Bioassay Development for Complex Biologics: A Case Study for a Prodrug

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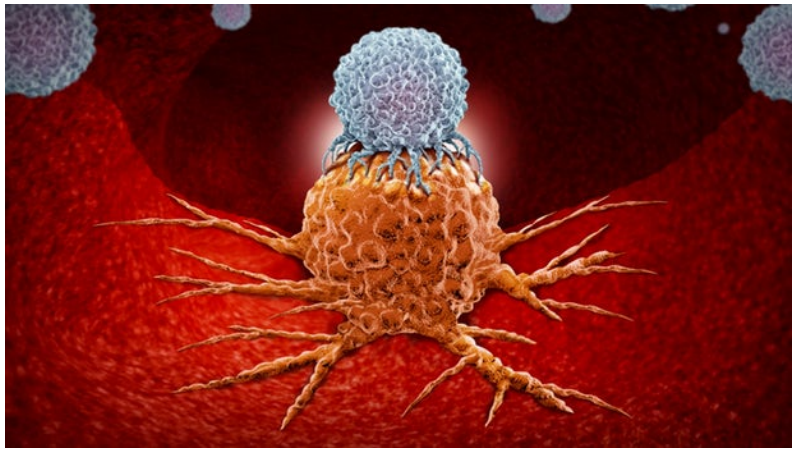
Immuno-Oncology

- Harness the immune system to attack cancer by targeting Immune Checkpoint Inhibitors

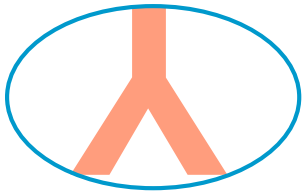


- Nivolumab (anti-PD1) and Yervoy (anti-CTLA-4) are approved I-O drugs for treatment of many cancer types
- During I-O treatment, some patients experience immune related Adverse Events (irAEs)
- irAEs are thought to be due to ‘on-target, off-tumor’ effects
- New protein engineering approaches are being evaluated to reduce irAEs

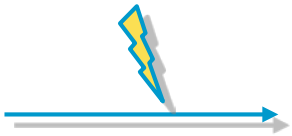
What is a Prodrug?

Prodrug: a biologically inactive compound that can be metabolized to produce a drug

Prodrug



Minimal activity



activation

Bioactivation

- **Enzymatic OR chemical activation**
- **Intra- or Extracellular**
- **Location of bioactivation:**
  - **Target tissues/cells**
  - **Metabolic Tissues**
  - **GI fluids**
  - **Systemic circulation**

Activated Prodrug

Full activity upon activation

10% of small molecular weight drugs are prodrugs

Example:
- **Aspirin**

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Prodrugs in Biologics
Example: Probody™ Therapeutic

Example: CytomX Probody™ Therapeutic

- **Masking peptide covers the active antigen-binding site of the antibody**
- **Masking peptide cleaved by proteases enriched within the diseased tissue**
- **Probody™ Therapeutic designed to provide 'On Target, On-tissue' activity**

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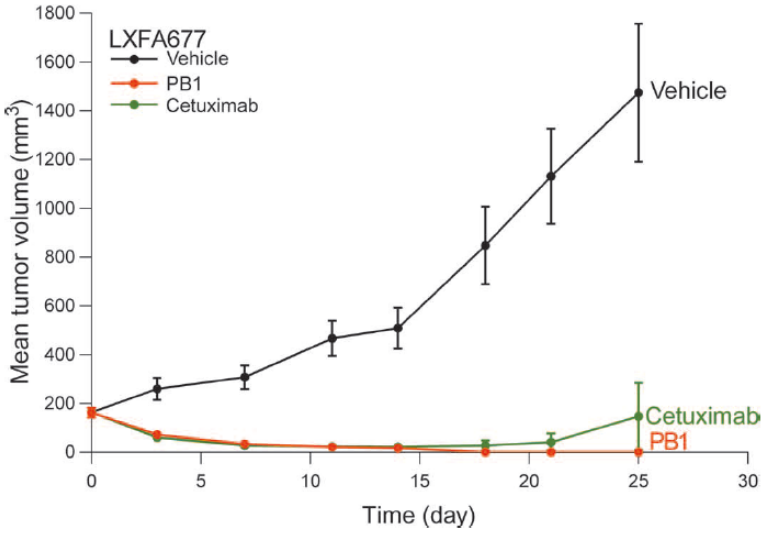
Probody™ Therapeutic Expands the Therapeutic Window of an Antibody Therapy

Example: Probody molecule targeting EGFR compared to Cetuximab (Anti-EGFR)

Comparable Efficacy
to Cetuximab in mouse models



Decreased Toxicity
to Cetuximab in nonhuman primates

Test article*	Dose (loading/ weekly), mg/kg	Dermatologic findings	
		Time to onset (study day)†	Extent and severity
Cetuximab	40/25	22, 23, 25	Mild to moderate
PB1	40/25	NO, NO, NO	Not applicable
PB1	120/75	9, 19, NO	Mild

