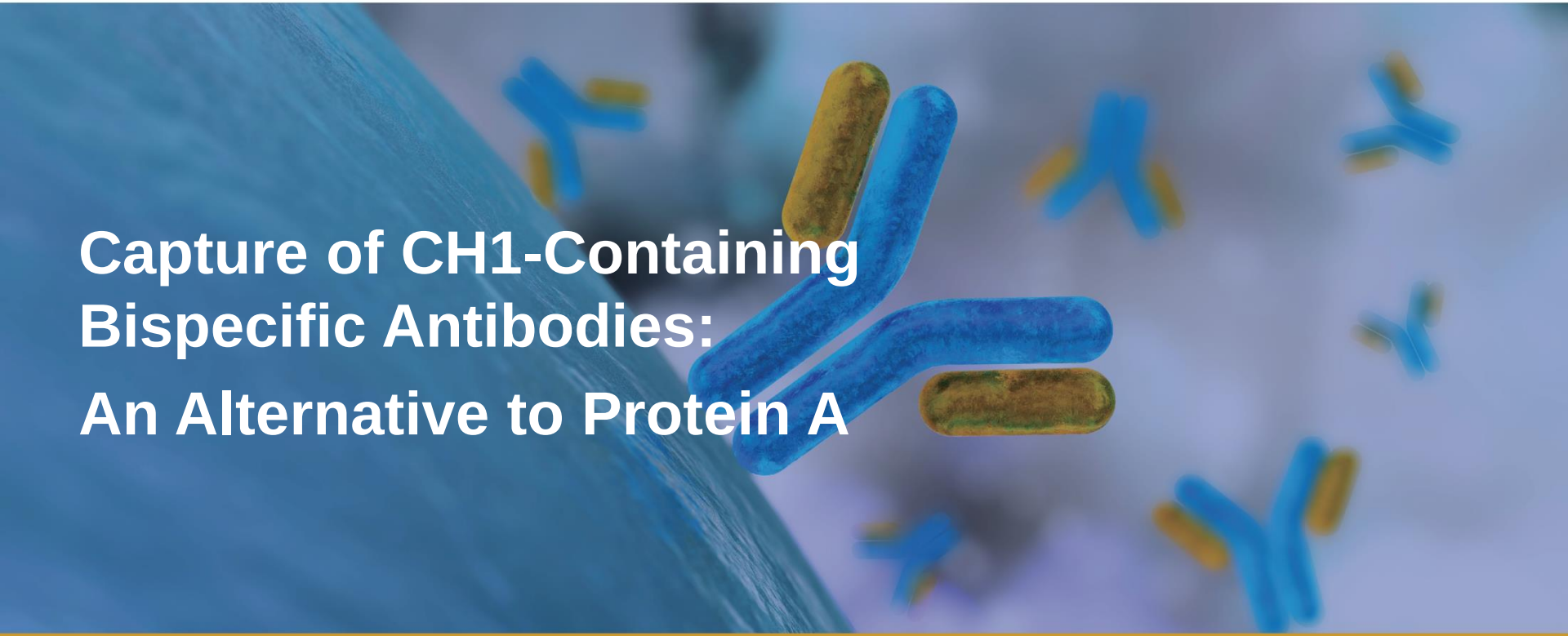
*Virtual BPI*
*Wednesday, 23 September 2020*



# Capture of CH1-Containing Bispecific Antibodies:

# An Alternative to Protein A

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CHAMOW & Associates
Biopharmaceutical Product Development

# Overview

- Introduction to CD3-TAA bispecific antibody Teneo-BsAb
- How to capture Teneo-BsAb?
- Initial test: Protein A
- Finding a better option: CaptureSelect CH1-XL
- Summary

