

*The Three A's of Biologicals:
Activity, Activity, Activity:*

*Guiding Process Development Using Bioactivity
Measurements*

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Vice President, Laboratory Services*

August 25, 2011



Aragen Bioscience, Inc.

- CRO based in Morgan Hill, CA
- Founded in 2006 with continuous operations dating back to 1993
- Diverse service offering
 - Manufacturing cell line development
 - Upstream and downstream process development
 - In vivo services
 - Cell-based assays
 - Custom studies

A Case Study

- Client had reference product (“Lot 1”) from an alternate expression system and purification process.
- Aragen Bioscience was to:
 - Create a stable, scalable CHO cell line expressing the product of interest for cGMP production.
 - Create a scalable purification scheme for the purification of product for pre-clinical studies and to serve as a basis for cGMP process development.

Production System

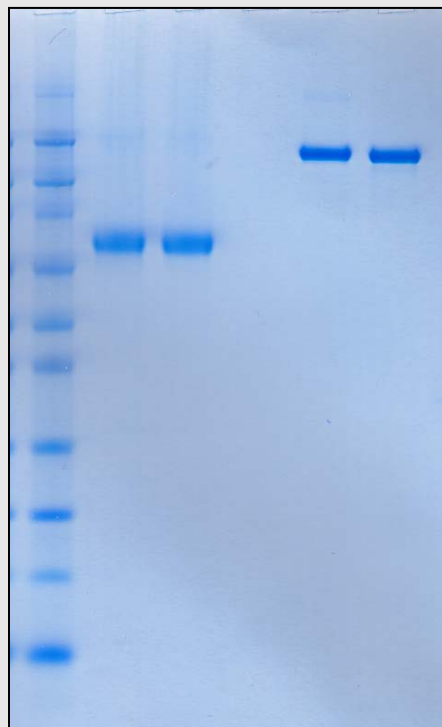
- Cloned protein of interest into Aragen's CHO dhfr expression vectors.
- Identified and selected high expressing CHO-DG44 clones.
- During cell line development, developed a high yield two column process.
- Developed analytical assays for comparison to Lot 1 reference material provided by the customer.

