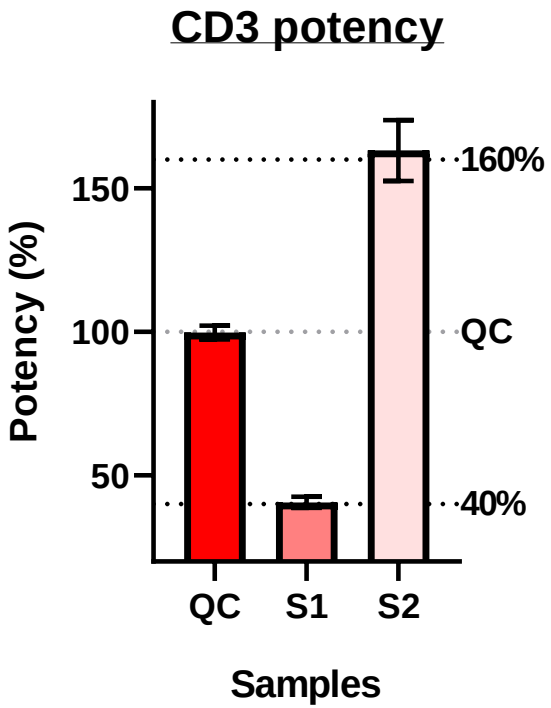


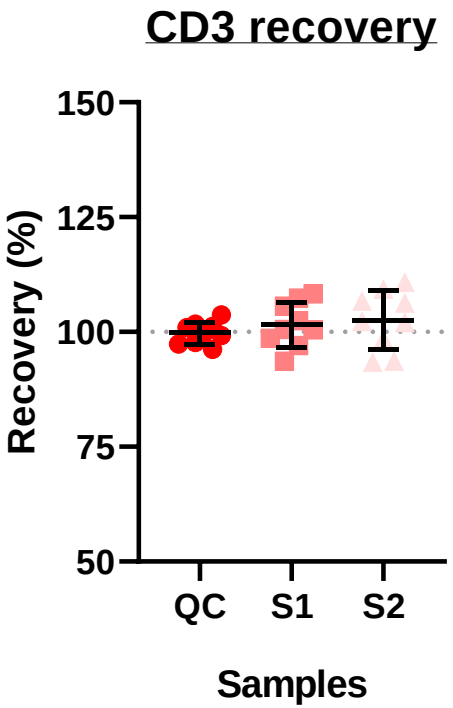




Performance of FLISA: CD3 binding







FLISA produces comparable accuracy and precision as ELISA

FLISA

	αPrX Recovery			αCD3 Recovery		
Condition	100%	40%	160%	100%	40%	160%
N	*9*	*9*	*9*	*9*	*9*	*9*
Average	100	101	103	100	102	103
STD Dev	*3.3*	*3.5*	*4.4*	*2.3*	*4.6*	*6.0*
%RSD	*3.3*	*3.5*	*4.3*	*2.3*	*4.5*	*5.9*

ELISA

Condition	100%	40%	160%	100%	40%	160%
N	*14*	*14*	*14*	*14*	*14*	*14*
Average	98	98	98	100	101	103
STD Dev	2.3	4.2	2.3	2.1	3.8	3.0
%RSD	2.4	4.2	2.3	2.1	3.7	2.9

Summary: FLISA is a novel ELISA alternative

- Novel use of fluorescence applied to measure bispecific antibody binding
- FLISA offers multiple advantages over ELISA
  - Simultaneously reports on both targets
  - Halves hands-o
- FLISA can be a su rable accuracy and precision compar
- FLISA can potent

Is the FLISA a stability-indicating assay?

Post-translational modification within aPrX binding domain demonstrates stability-indicating properties of FLISA