# Teneo-BsAb:

A CD3-tumor associated antigen (TAA) bispecific antibody

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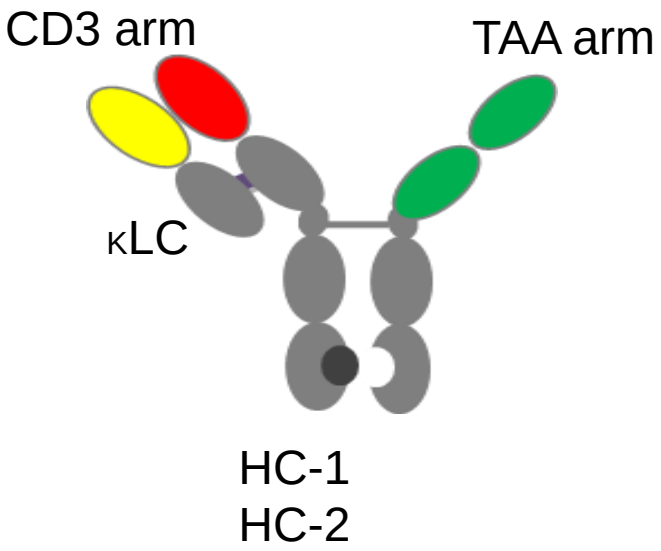
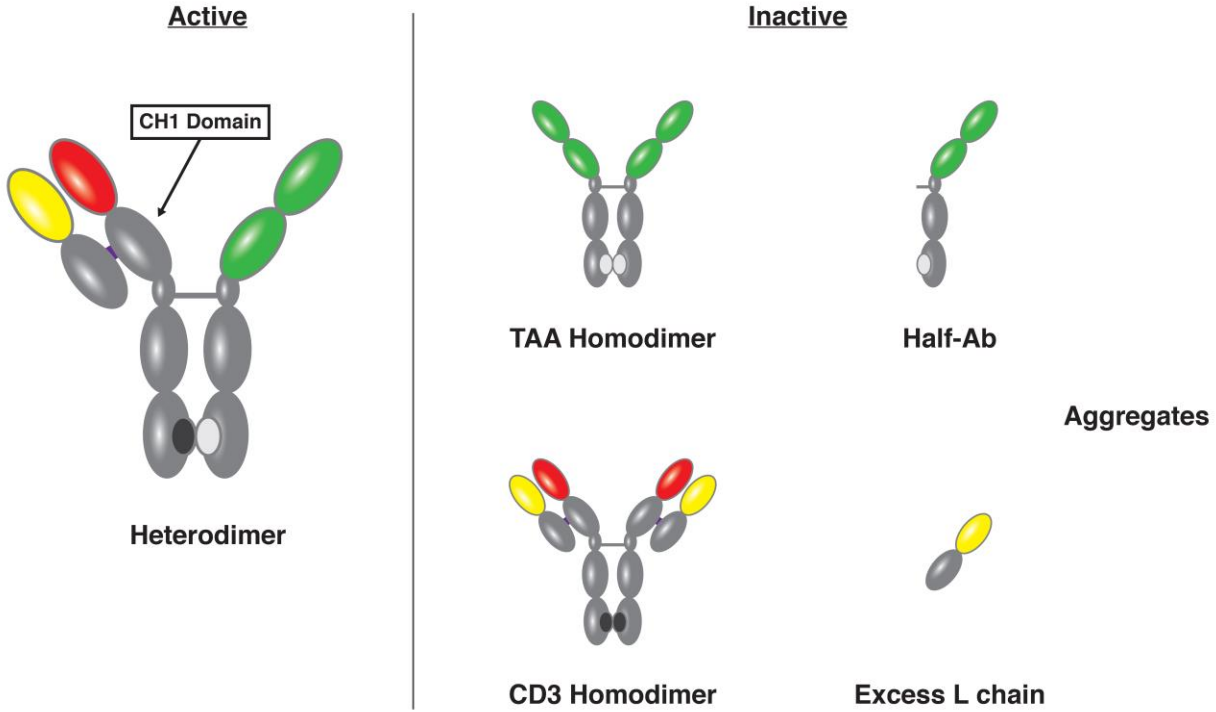
Biopharmaceutical Product Development

# Teneo-BsAb CD3-TAA bispecific antibody

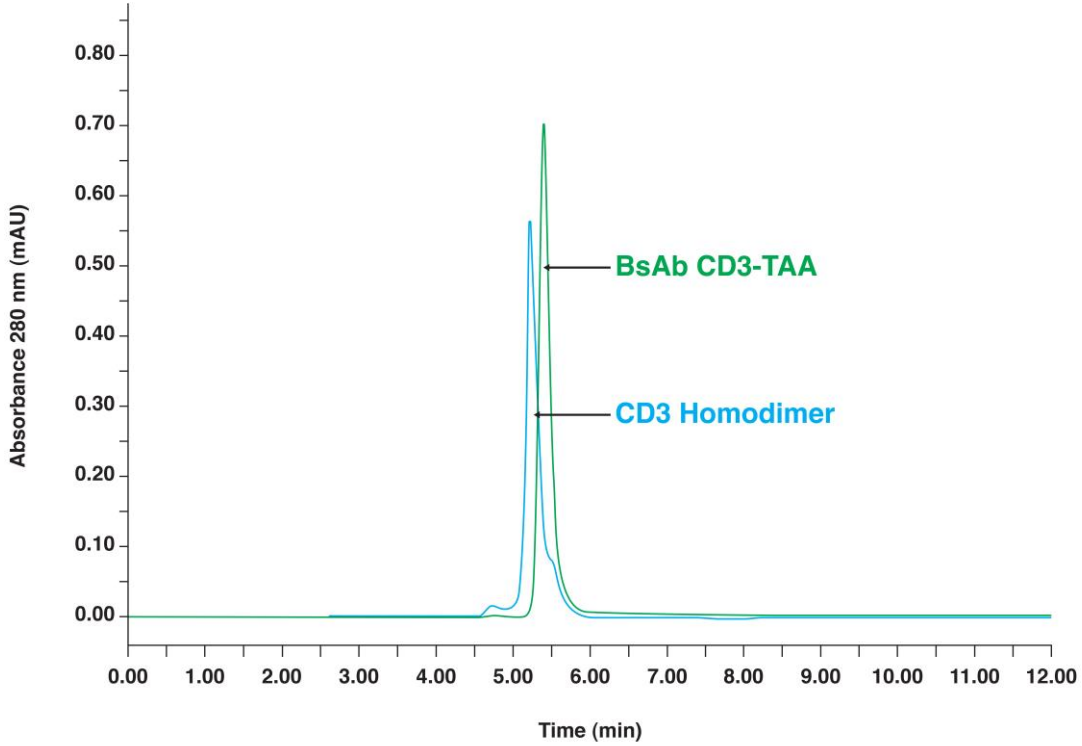



- Fully human IgG4 bispecific monoclonal antibody
  - 2 heavy chains (HC-1 and HC-2) and 1 kappa light chain (KLC)
  - Knobs into holes technology
  - Acid labile
- CD3 arm
  - HC-1 + KLC
  - T-cell receptor CD3
- TAA arm
  - HC-2 only
  - Consists of 2 identical VH domains recognizing TAA
  - Bivalent for increased avidity (<1 nM)
  - Derived from Teneobio's proprietary UniRat™ technology