Initial Physical Analysis

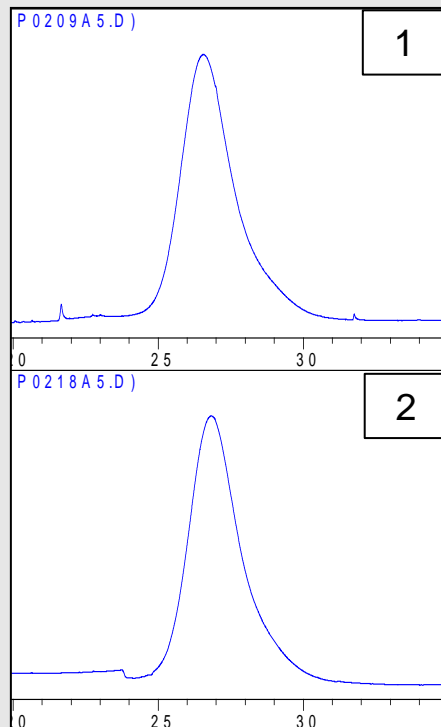
Two Column Process

SDS-PAGE

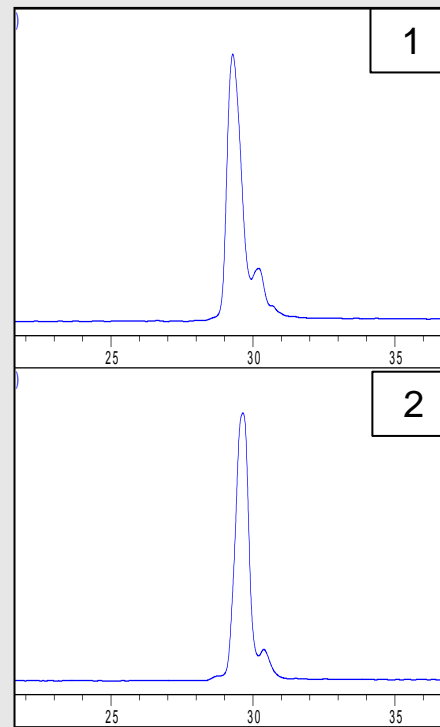
+DTT -DTT
1 2 1 2



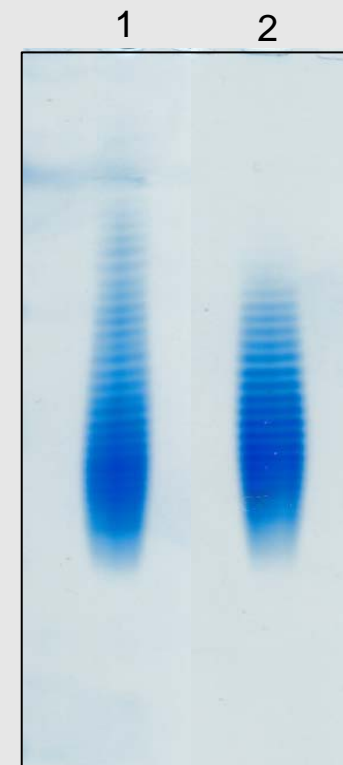
SEC-HPLC



RP-HPLC

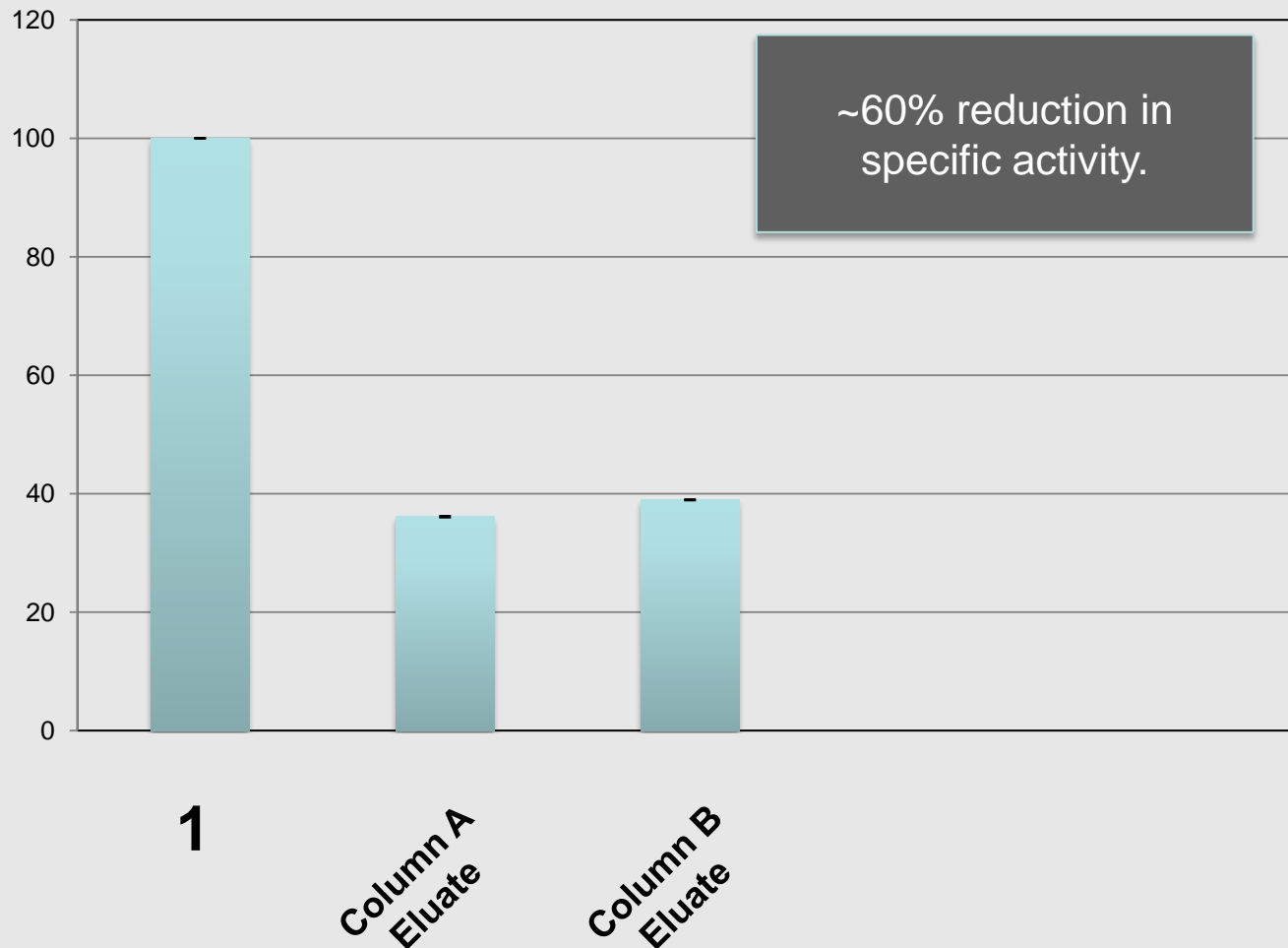


IEF

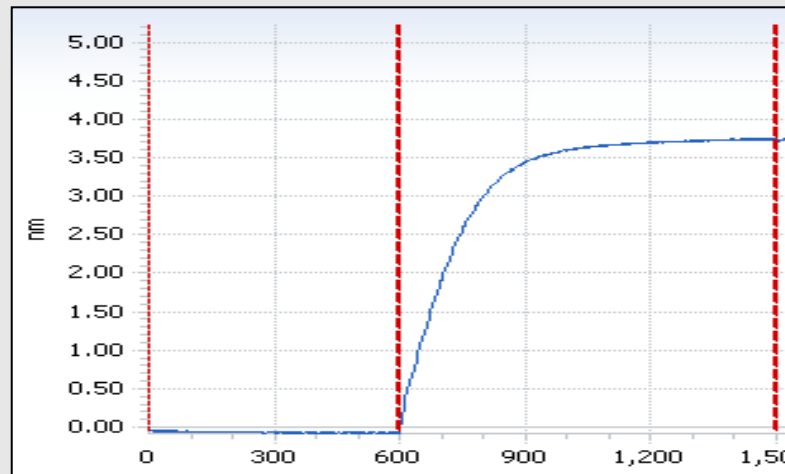
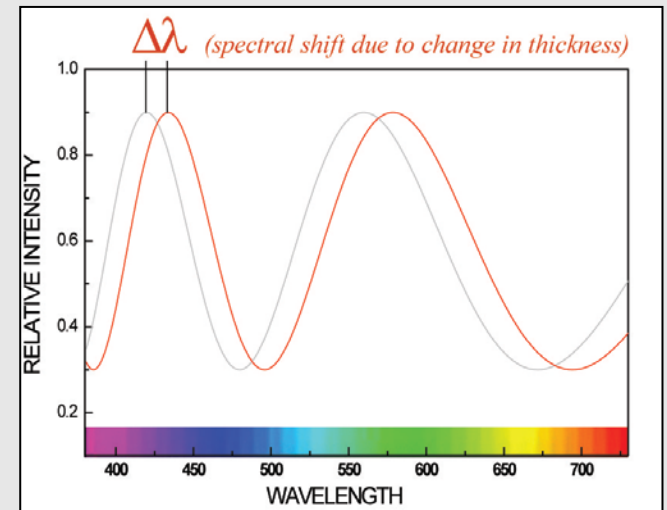
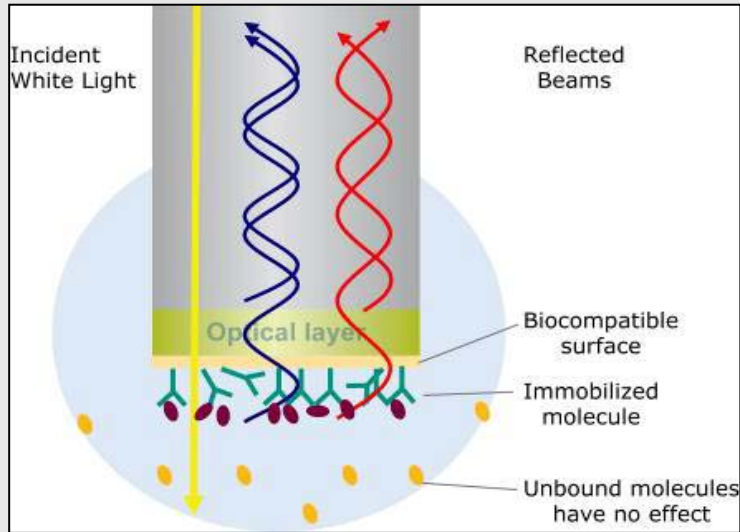


Functional Characterization

ELISA-Based Binding Analysis

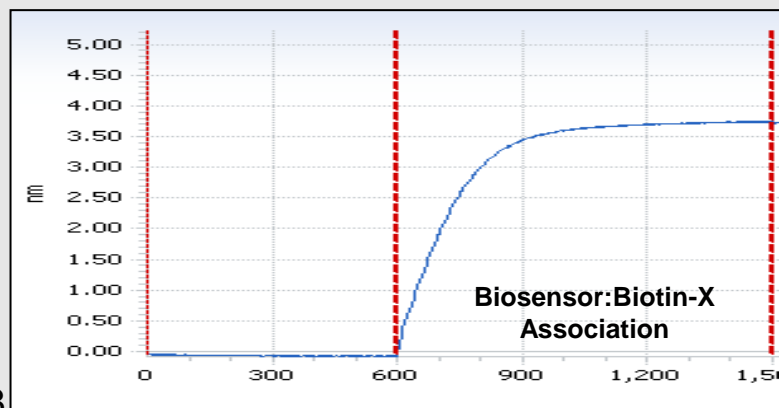
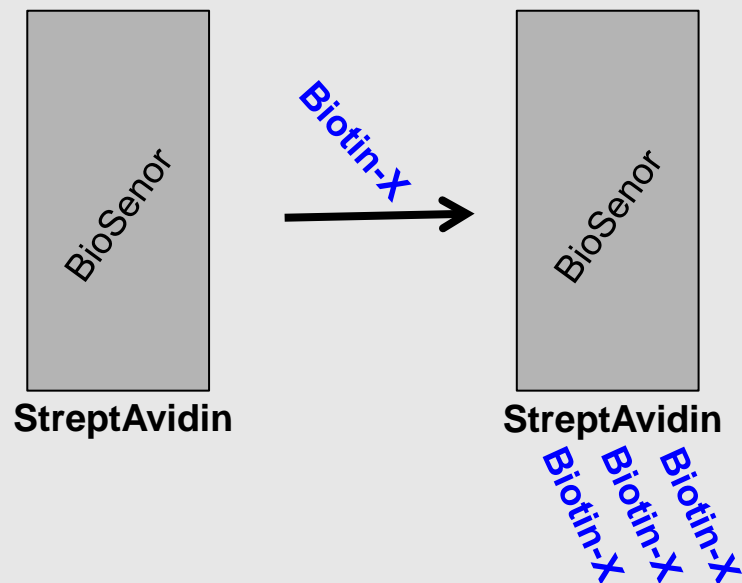