Luc R. Desnoyers, Olga Vasiljeva, Jennifer H. Richardson, Annie Yang, Elizabeth E. M. Menendez, Tony W. Liang, Chihunt Wong, Paul H. Bessette, Kathy Kamath, Stephen J. Moore, Jason G. Sagert, Daniel R. Hostetter, Fei Han, Jason Gee, Jeanne Flandez, Kate Markham, Margaret Nguyen, Michael Krimm, Kenneth R. Wong, Shouchun Liu, Patrick S. Daugherty, James W. West and Henry B. Lowman (2013) **Tumor-Specific Activation of an EGFR-Targeting Probody Enhances Therapeutic Index,** *Sci Transl Med* **5**, 207ra144207ra144

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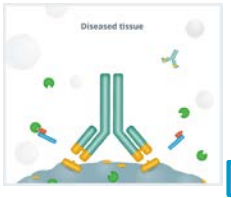
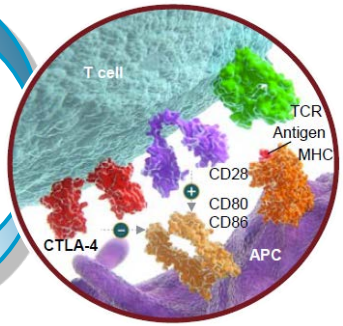
Probody™ Therapeutic use in I-O

- Activated by proteases enriched at tumor microenvironment
- Minimal activity within non targeted tissues (minimize toxicity)

Two activity states
Complex Signaling events



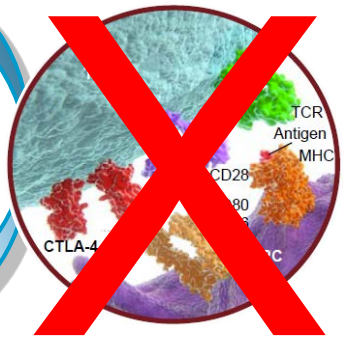
Tumor Micro Environment



Activated T-Cell

- Activated
- Binds to target
- T-Cells are activated

Non-tumor Tissue



Non-activated T-Cell

- Remains Masked
- Does not bind to target
- T-Cells are not activated

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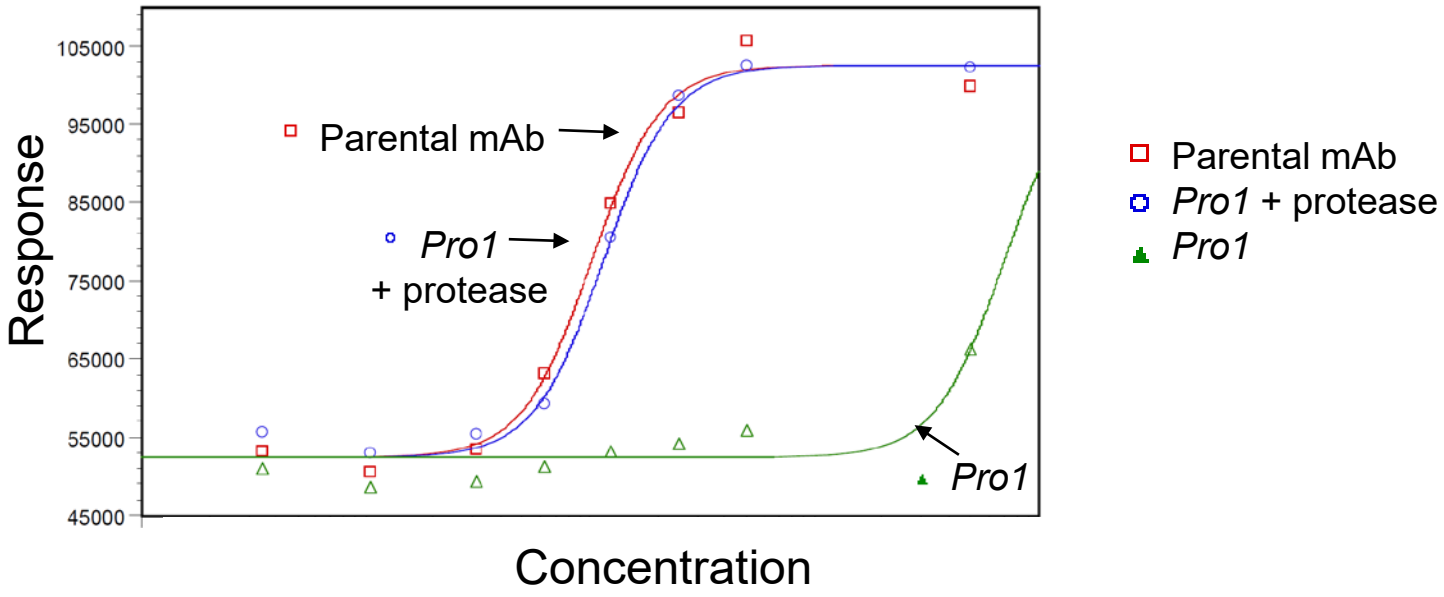
T-Cell Activation with Prodrug-1 (*Pro1*)

Prodrug-1 (*Pro1*)