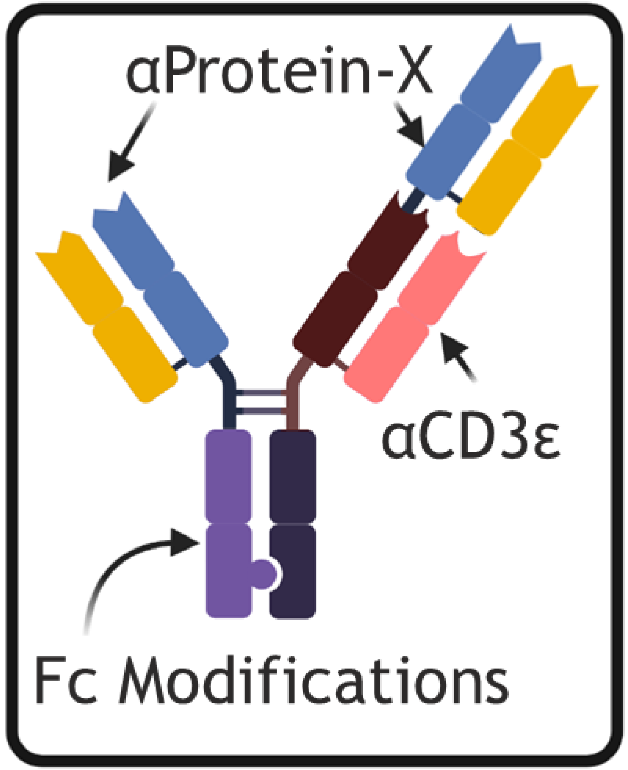
PTM-Z as a pCQA in BMS-X

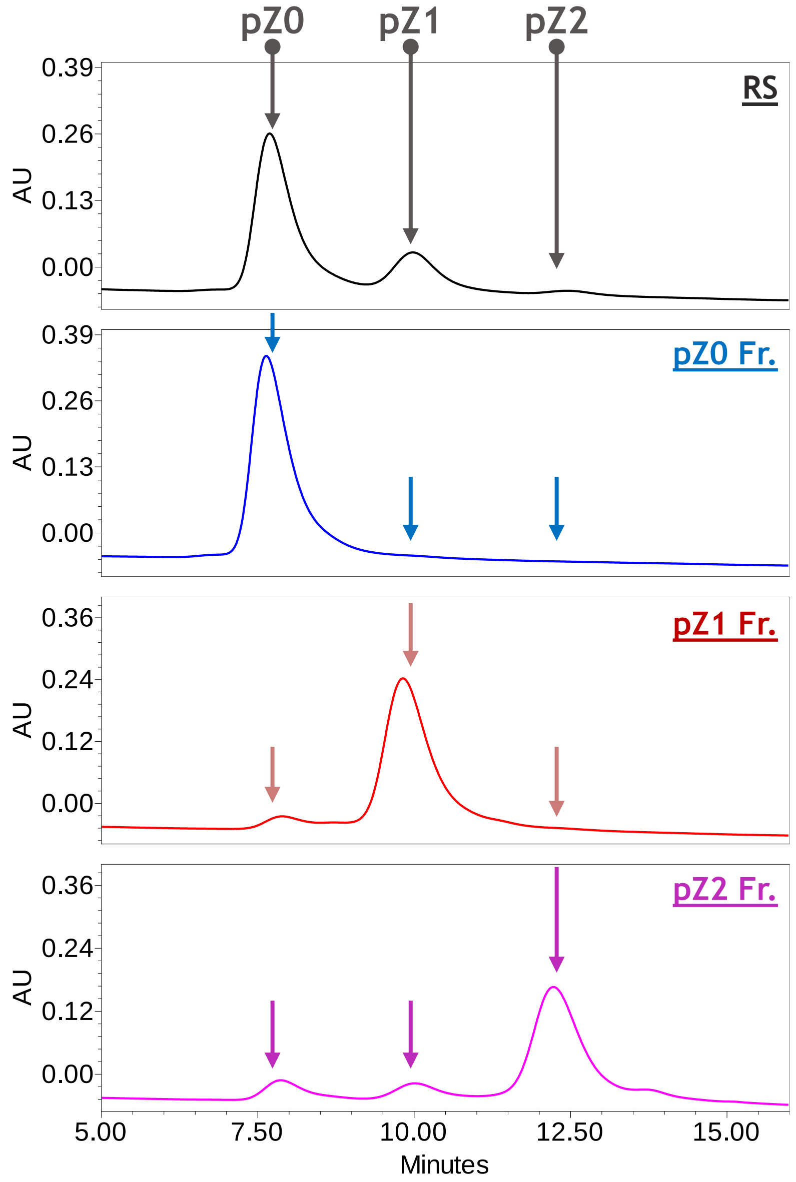
Heavy Chain 1:

.... 50
.... 100
....Z..... 150
.... 200
.... 250
.... 300
.... 350
.... 400
.... 461

Heavy Chain 2:

.... 50
.... 100
....Z..... 150
.... 200
.... 250
.... 300
.... 350
.... 400
.... 450
.... 500
.... 550
.... 600
.... 650
.... 694