ForteBio: Bilayer Interferometry Technology Overview



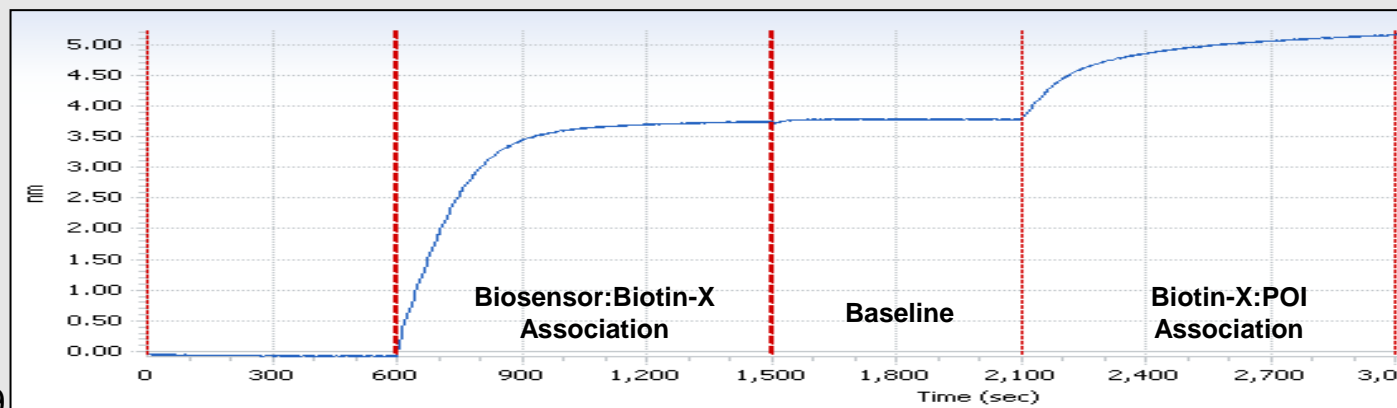
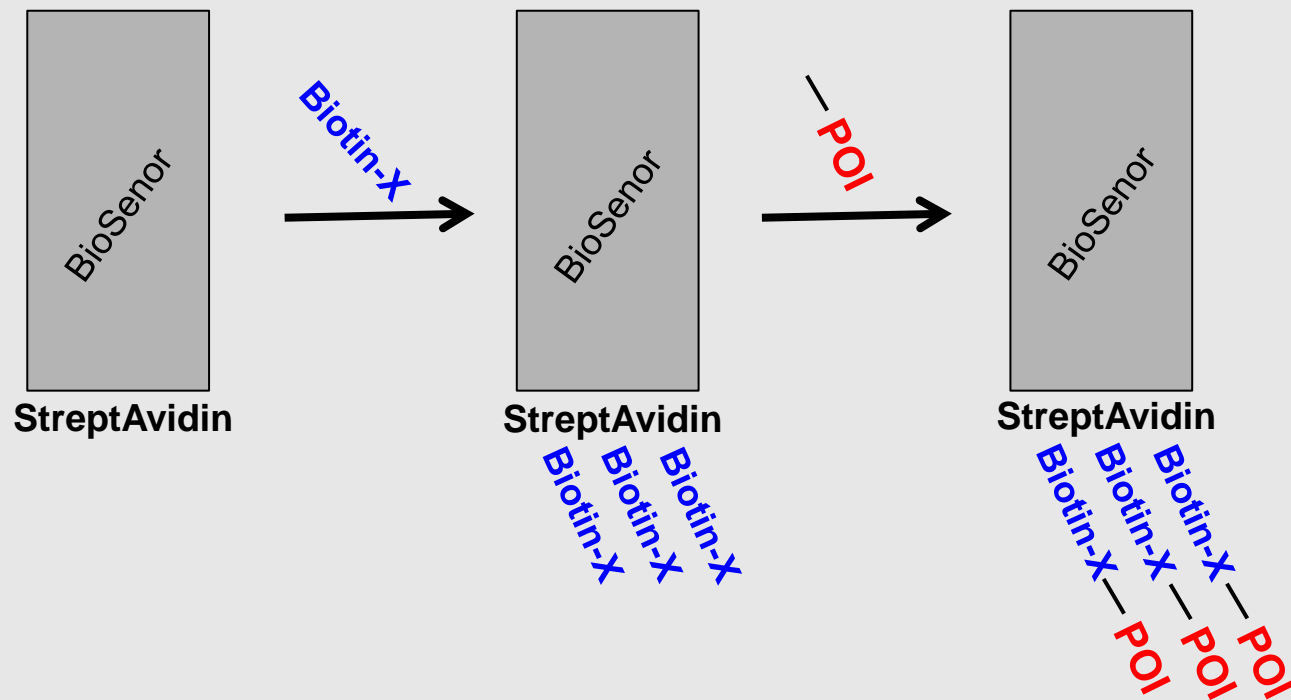
Functional Characterization