- **Designed to activate T-Cells specifically within tumor**
- **Probody™ Therapeutic, activated by proteases enriched in tumor site**



- ***Pro1* demonstrates minimal T-Cell activation**
- **Protease treated *Pro1* has similar activity to parental mAb**
- **TWO distinct activities**

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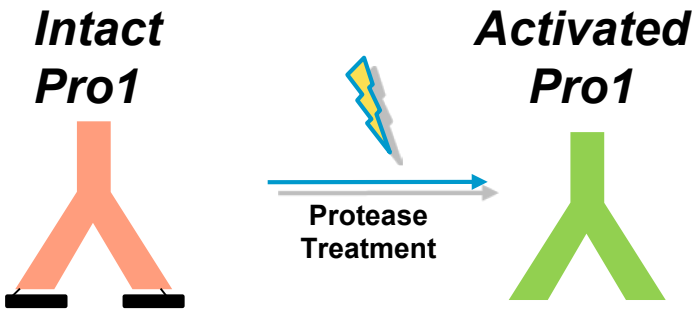
How Do We Define Potency of *Pro1*?

- **Two distinct activities due to <u>two distinct states</u> of the molecule**



- **Which state do we measure to assess potency?**

From ICH Q6B:

Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties.

Measuring Potency of *Pro1:*
Attributes linked to Biological Properties

Attributes Contributing to Full Activity

1. **Substrate Linker**
   - **Cleavable site for activation**

2. **Parental Antibody**
   - **Target Binding, Fc interactions**

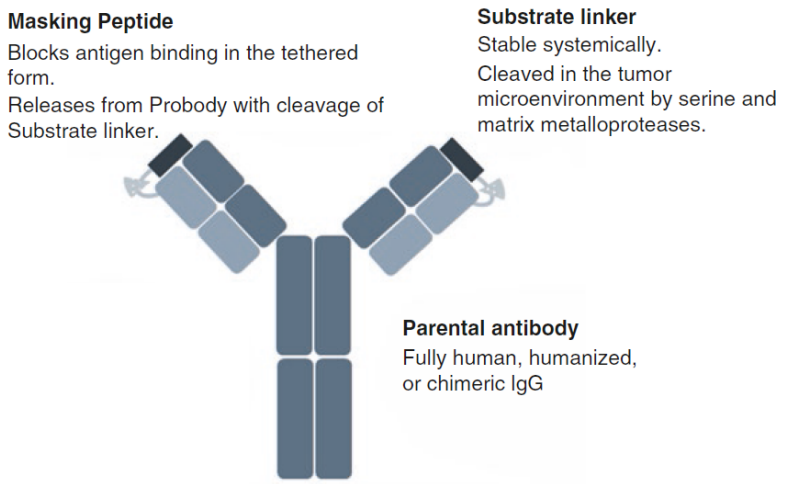
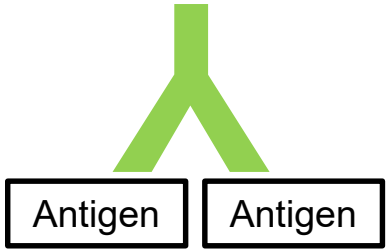


Figure from: Lin J., Sagert J. (2018) Targeting Drug Conjugates to the Tumor Microenvironment: Probody Drug Conjugates. In: Damelin M. (eds) Innovations for Next-Generation Antibody-Drug Conjugates. Cancer Drug Discovery and Development. Humana Press, Cham

