Bioproduction Congress
Dublin, Ireland
Tuesday, 09 October 2018

An alternative to Protein A for capture of Fc-containing bispecific antibodies

Steven M. Chamow[1], Brett Jorgensen[2] and Ute Schellenberger[2]

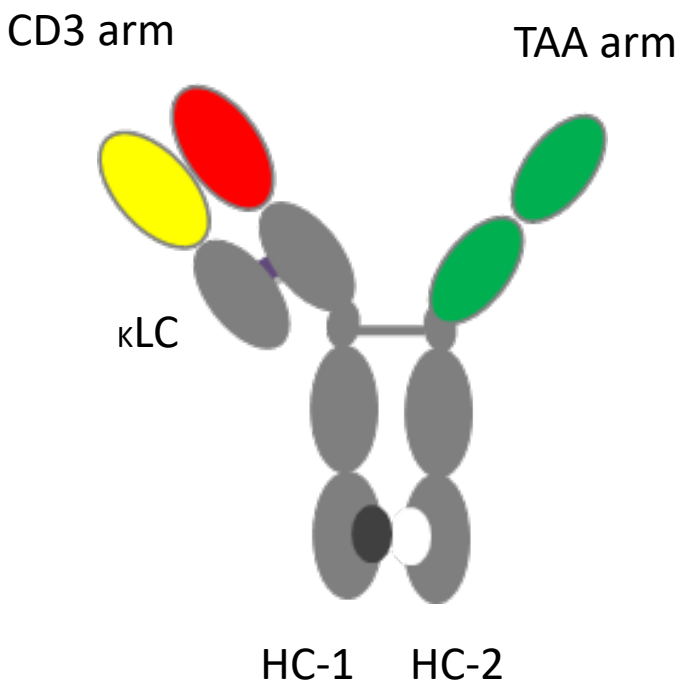
[1]Chamow & Associates, Inc., San Mateo, CA
[2]Teneobio, Inc., Menlo Park, CA

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



TNB-BsAb CD3-TAA bispecific antibody

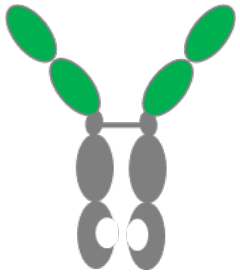
- Fully human IgG4 bispecific monoclonal antibody
  - 2 heavy chains (HC-1 and HC-2) and 1 kappa light chain (κLC)
  - Knobs into holes technology
  - Acid labile
- CD3 arm
  - HC-1 + κLC
  - T-cell receptor CD3
- TAA arm
  - HC-2 only
  - Consists of 2 identical VH domains recognizing TAA
  - Bivalent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