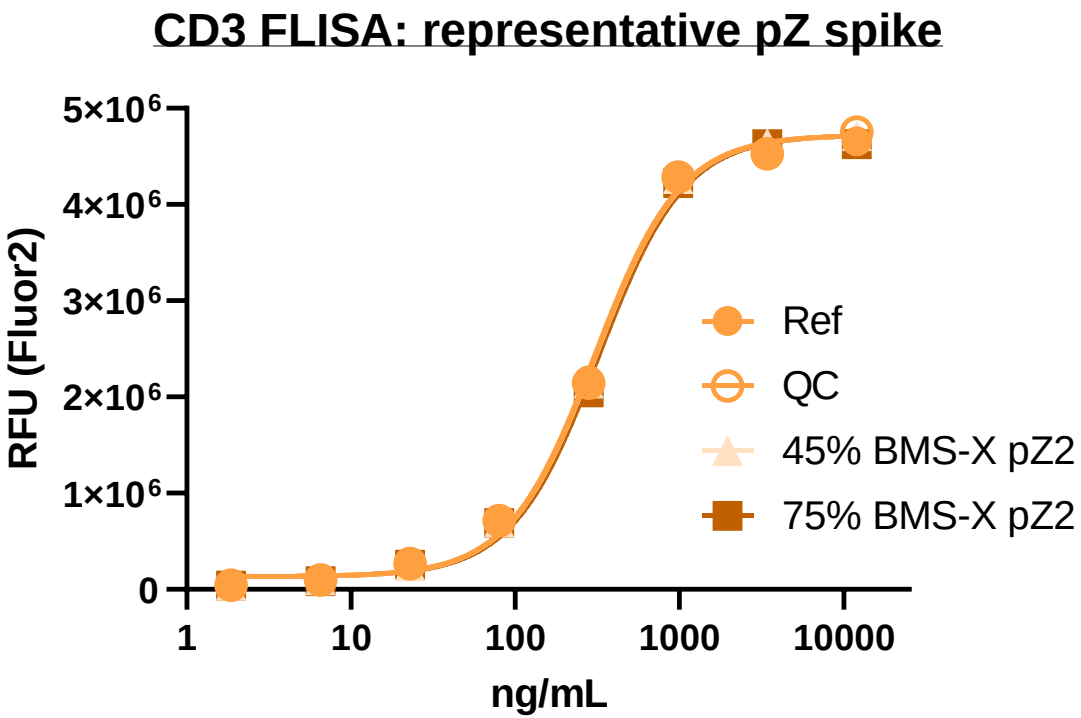
PTM-Z (pZ) Spike Study Overview

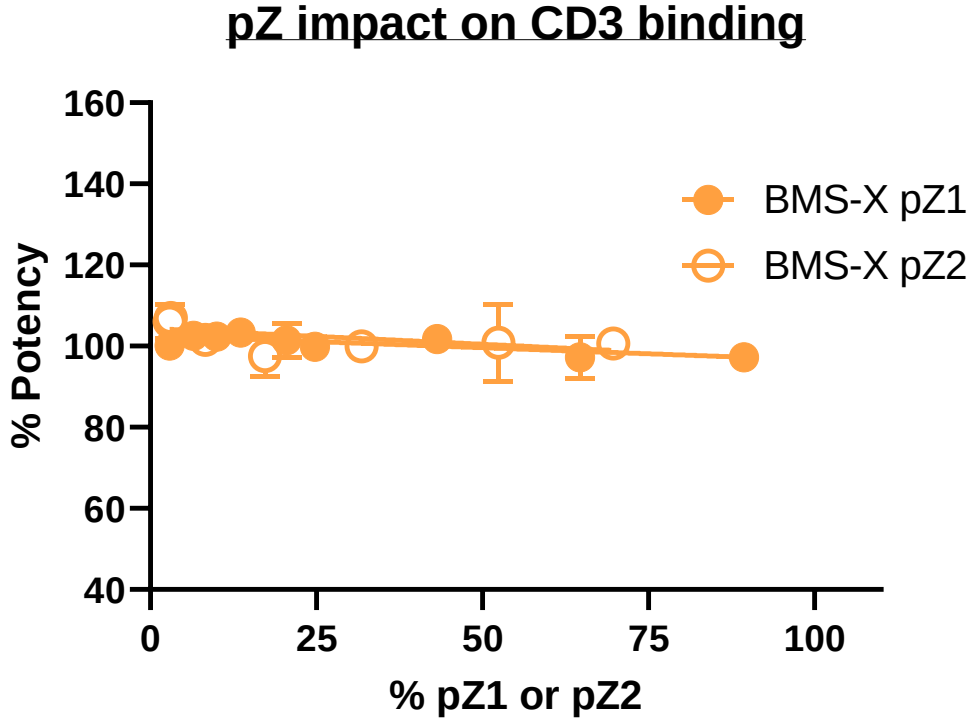
- Impact on BMS-X binding by pZ demonstrated by a correlative study
  - Samples fractionated from reference standard (RS) using preparative scale chromatography column
  - Multiple levels of pZ targeted for
    1) BMS-X containing a single moiety of pZ on *one arm* (pZ1)
    2) BMS-X containing a single moiety of pZ on *both arms* (pZ2)
  - Content verified using analytical scale chromatography column and intact MS

Target	*Species*	
pZ (%)	pZ1	pZ2
0	2.9	2.9
4	6.5	3.1
8	10.0	-
12	13.6	8.3
18	20.5	-
24	24.8	17.3
45	43.2	31.8
75	64.7	52.4
100	89.4	69.7

Majority of BMS-X with pZ contains a single-moiety of pZ (pZ on just one arm)