ForteBio Assay Development



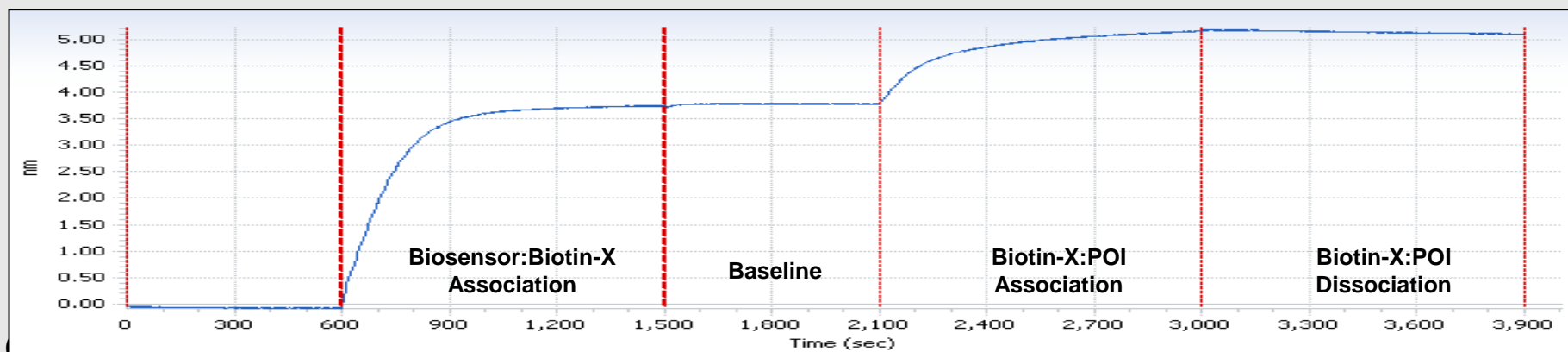
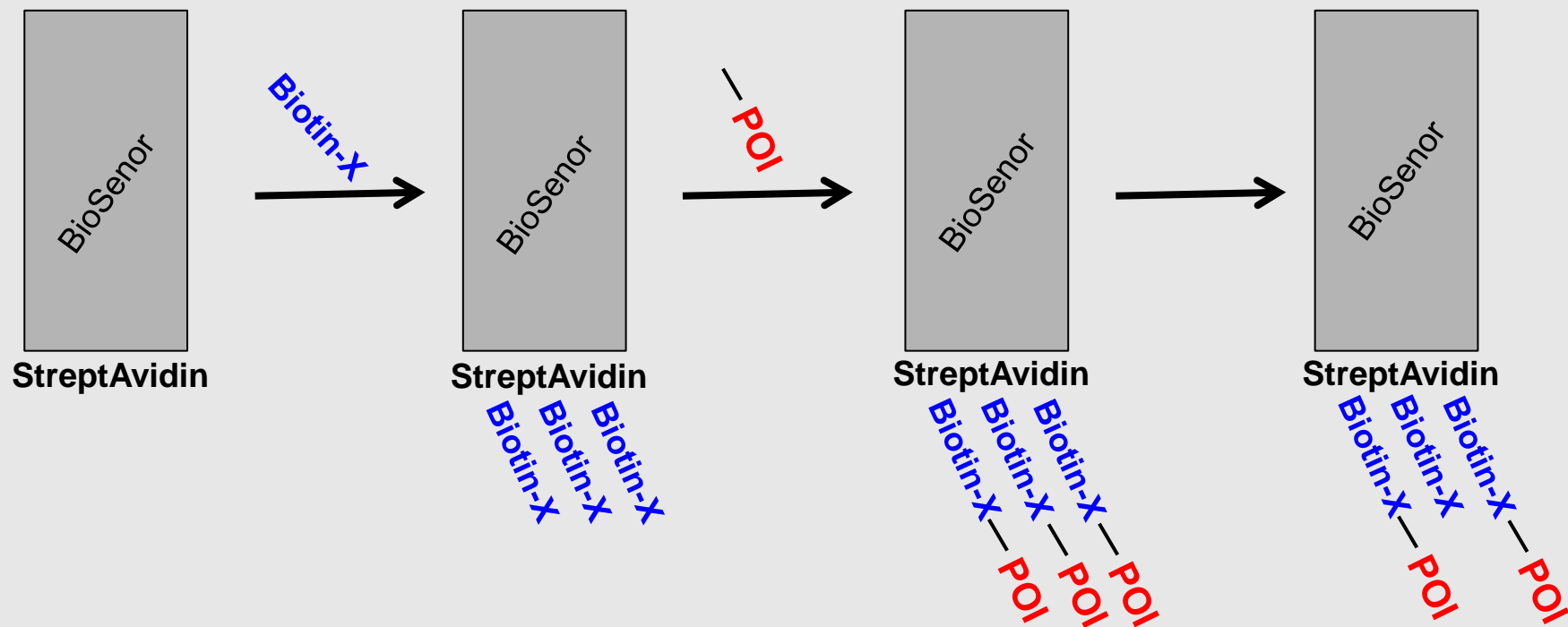
Functional Characterization

ForteBio Assay Development



Functional Characterization

ForteBio Assay Development



Functional Characterization

ForteBio Binding Data

Test Material	K_D
Lot 1	9.8 nM
Column B Eluate	17.3 nM

ForteBio analysis revealed approximately two-fold reduction in binding affinity consistent with plate based binding assay

Functional Characterization

Reduced Product Binding Activity

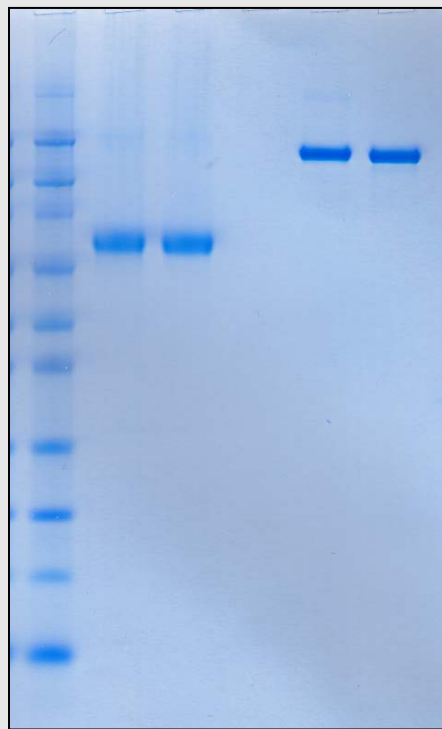
- Inactivation of the product was not due to the purification process. Lot 1 reprocessed using the same column steps retained 100% of its original activity.
- Reduced specific activity was observed in the conditioned medium.
- A significant fraction of the material being produced by the CHO cells was inactive.
- Challenge: Identify and remove the inactive protein species