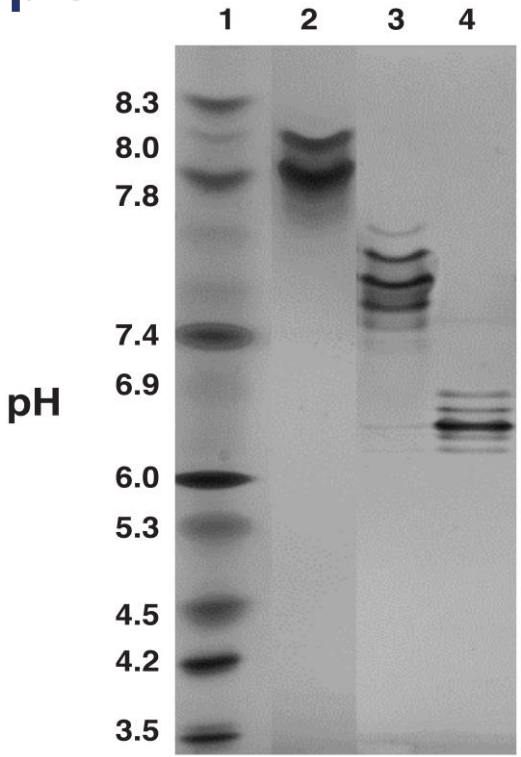
# Teneo-BsAb CD3-TAA expressed products



# Teneo-BsAb heterodimer is similar in size to CD3 homodimer



# Teneo-BsAb CD3-TAA heterodimer and homodimers have distinct pIs




Lanes:

1) IEF, pI stds
2) CD3 homodimer(knob-knob), pI = 7.6-8.0
3) Teneo-BsAb (heterodimer) pI = 7.4-7.6
4) TAA homodimer (hole-hole), pI = 6.2-6.9

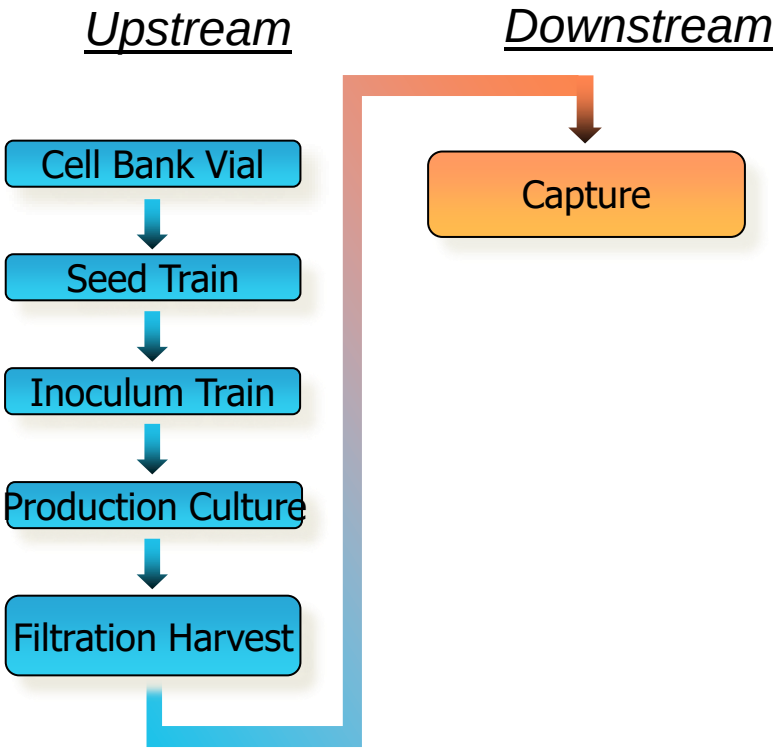
5ug/lane loaded

pH 3-10 IEF gel(Invitrogen)
Instant Blue Stain(Expedeon)
Serva IEF Markers 3-10 mix
IEF Gel Program

| | | | |
|---|---|---|---|
| 1hr | 100V | 18mA | 2.0W |
| 1hr | 200V | 18mA | 3.5W |
| 30min | 500V | 18mA | 9.0W |