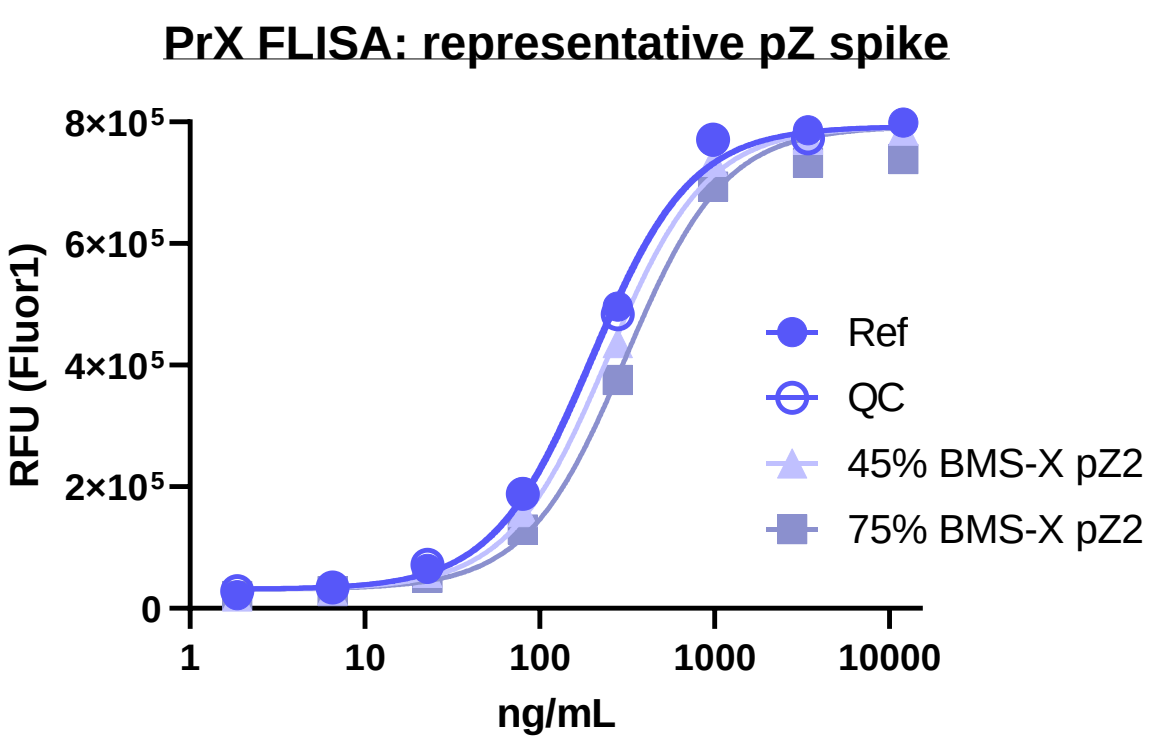
PTM-Z minimally impacts CD3 binding

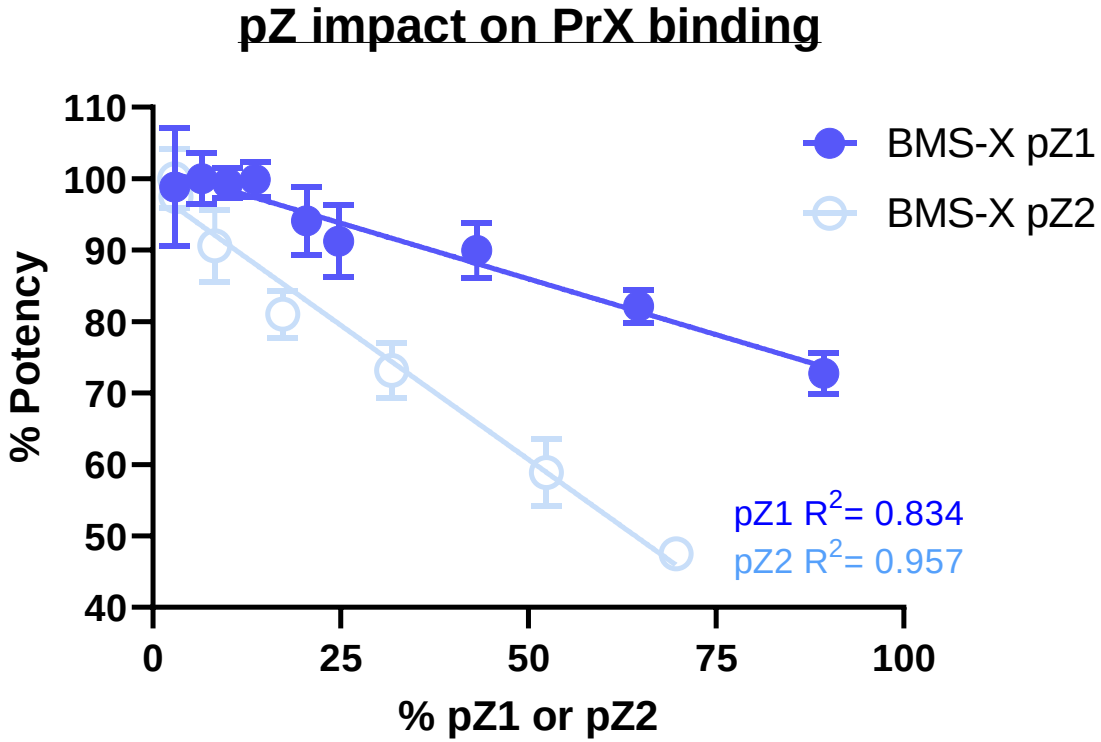




- pZ located in αPrX CDR is not expected to impact CD3 binding

PTM-Z impacts binding of BMS-X to PrX





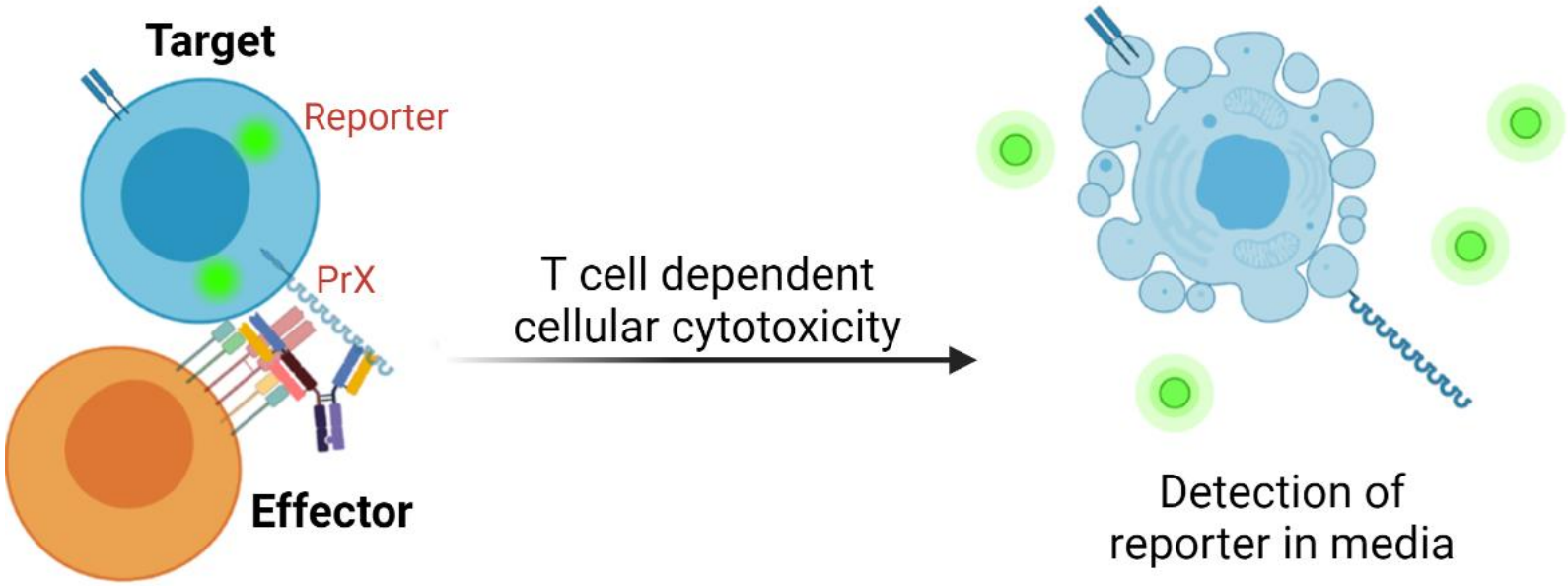
	1% pZ change corresponds to change in potency of y% (slope):
pZ1	-0.312
pZ2	-0.752