Initial Physical Analysis

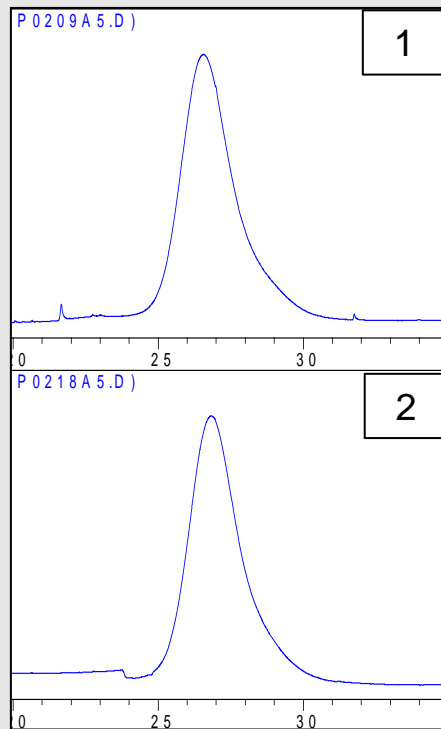
Two Column Process

SDS-PAGE

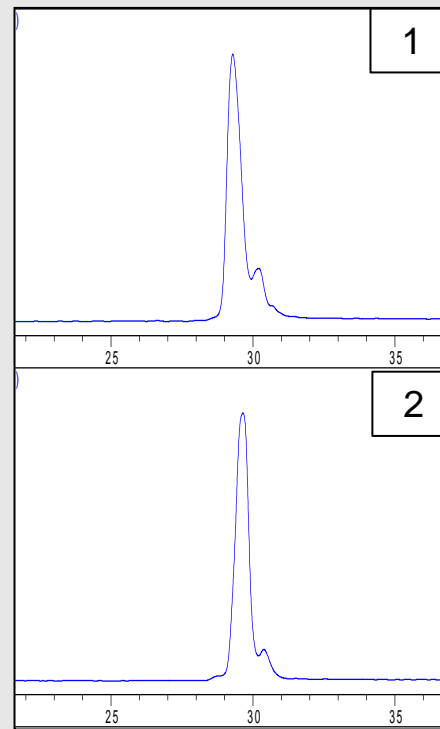
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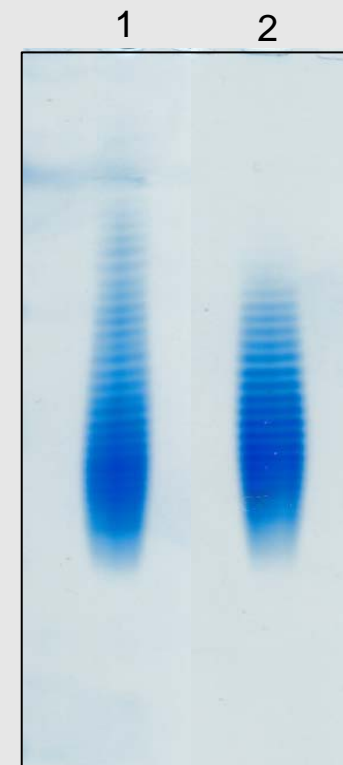
SEC-HPLC



RP-HPLC



IEF

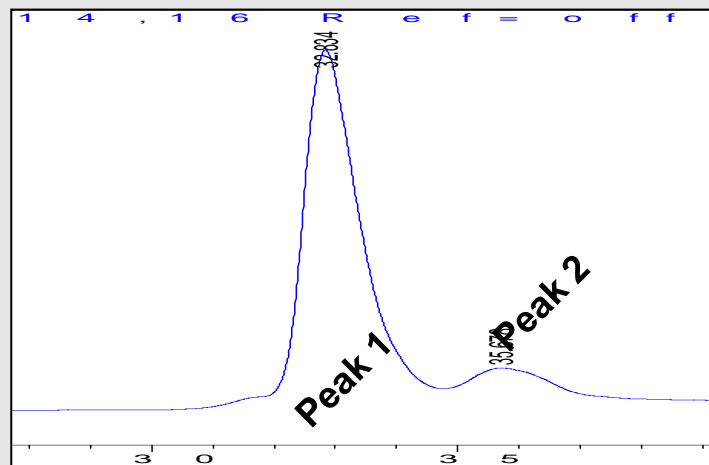


Identification of Inactive Species

Column C

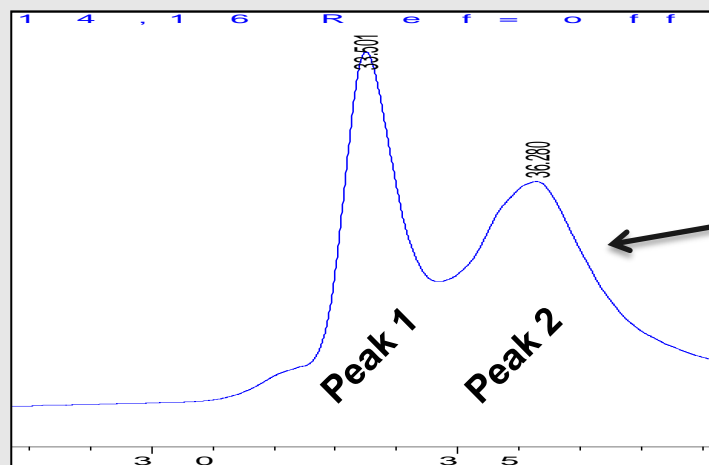
Lot 1

High Specific Binding Activity



Lot 2

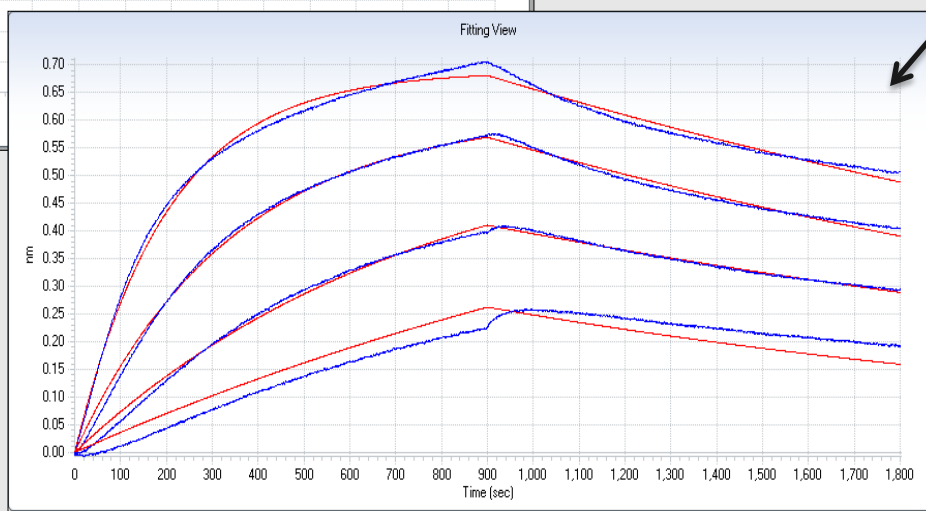
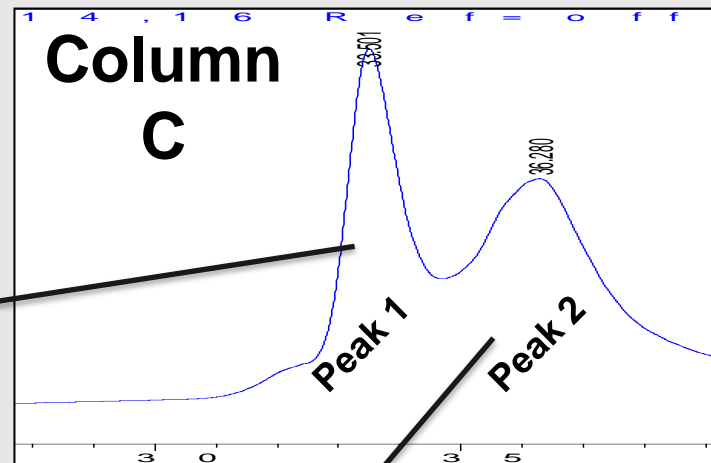
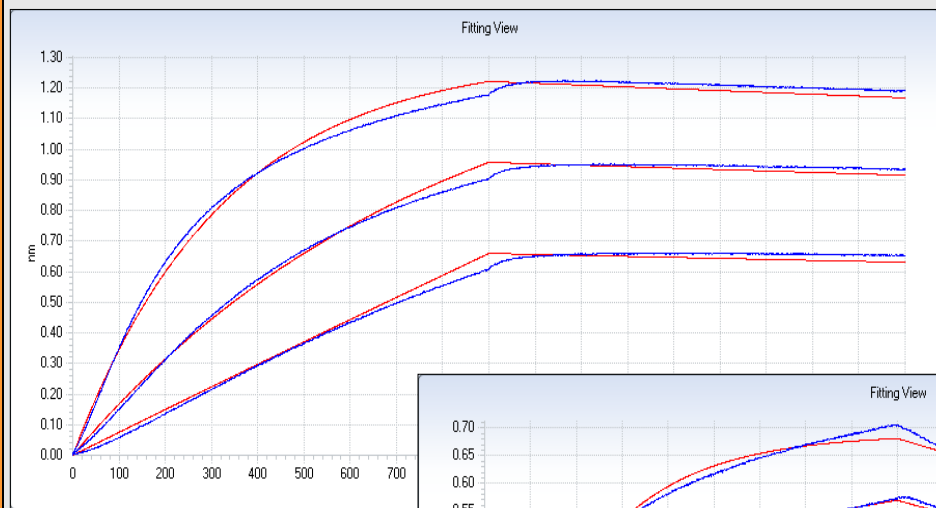
Low Specific Binding Activity