# How to capture Teneo-BsAb?

# Process for production of Teneo-BsAb CD3-TAA

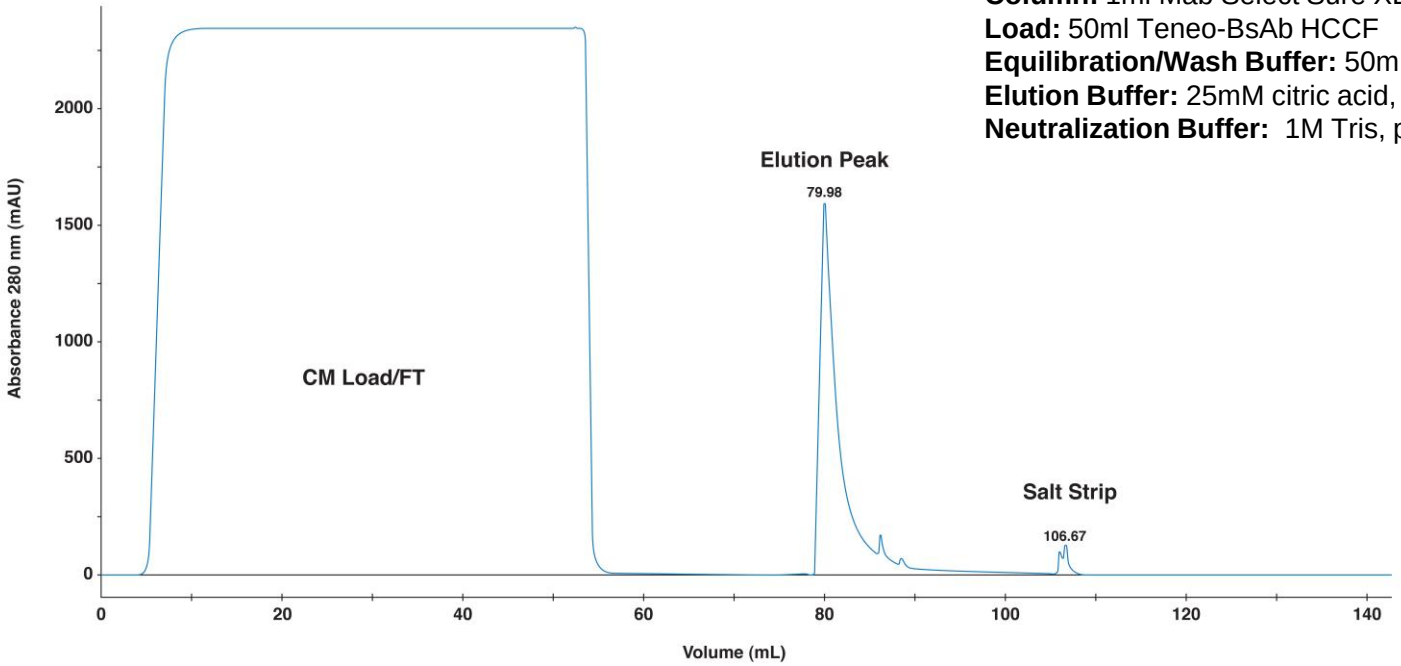


# Initial test for capture:
Protein A

# Elution of Teneo-BsAb from Protein A at pH 3.6 is efficient…

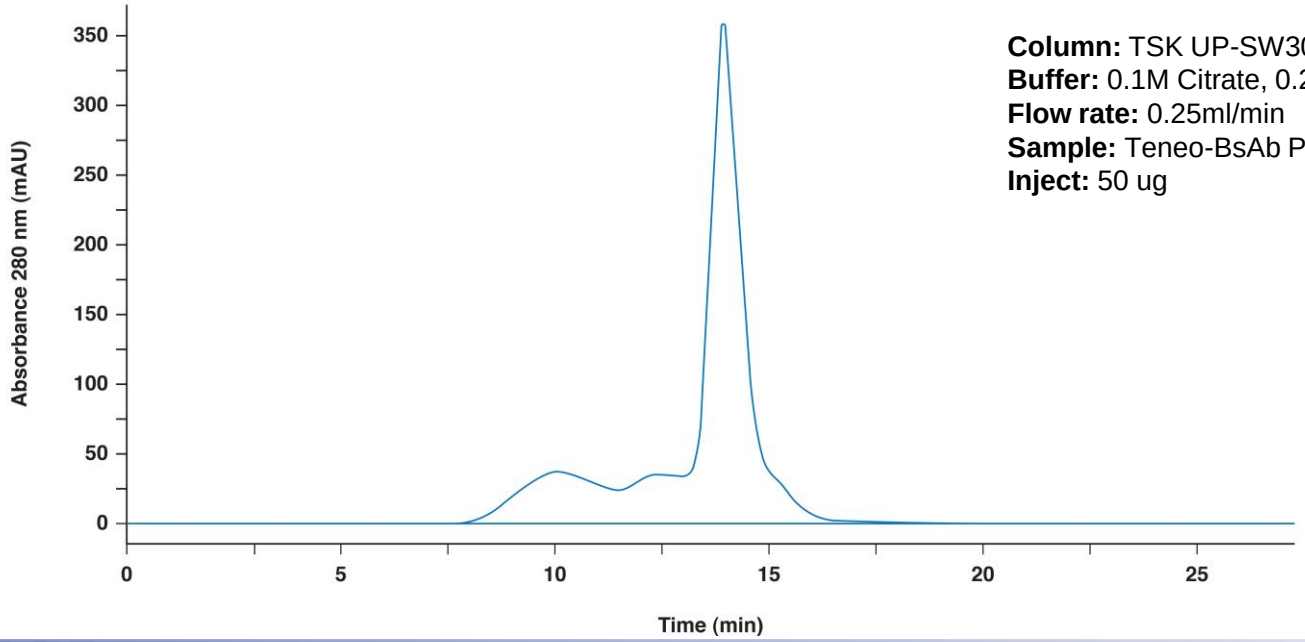
**Result:**
**Eluted peak is 96% of total integrated area**

**Column:** 1ml Mab Select Sure XL HiTrap
**Load:** 50ml Teneo-BsAb HCCF
**Equilibration/Wash Buffer:** 50mM Tris, pH 7.0
**Elution Buffer:** 25mM citric acid, pH 3.6
**Neutralization Buffer:** 1M Tris, pH 9.0