Normalized to potency with 0% pZ sample

Summary: FLISA is a stability-indicating assay

- Novel use of fluorescence applied to measure bispecific antibody binding
- FLISA offers multiple advantages over ELISA
  - Simultaneously reports on both targets
  - Halves hands-on time
- FLISA can be a suitable, innovative alternative for potency assays with comparable accuracy and precision compared to ligand-binding ELISA
- FLISA can potentially be repurposed for other multi-specific antibody drugs
- FLISA accuracy and precision enables understanding of pZ impact via correlation study

Designing a TDCC (T cell dependent cellular cytotoxicity) early-stage characterization assay

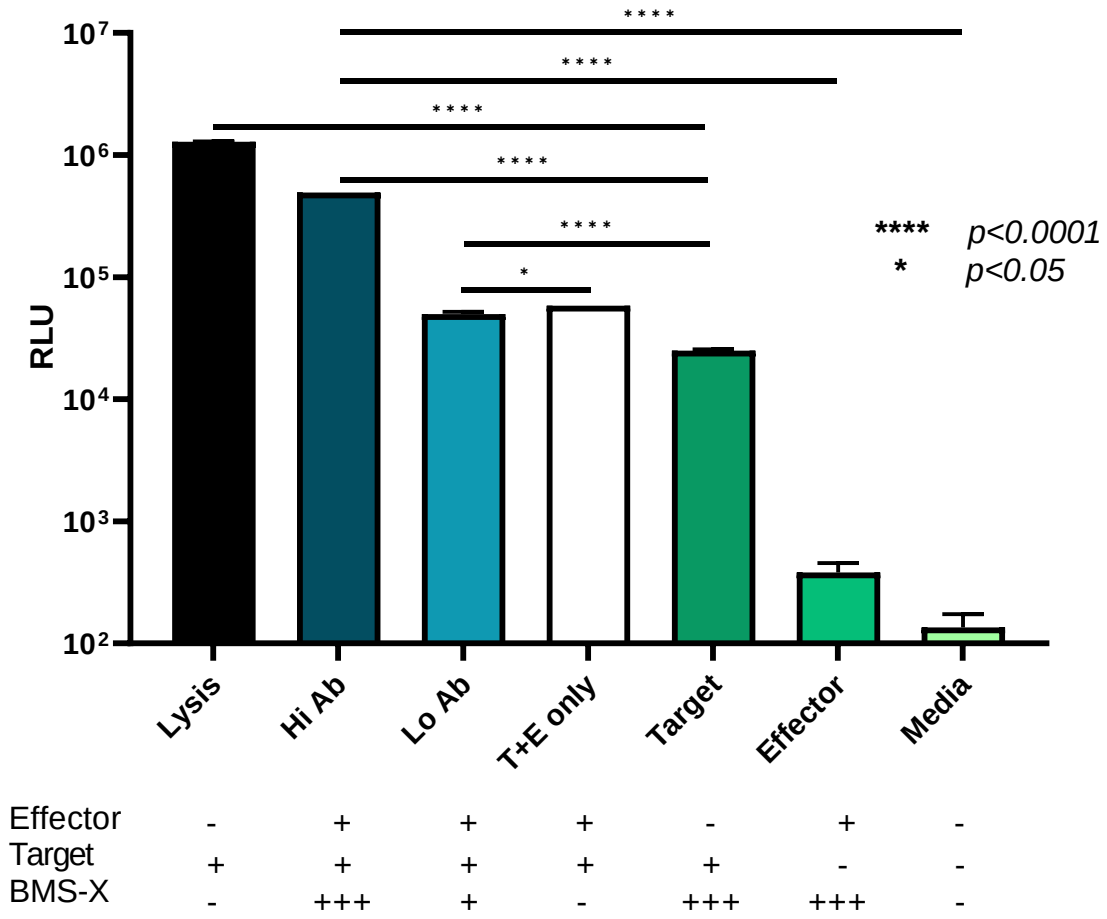


BMS-X utilizes T cell dependent cellular cytotoxicity (TDCC) as the mechanism of action (MoA)