Presence of large second peak correlated with reduced specific binding activity.

Identification of Inactive Species

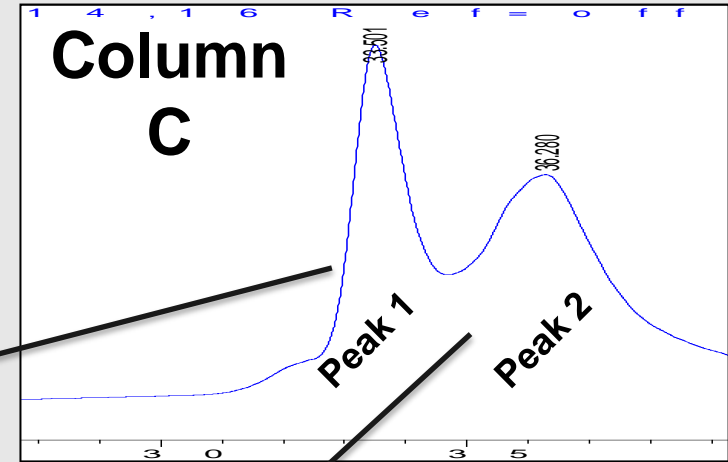
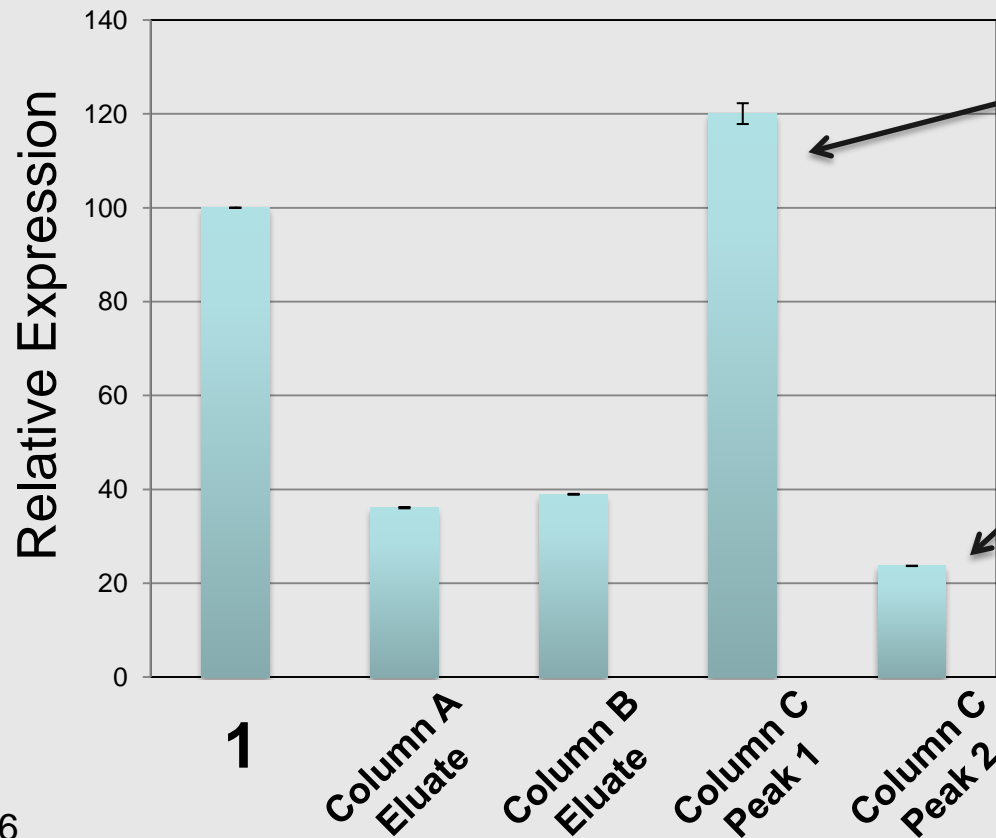
Kinetic Binding Analysis



An increased off-rate was clearly evident in the non-active fraction

Identification of Inactive Species

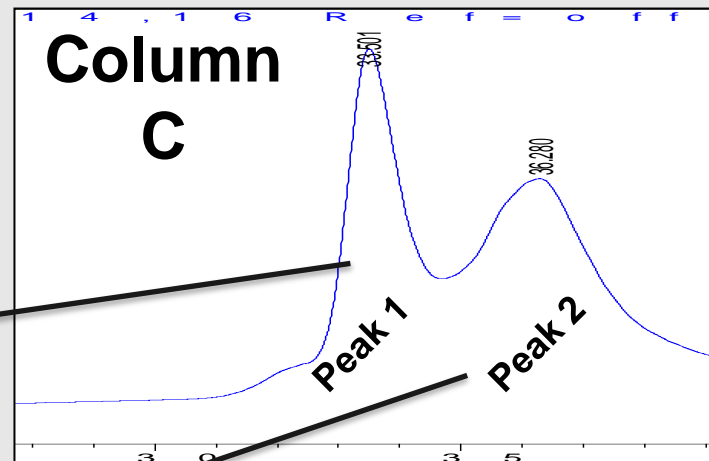
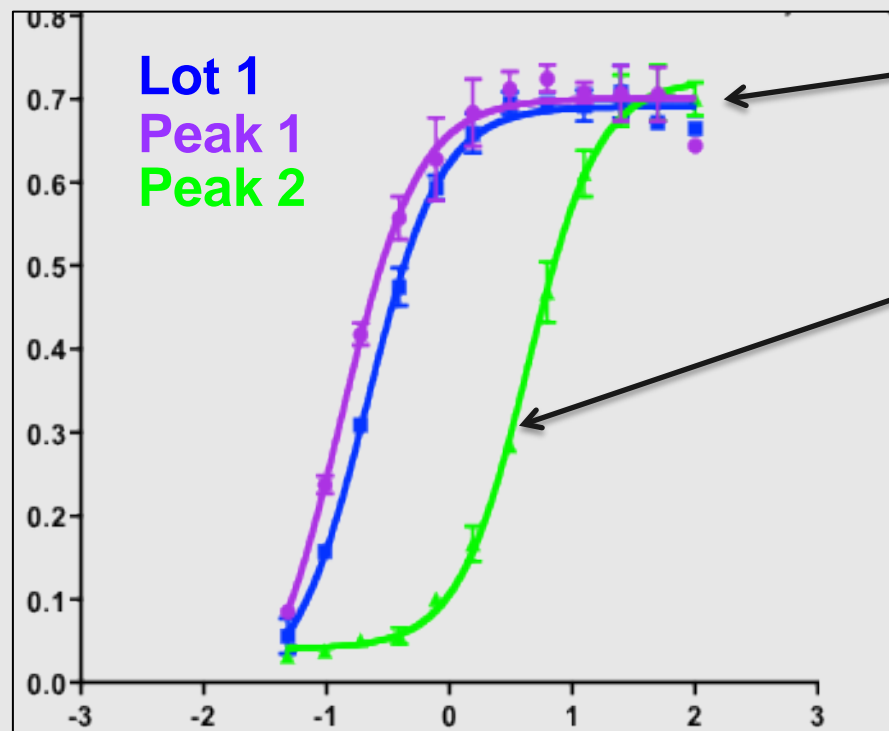
ELISA-Based Binding Analysis