Potency Assay Strategy

Step 1. Activate *Pro1*

Step 2. Assess Biological Activity



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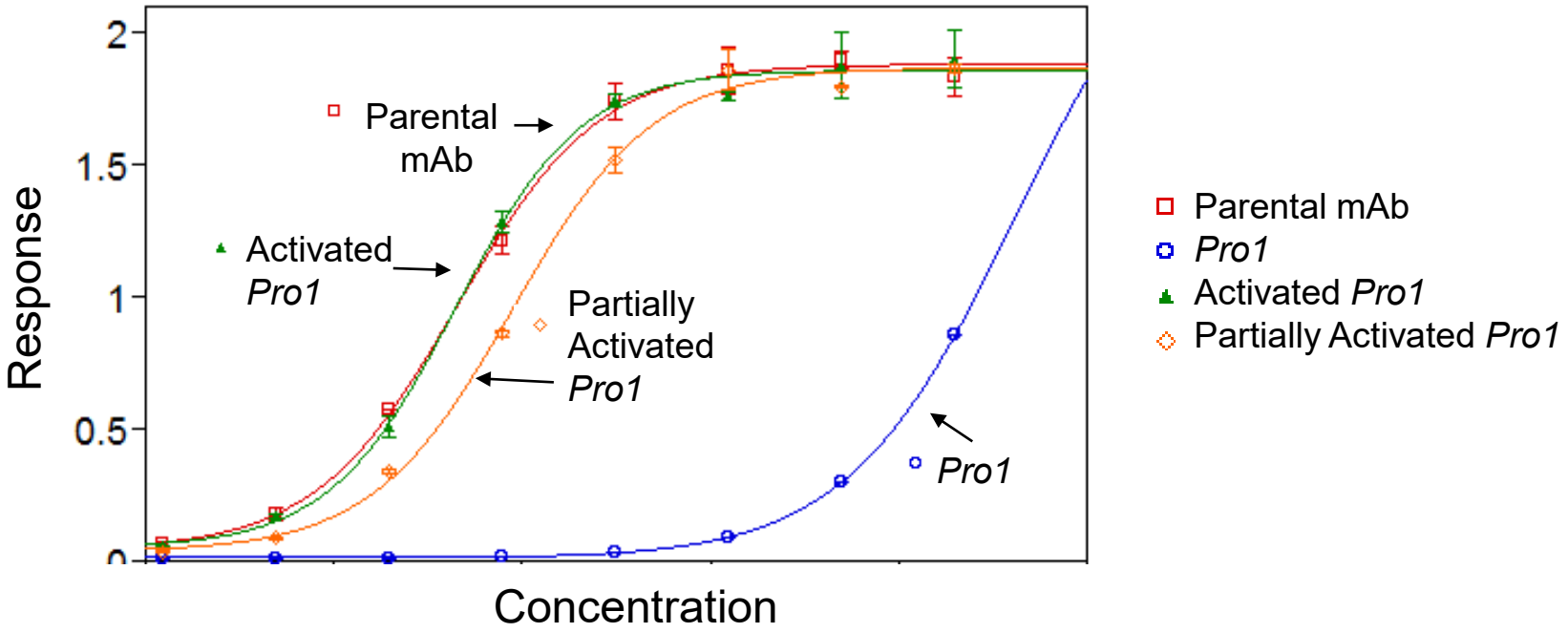
Challenge for Assay Development:
Activation Conditions Impact Sample Activity



- **Partially activated *Pro1* demonstrates reduced antigen binding**
- **Partial activation of *Pro1* COULD be due to insufficient activation conditions and NOT sample properties**

Ensure Activation Conditions are Reliable and Robust

Goals during assay development and beyond:

1. Understand Unique Critical Reagent (Protease)

2. Define Robust Activation Reaction Conditions

3. Maintain Controlled Conditions during assay Lifecycle

Understand Unique Critical Reagent

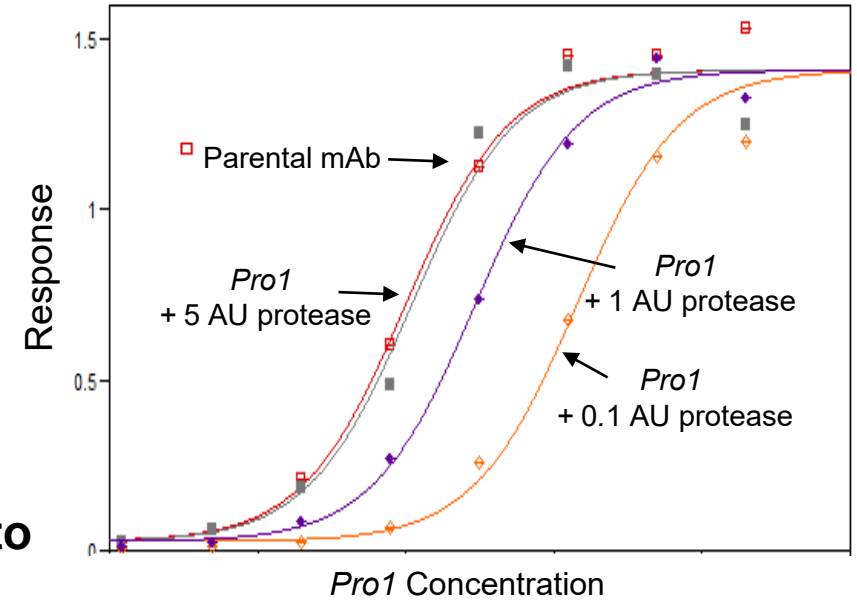
Protease Considerations

- **Supplied with guaranteed activity using manufacturer's substrate, Specific activity listed = "> XX units/min/ug"**
- **How does this relate to *Pro1?***
- **How do we determine working concentration of protease?**

Approach:

Use *Pro1* Activity Assay to measure Protease activation

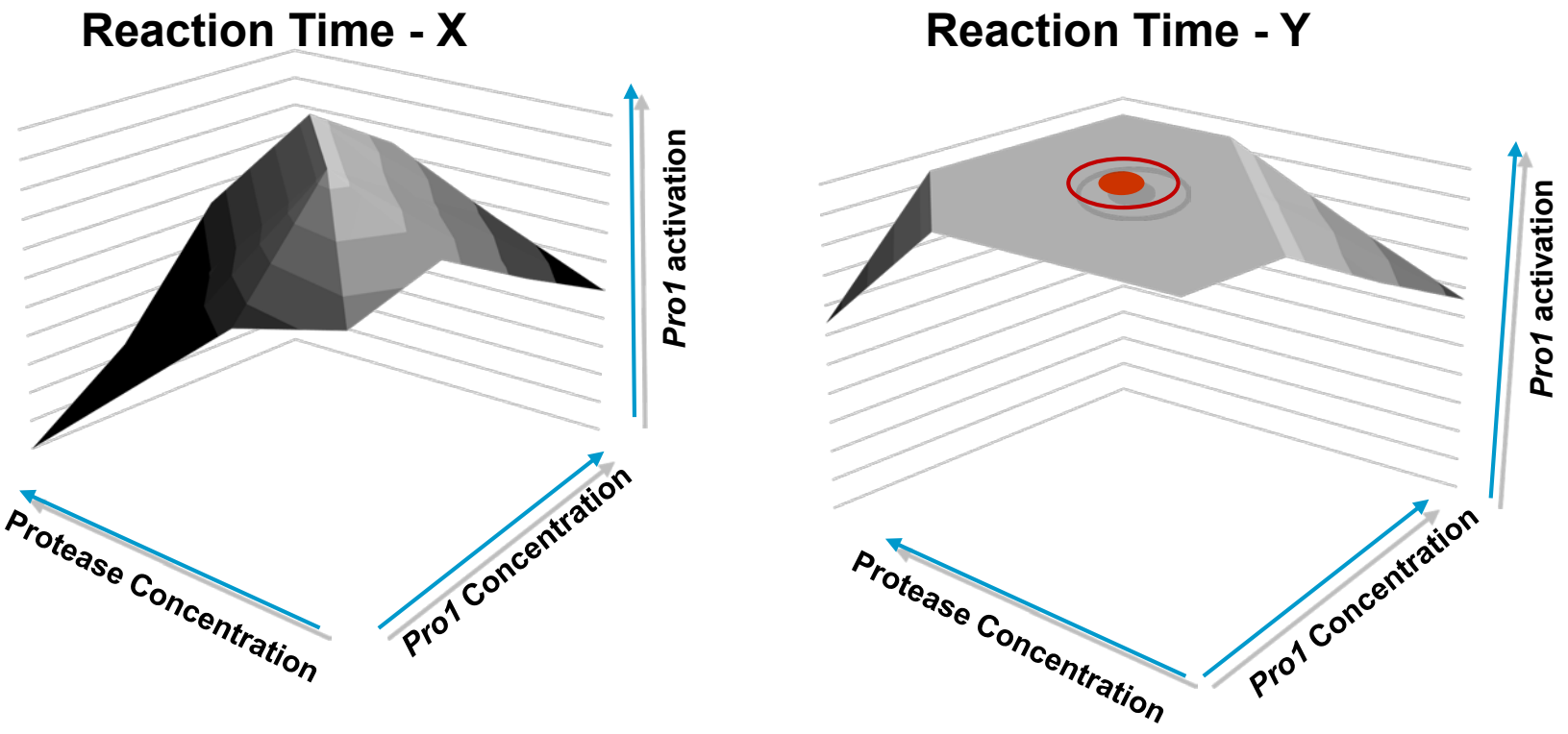
- **Protease activity is not binary**
- **Readout of protease activity requires**
  - **Full *Pro1* dose curve**
  - **Comparison to Parental mAb**
  - **Several concentrations of protease to understand working range**



Define Robust Activation Conditions

- **Enzyme Kinetics are influenced by interacting factors (Reaction time, substrate (*Pro1*) and enzyme concentration)**

Hypothetical Interactions



- **Goal: Find multi-parameter conditions which meet assay needs**

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Define Robust Activation Conditions

Experimental Approach to optimize conditions for *Pro1*

Highly influential factors optimized simultaneously

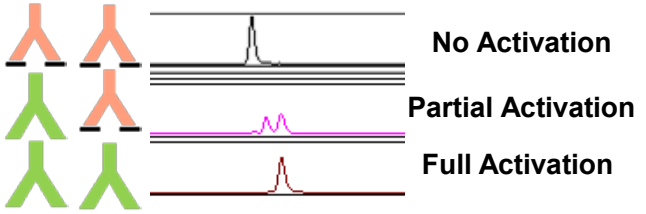
Experimental Design

Condition	Incubation Time (Low, Med, High)	Protease Concentration (Low, Med, High)
1	Low	Low
2	Med	
3	High	
4	Low	
5	Med	Med
6	High	
7	Low	High
8	Med	
9	High	