- Target: Cell line expressing both PrX and constitutive, cytoplasmic reporter
- Effector: Human T cell line

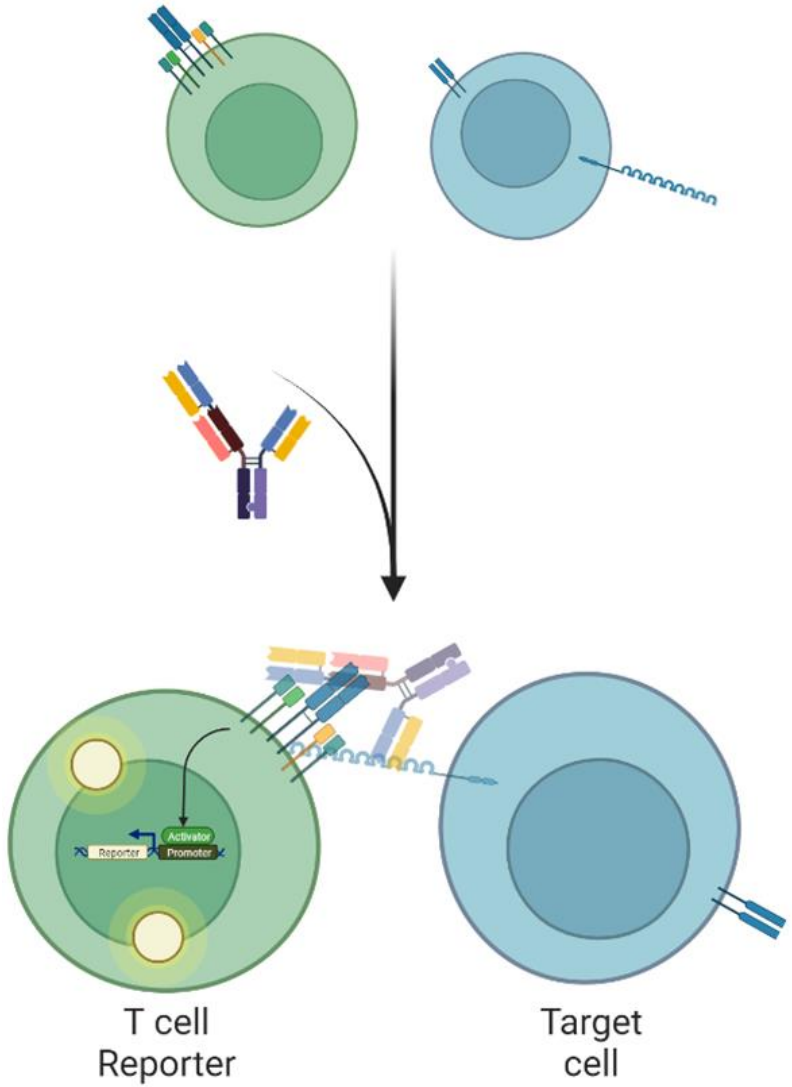
Early-stage characterization assay focused on modeling a critical aspect of the mechanism-of-action for a given asset and in a physiologically relatable system

TDCC effectors specifically lyse target cells in the presence of BMS-X



- TDCC demonstrates roughly ~30% of complete cell lysis
- In the absence of BMS-X, target and effector cells generate minimal signal, equal to background from target alone