Column C Peak 1
contained the
high specific
binding product

Identification of Inactive Species

Cell-Based Activity Assay



The reduced binding fraction had 100-fold reduced cell based activity

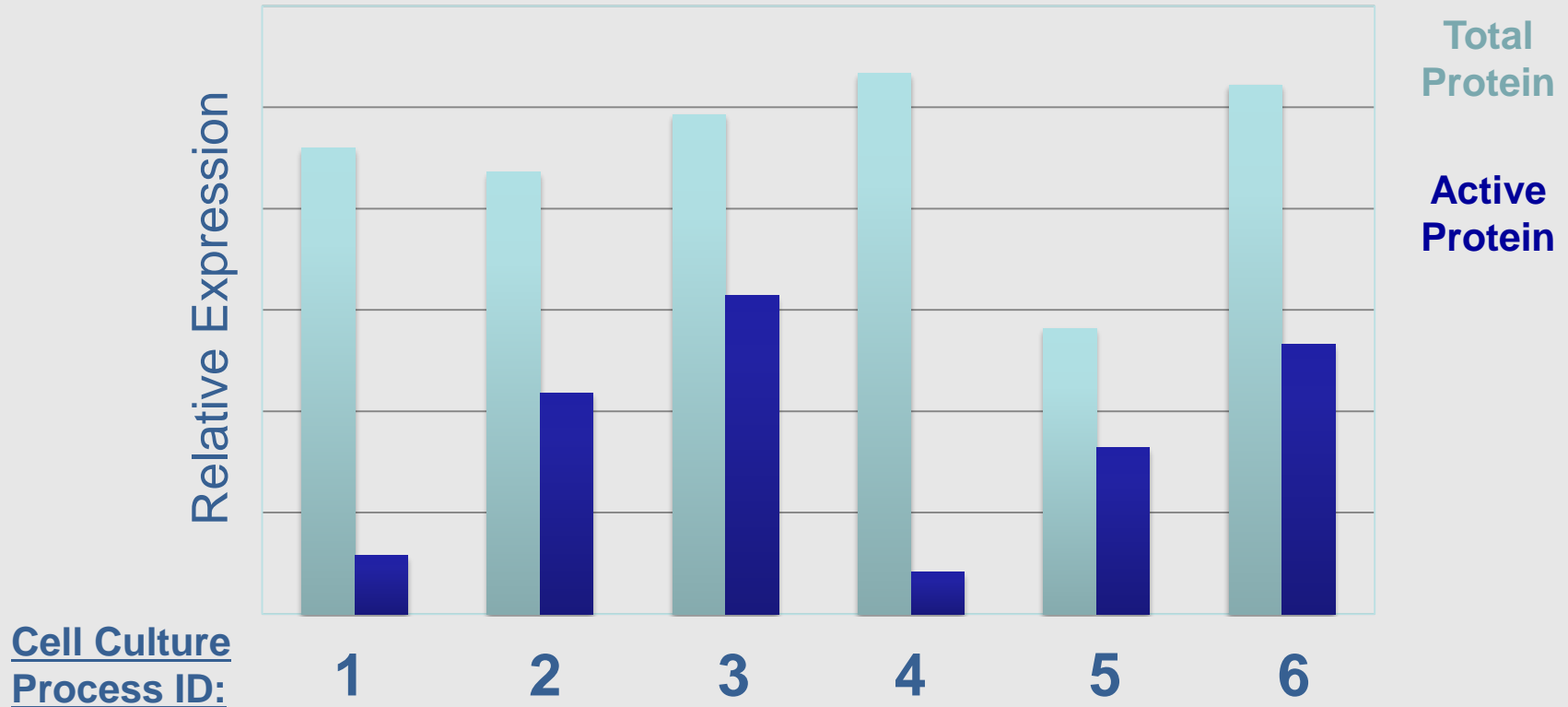
Identification of Inactive Species

Summary

- Identified and developed a process to separate inactive product.
- Resulted in significantly reduced yield of active protein.
- Challenge: Can we identify an upstream process and/or alternate clones that result in higher proportion of active product?

Tissue Culture Process Development

Increasing Expression of Active Protein

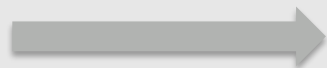


Tissue culture process development identified conditions resulting in higher levels of active protein