# … But causes Teneo-BsAb to aggregate

**Result:**
**SEC analysis indicates substantial amount of aggregated product after pH 3.6 elution**

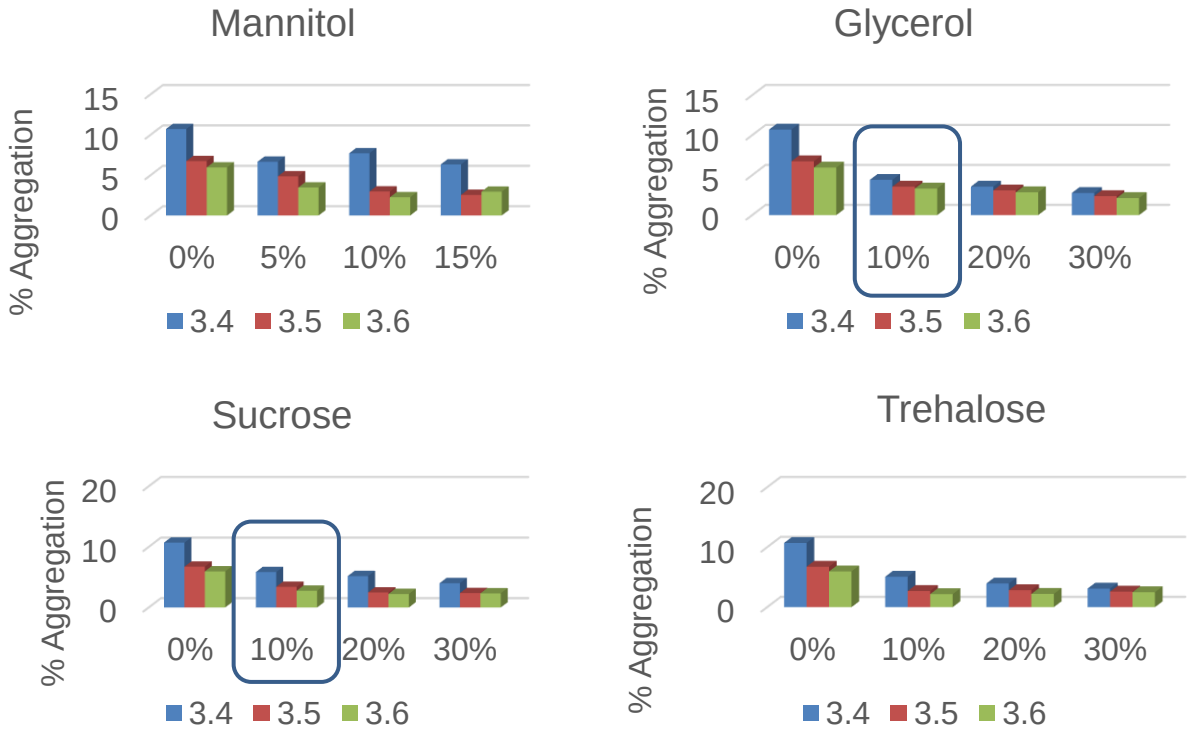



**Column:** TSK UP-SW3000
**Buffer:** 0.1M Citrate, 0.2M Arg, 0.5M NaCl, pH 6.2
**Flow rate:** 0.25ml/min
**Sample:** Teneo-BsAb Prot A eluate pool
**Inject:** 50 ug

# Can additives protect Teneo-BsAb at low pH?

- DOE in GE Predictor Plates
- Elution buffer supplemented with polyols
- Test of three factors
  - Mannitol, glycerol, sucrose, trehalose
  - 5% - 30%
  - pH 3.4, 3.5, 3.6

# Additives can reduce aggregation of Protein A-eluted Teneo-BsAb CD3-TAA



# Summary of issues with Protein A for capture

- Acid lability of Teneo-BsAb
  - Low pH elution will induce aggregation of product even with the addition of polyols to elution buffer
  - Cannot use low pH for virus inactivation
- Copurification of TAA - homodimer

# **Finding a better option:**
CaptureSelect CH1-XL

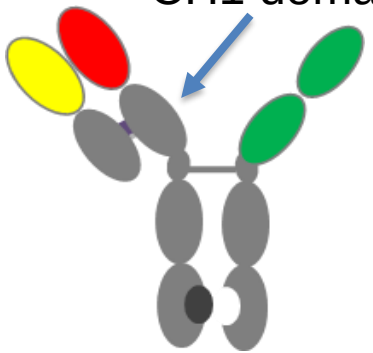
# CaptureSelect CH1-XL

- **General properties**
  - Ig heavy chain CH1-specific nanobody ligand
    - Eifler N., Biotechnol. Prog. 30, 1311-1318, 2014
  - Recognizes all four subclasses of IgG (IgG1, IgG2, IgG3, IgG4)
  - Ligand immobilized on agarose 65 um
  - Binding capacity < 20 mg/mL of IgG
  - Flow 5 – 200 cm/hr
  - Stable to base (25 – 50 mM NaOH) for sanitization
  - Commercially available from Thermo
- **For our specific need**
  - Binds bispecific heterodimer but NOT TAA homodimer
  - Elutes under less stringent acidic (pH 4) condition