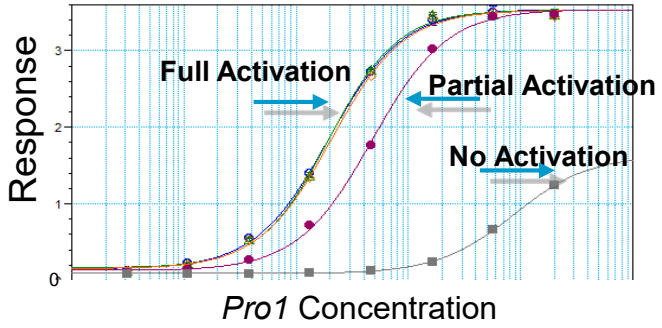
*

Assessment with two orthogonal methods

Readout#1 –Activation Detected by Chromatography



Readout#2 –Activation Detected by Antigen Binding Activity



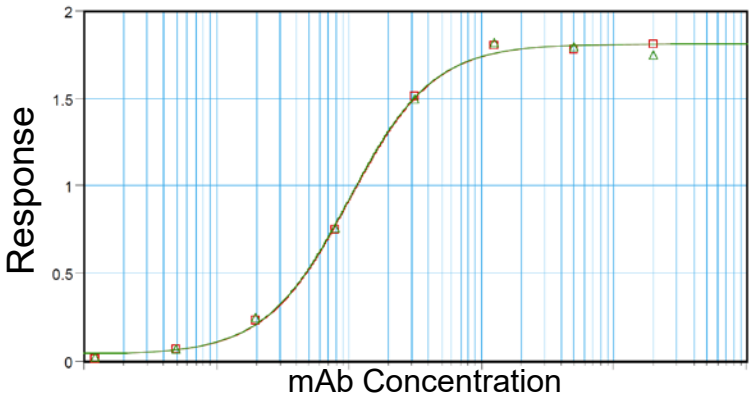
***Optimal conditions require finding the right combination of parameters**
Strategy ensures Robustness with a QC friendly allowance for Incubation Time

Maintain Controlled Activation Conditions During Assay Lifecycle

Objective: Design bioassay to ensure proper *Pro1* activation conditions

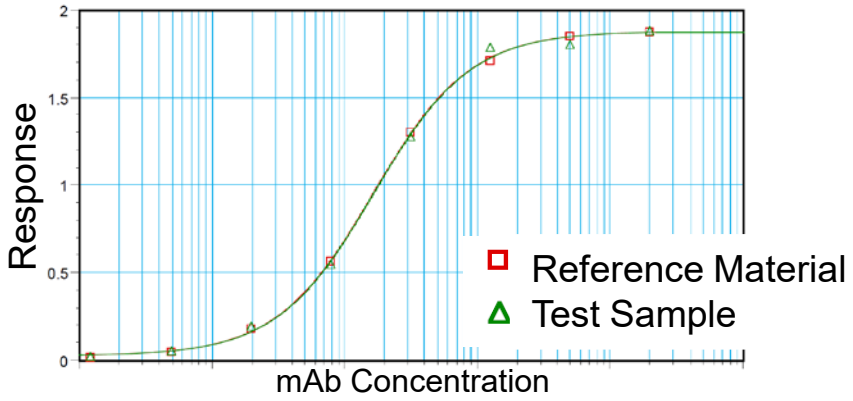
Typical Bioassay:

Assay #1



- Assay and Sample Acceptance Criteria Met

Assay #2



- Assay and Sample Acceptance Criteria Met

Absolute curve parameters vary between assays

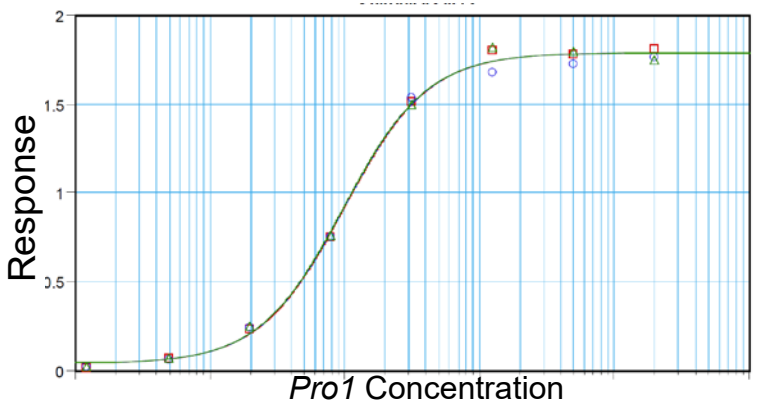
Challenge: How do we control assay for activation conditions without dependence on absolute curve parameters?

Maintain Controlled Activation Conditions During Assay Lifecycle

Challenge: How do we control assay for activation conditions without dependence on absolute curve parameters?

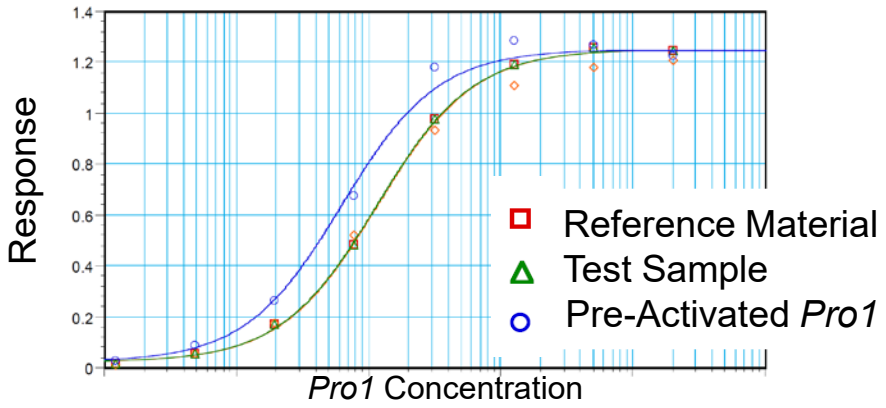
***Pro1* Bioassay: Include a pre-activated *Pro1 control* in all assays**