Clone Identification

Using New Culture Process

Seed
Flask



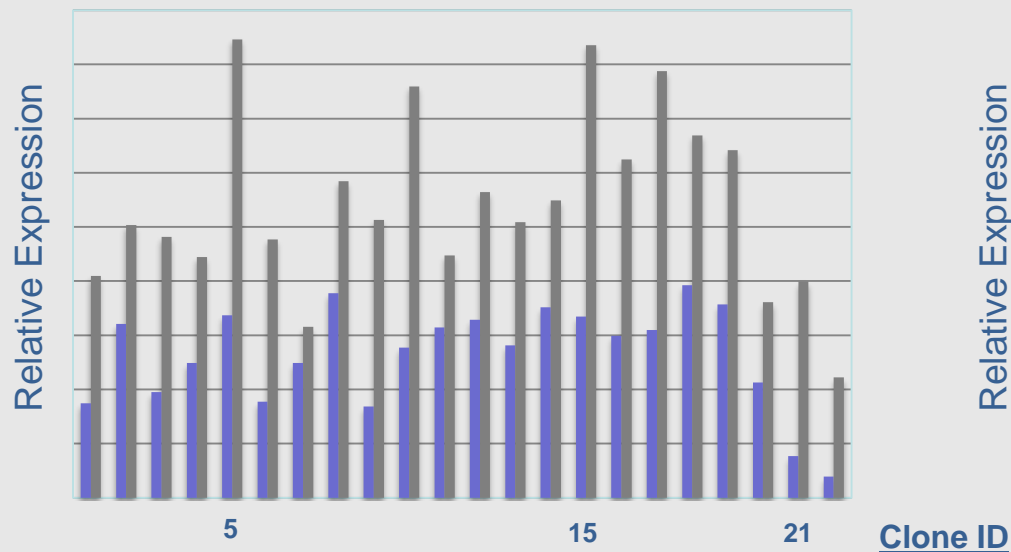
T_1



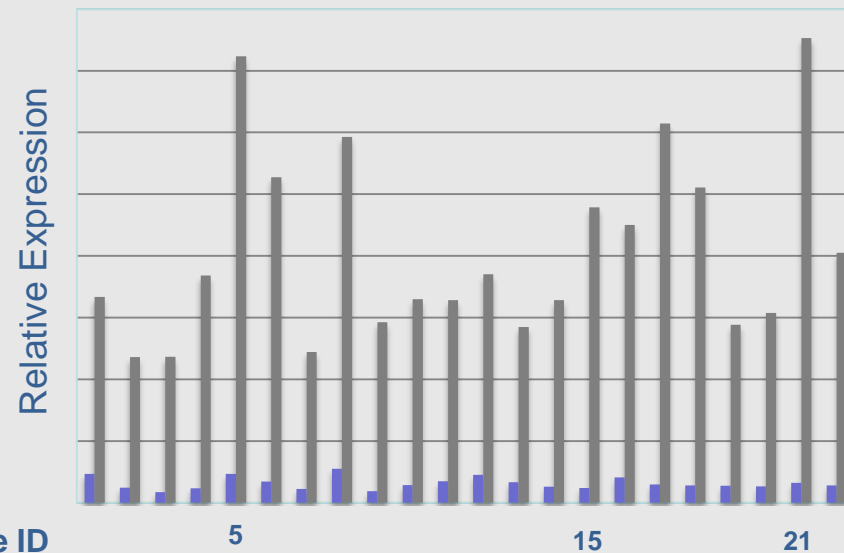
Temp Shift

T_2

Total Protein



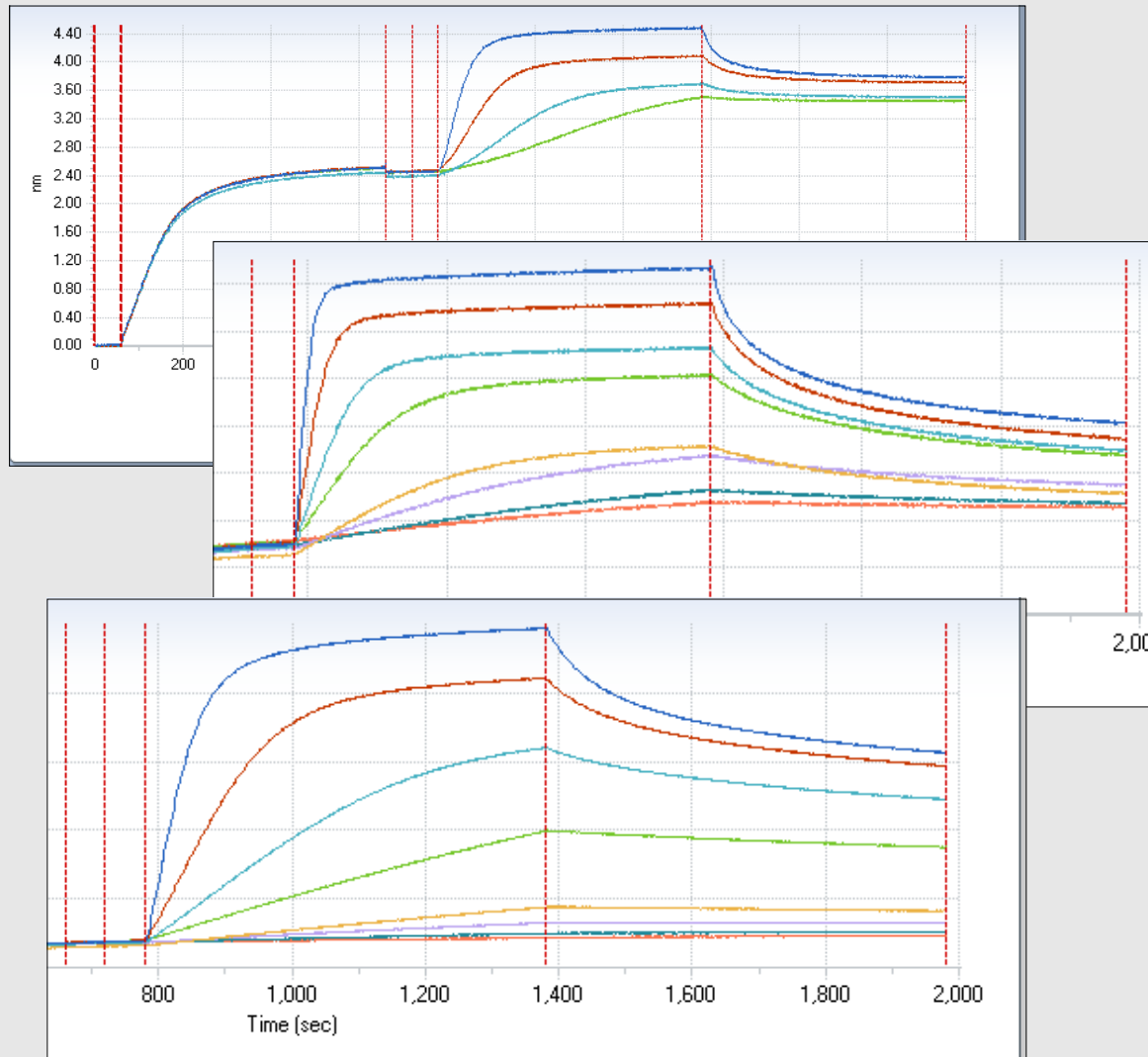
Active Protein



A Case Study: Summary

- New expression platform resulted in reduced specific activity of expressed protein.
- Application of various techniques used to characterize and identify the inactive form of protein being produced.
- Purification process development resulted in ability to remove low activity species.
- Used the alternative cell culture process in a screen to identify clones expressing high specific activity protein product.
- Current shake flask process generates $>1.5\text{g/L}$ with high yield, three column purification process.

Other ForteBio Assays



- Fc Receptor Binding
 - FcRN
 - CD16
 - CD32
- Antibody – Antigen
 - Intact
 - Peptides
- Cytokine – Receptor
- Antibody Titering
- Fusion Protein Titering
- Etc.



In Vitro

- Gene Cloning
- Protein Expression/Purification
- Protein Characterization
- Cell Line Development
- Process Development
- Bioproduction
- Cell-based Assays
- Cytokine Expression
- Methylation Studies
- Genotyping
- qPCR
- Other ...

In Vivo

- Hybridoma Development
- Animal Husbandry
- Ascites Production
- PK/Tox studies
- Disease Efficacy Models
 - Asthma
 - Oncology
 - Fibrosis
 - Wound Healing
- Microsurgery
- Immunohistochemistry
- Custom Disease Models
- Other