# Only Teneo-BsAb contains a CH1 domain

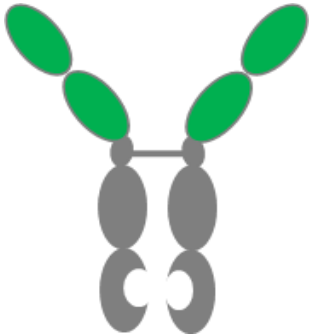
*Active*

CH1 domain



Heterodimer

*Inactive*

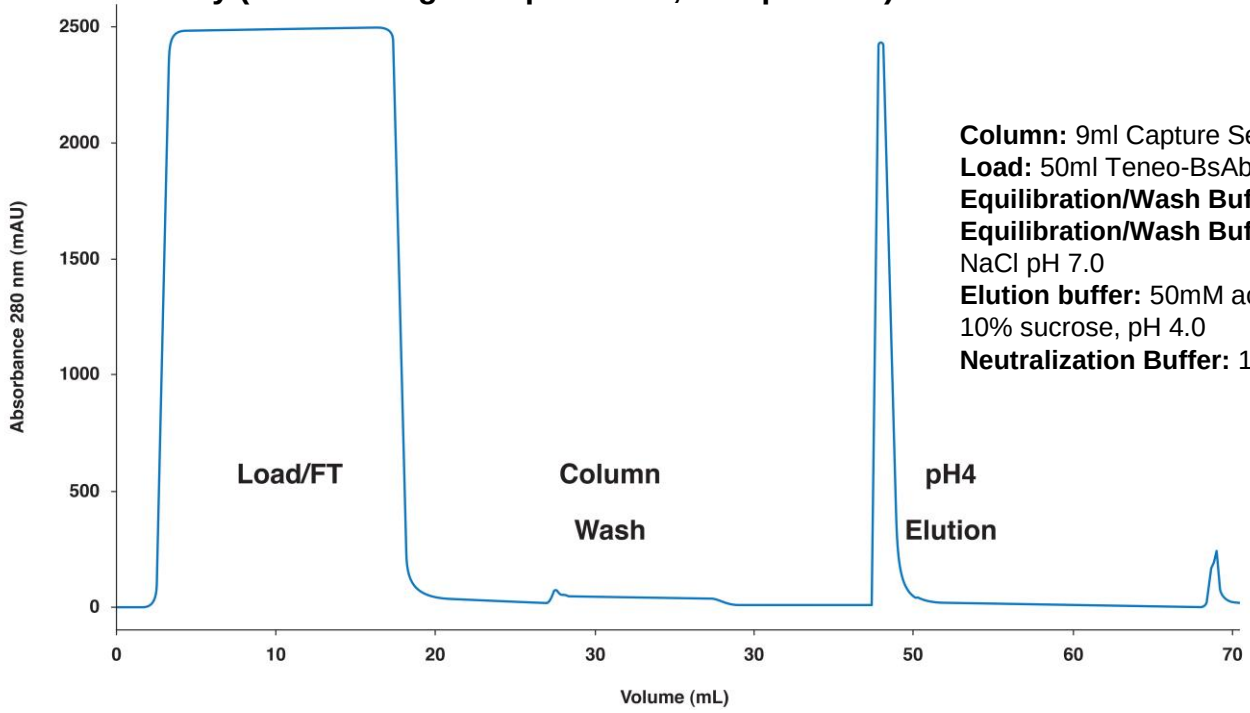


TAA Homodimer

# CaptureSelect CH1-XL elution of Teneo-BsAb at pH 4

**Result:**

**Teneo-BsAb eluted efficiently (93% of integrated peak area, 2CV pool vol)**

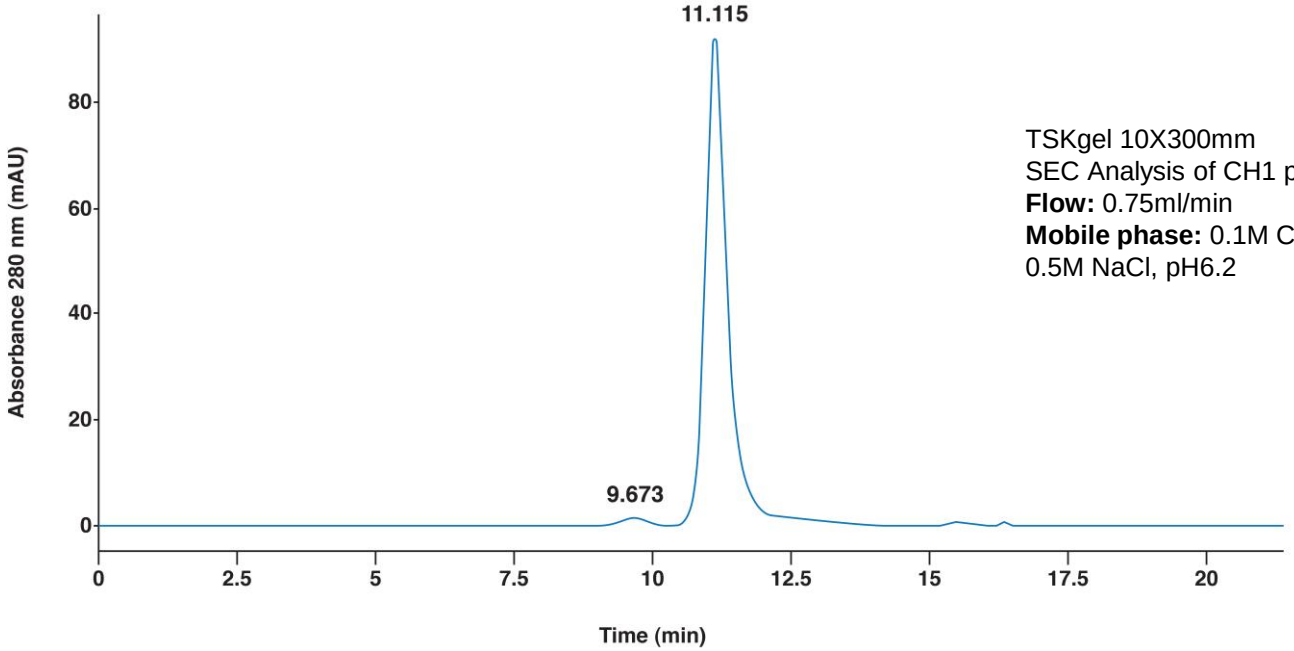



**Column:** 9ml Capture Select
**Load:** 50ml Teneo-BsAb HCCF
**Equilibration/Wash Buffer #1:** 50mM Tris, pH 7.0
**Equilibration/Wash