Designing a late-stage T cell reporter assay

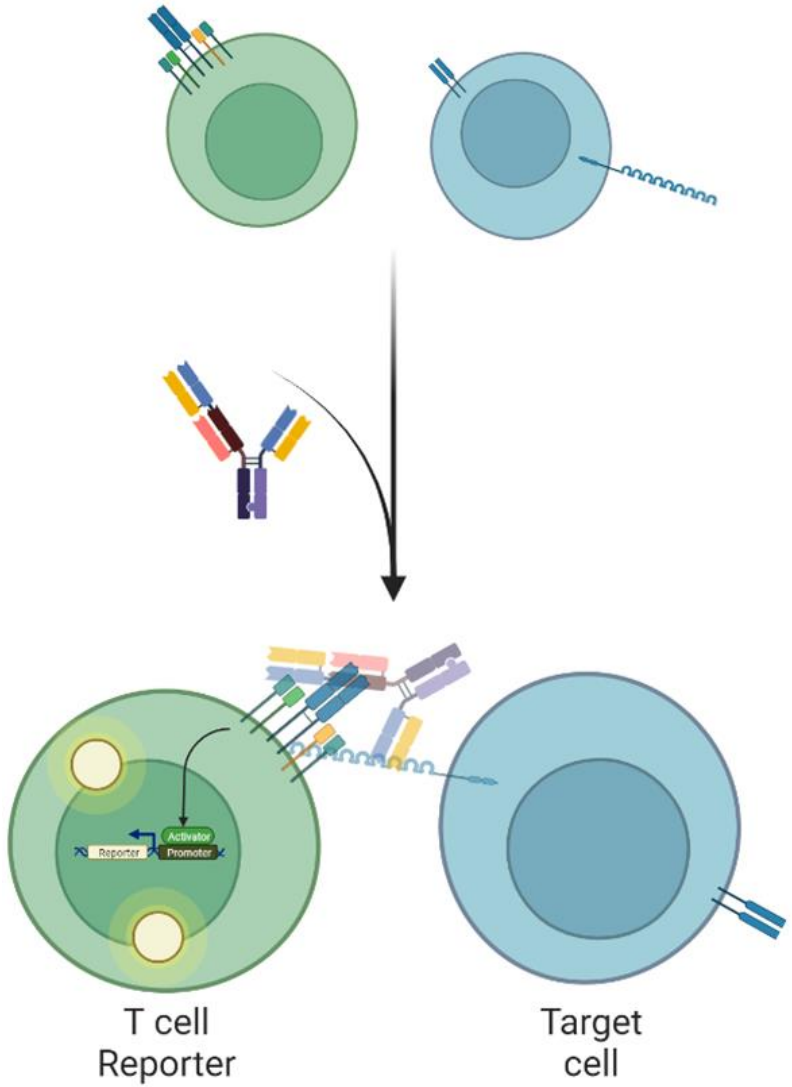


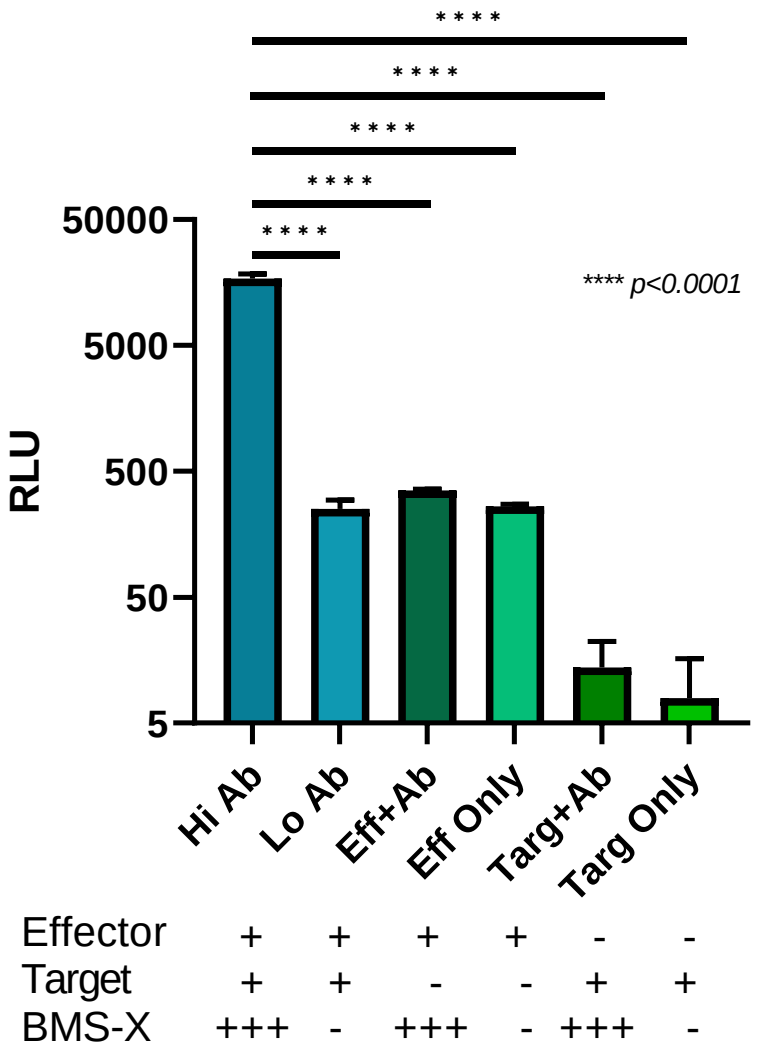
Late-stage bioassay for lot release

- Reflective of mechanism of action
- Analytical profile must support late-stage specification
- Must demonstrate stability-indicating properties
- Operationally friendly
  - Suitable performance in a QC lab
  - Suitability of reagents
  - Accessible instrumentation

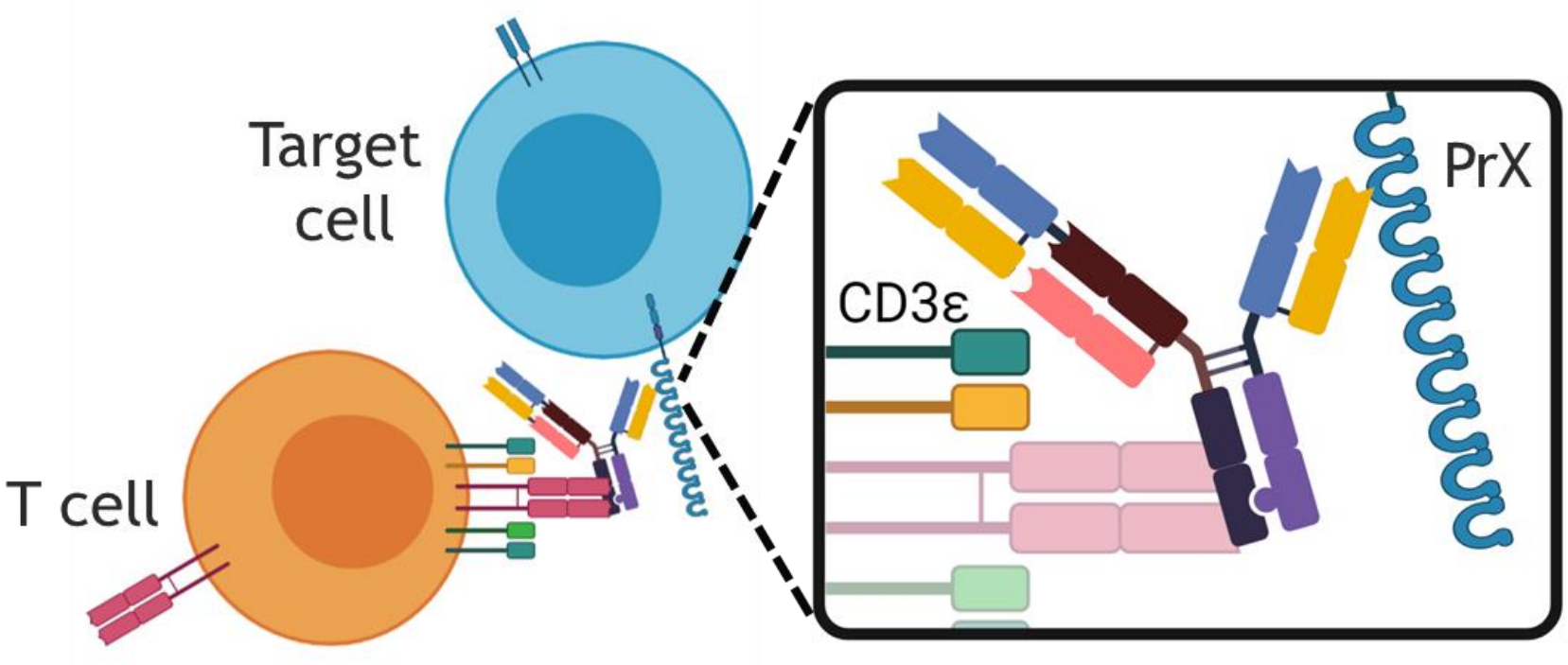
- Target: PrX-expressing cell line
- Effector: T cell line with activation-inducible reporter