Assay #1



- Assay Acceptance Criteria Met

Assay #2



- Assay Acceptance Criteria NOT Met

Complexities:

- **Generation of pre-activated *Pro1* in large scale**
- **Qualification activities of pre-activated *Pro1***

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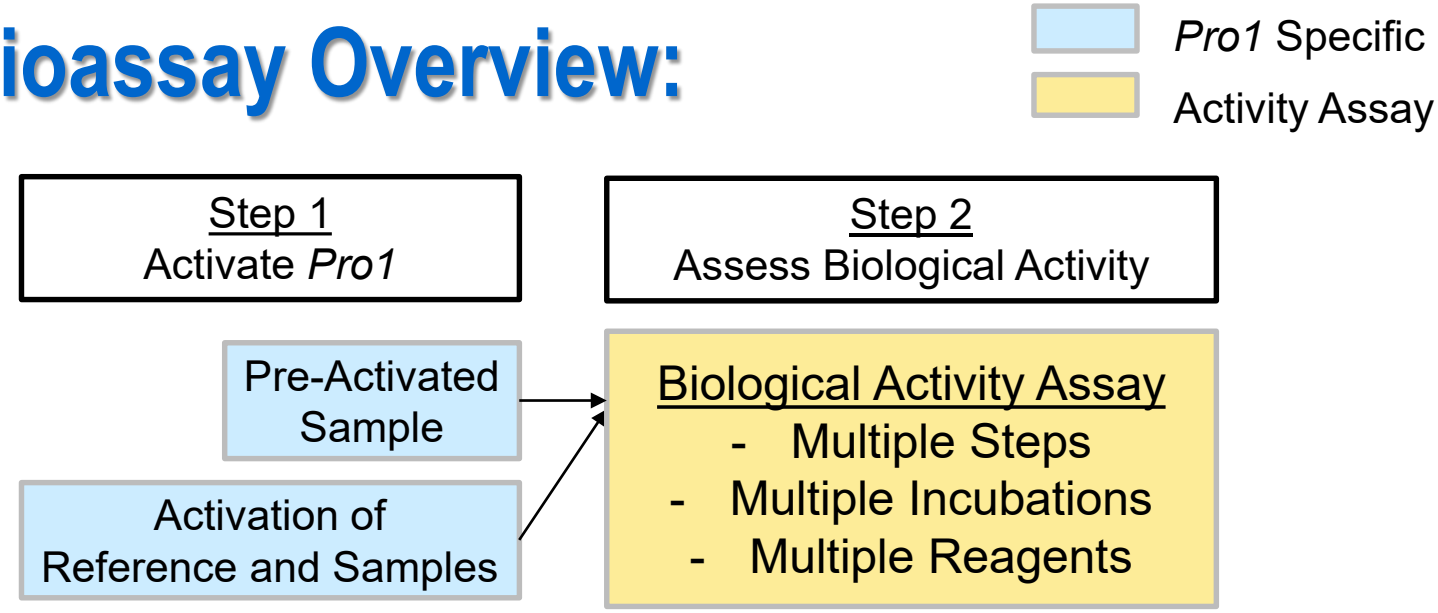
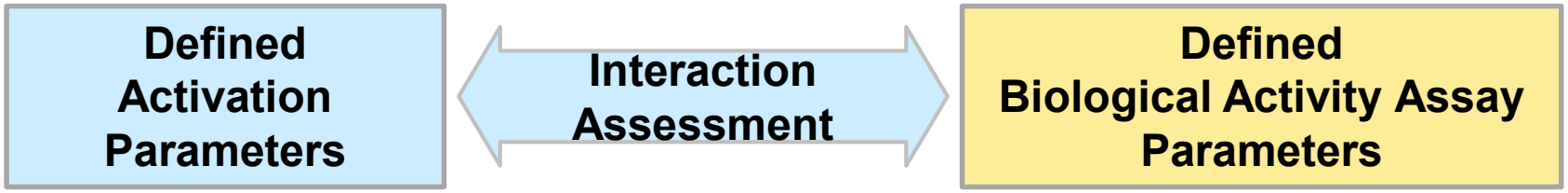
Overall Activity Assay: Potency Assay

Pro1 Bioassay Overview:



Pro1 Bioassay Development:

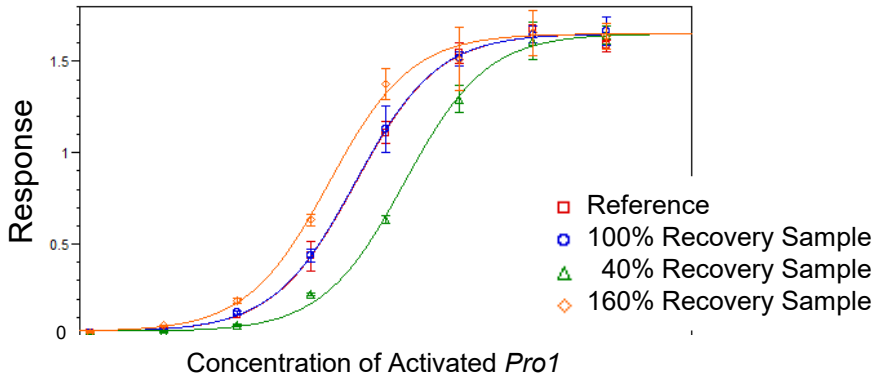


Multifactorial Approach:
Define Suitable Parameters for Each Incubation

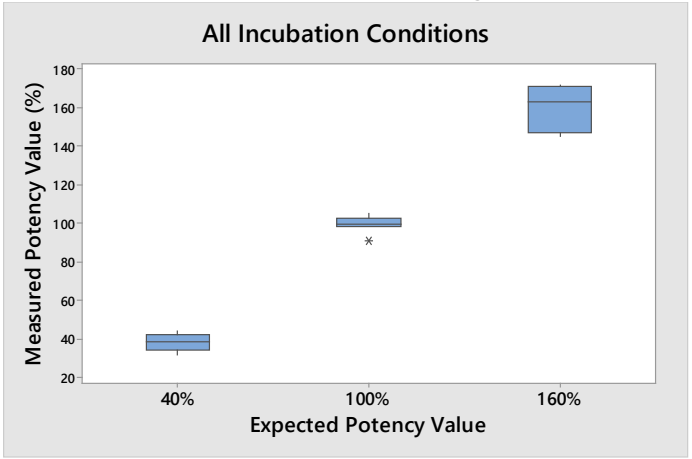
Condition Number	Experimental Parameter		
	Incubation A	Incubation B	Incubation C
1	-	-	-
2	-	-	+
3	-	+	-
4	-	+	+
5	+	-	-
6	+	-	+
7	+	+	-
8	+	+	+

Shortest time allowable (-)
Longest time allowable (+)

Representative Data from Condition #4



ALL Conditions - Sample Potency Determinations



Defined parameters provide suitable assay performance

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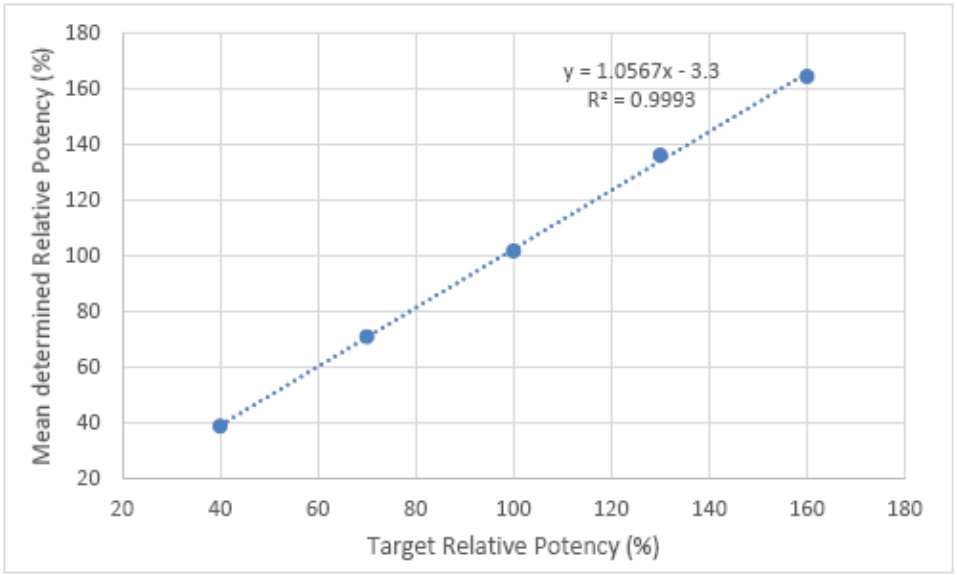
Potency Assay for *Pro1* Meets Performance Expectations

Linearity



Accuracy / Precision

Nominal % Potency		Mean % Recovery
Sample	40	97
	70	101
	100	102
	130	105
	160	103
Mean		102
% CV		4%

N = 30

Optimized assay meets performance expectations for accuracy, linearity, precision, specificity and range

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Summary and Conclusions

- **A significant challenge for Immuno Oncology product development has been modeling MoA complexity**
  - **This challenge is increasing with increased product complexity**

- ***Prodrug - 1* Potency Assay:**
  - **Was developed with incorporation of specific critical reagent performance in mind**
  - **Includes assay acceptance criteria to control for activation**
  - **Is accurate and precise and supports long-term product needs related to release and stability testing**

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Abstract

Next generation biologics are now being developed that are designed for more efficacy and less toxicity than parent molecules. By nature, these molecules have increased biological complexity with mechanisms of action (MOAs) often involving multiple steps to elicit true biological response. Biologics that are prodrugs are engineered to be in an inactive state during drug administration (non-antigen binding state) and then converted to an activated state (antigen binding state) in the tumor. Bioassays to measure potency of prodrug biologics therefore include an activation step which must be methodically controlled. Here we describe how suitable prodrug activation conditions were defined in conjunction with additional assay conditions to ensure consistent bioassay performance.