Buffer #2:** 50mM Tris, 0.5M NaCl pH 7.0
**Elution buffer:** 50mM acetic acid, 10% glycerol, 10% sucrose, pH 4.0
**Neutralization Buffer:** 1M Tris, pH 9.0

# Teneo-BsAb eluted from CH1-XL contains minimal aggregates

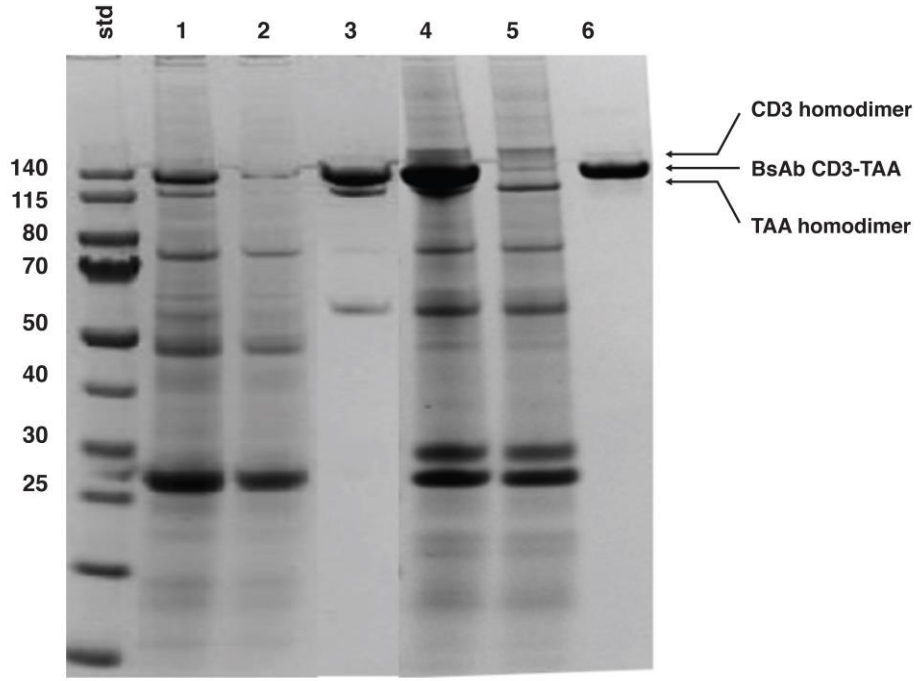
**Result:**

**CaptureSelect CH1-XL pool has HMW content at 2% with efficient binding of product out of HCCF.**




TSKgel 10X300mm
SEC Analysis of CH1 pool
**Flow:** 0.75ml/min
**Mobile phase:** 0.1M Citrate, 0.2M arginine, 0.5M NaCl, pH6.2