Designing a late-stage T cell reporter assay





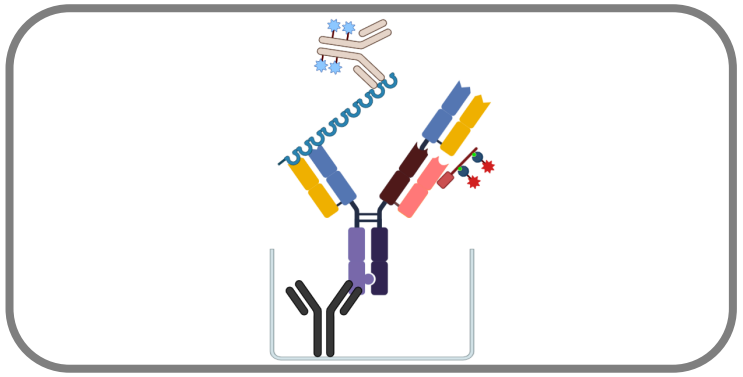
Tripartite, staged potency strategy for BMS-X



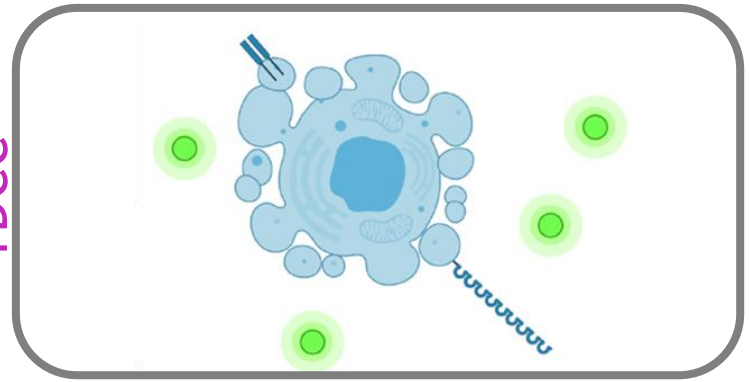
Tripartite, staged potency strategy for BMS-X

- Early-stage assay for lot release and stability via FLISA
  - novel design to address complex molecules while maintaining QC-friendly workflow
  - Demonstrates simultaneous binding of BMS-X to reflect mechanism-of-action
  - Stability-indicating via pZ correlative study
- MoA-reflective characterization bioassay spanning lifetime of asset via TDCC
- Late-stage cell-based bioassay for lot release and stability via T cell reporter

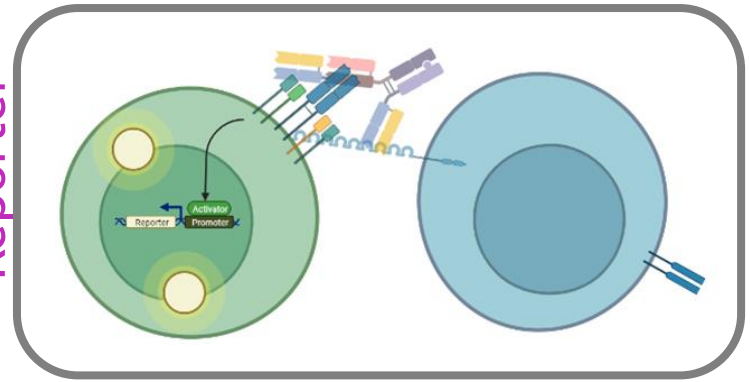
FLISA



TDCC



Reporter



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- MoA-reflective characterization bioassay spanning lifetime of asset via TDCC
- Late-stage cell-based bioassay for lot release and stability via T cell reporter

- Comprehensive approach to cover all relevant aspects of BMS-X MoA
- Phase-appropriate approach
- Informed by knowledge gained from successive assay development efforts

Acknowledgements

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Key Contributors

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