# TAA homodimer is separated from BsAb CD3-TAA by CaptureSelect CH1-XL but not by Protein A

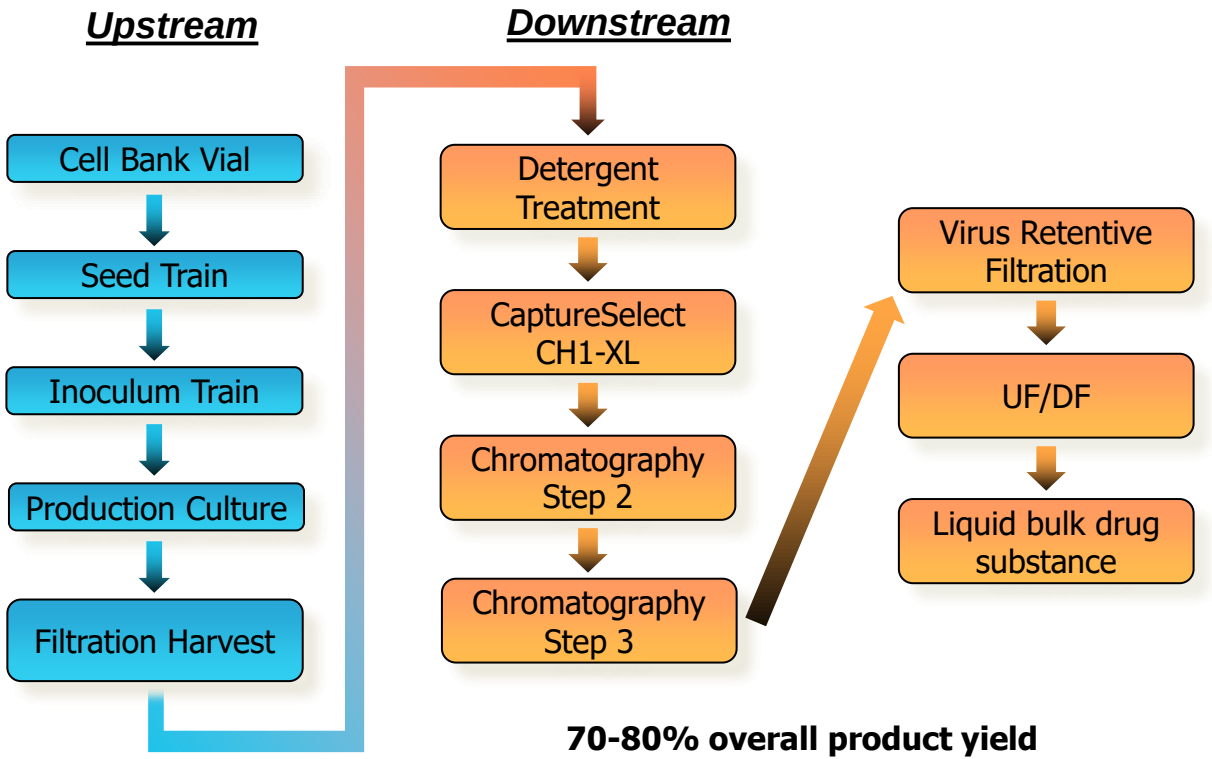



Lanes:

1) Protein A load 10 ul
2) Protein A flow-through 10 ul
3) Protein A pool 2 ug
4) CH1-XL load 10 ul
5) CH1-XL flow through 10ul
6) CH1-XL pool 2 ug

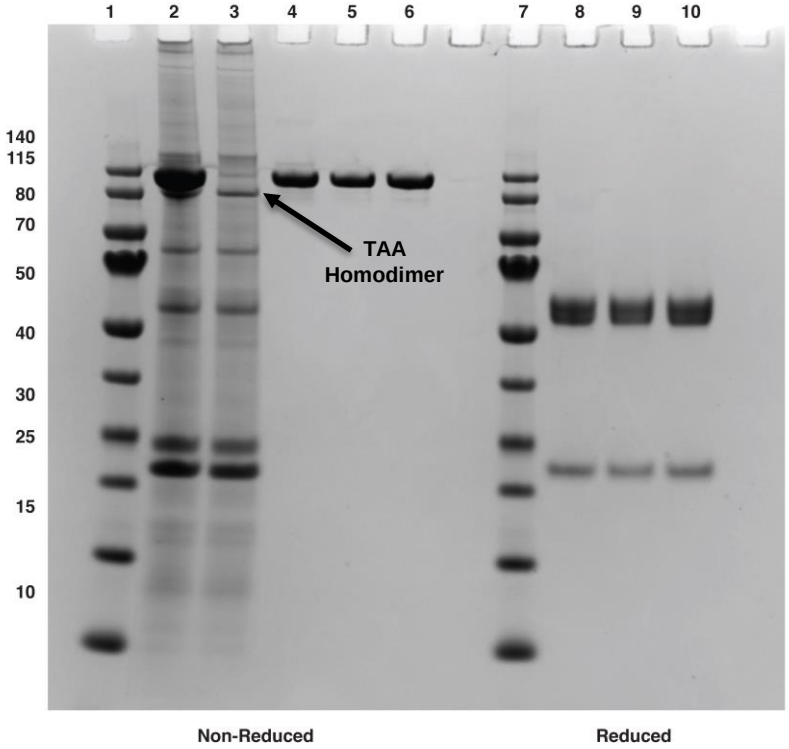
NuPAGE 4-12% Bis-Tris gel
MES Running Buffer
InstantBlue Stain(Expedeon)
PageRuler Prestained Protein Ladder
Run Conditions:
35min.
200V
120mA
25watts

# Process for clinical production of Teneo-BsAb drug substance




**70-80% overall product yield**

# Summary of Teneo-BsAb purification through downstream process




Lanes:

1) MW
2) HCCF 5ul
3) CH1 flow through 5ul
4) CH1-XL1 pool 2ug
5) Int. purification step- pool 2 ug
6) Polishing step- pool 2 ug
7) MW
8) CH1-XL pool 2ug
9) Int. purification step- pool 2 ug
10) Polishing step- pool 2 ug

NuPAGE 4-12% Bis-Tris gel
MES Running Buffer
InstantBlue Stain(Expedeon)
PageRuler Prestained Protein Ladder
Run Conditions:
35min.
200V
120mA
25watts

# Summary

- Teneo-BsAb is a CD3-TAA bispecific antibody that is trimeric, containing two H and one L chain
- We initially tested Protein A to capture
  - Low pH sensitivity of Teneo-BsAb was problematic
  - Copurification of TAA homodimer created additional downstream challenges
- CaptureSelect CH1-XL was identified as a better option
  - Mild elution condition (pH 4)
  - Good capacity (< 15 mg/mL)
  - High recovery (> 85% step yield)
  - However… low pH hold of eluted pool not an option for virus inactivation
- CaptureSelect could be more generally useful for bispecific constructs containing CH1 domains

# Questions

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