Teneo-BsAb CD3-TAA expressed products

Active

Heterodimer > 90%

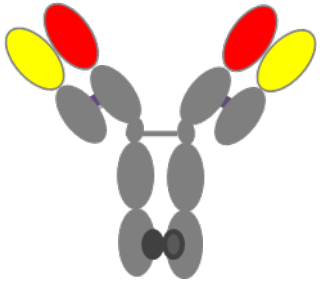
Inactive



TAA Homodimer < 5%

Half-Ab < 1%

Aggregates <2%



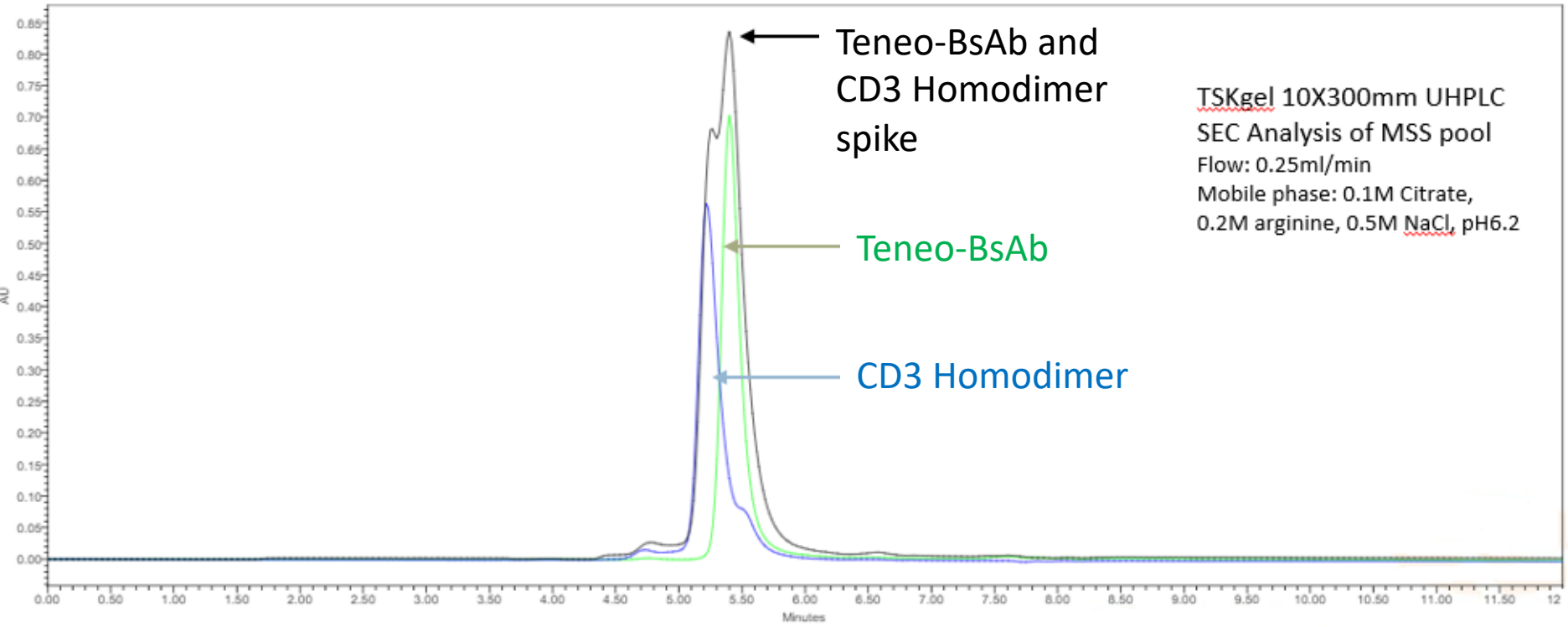
CD3 Homodimer < 5%

Excess LC

CHAMOW & Associates

Biopharmaceutical Product Development

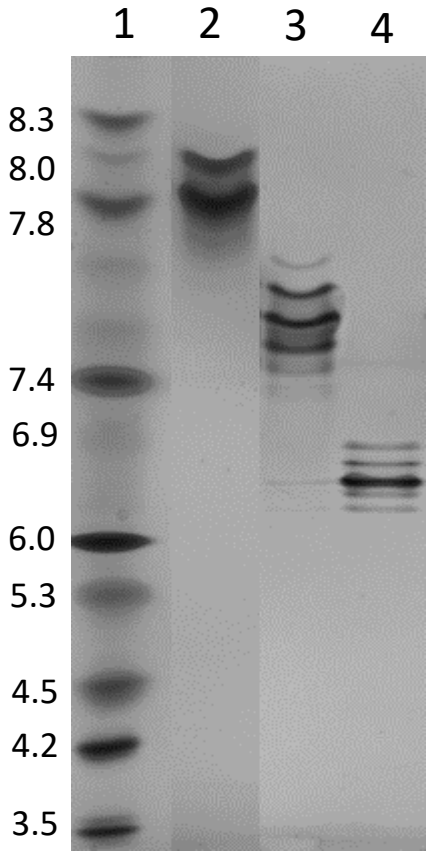
Teneo-BsAb heterodimer is similar in size to CD3 homodimers



CHAMOW & Associates

Biopharmaceutical Product Development

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



Lanes:

1) IEF, pI stds
2) CD3 Homodimer(knob-knob), pI = 8
3) Teneo-BsAb (Heterodimer) pI = 7.4-7.6
4) TAA Homodimer(hole-hole), pI = 6.2

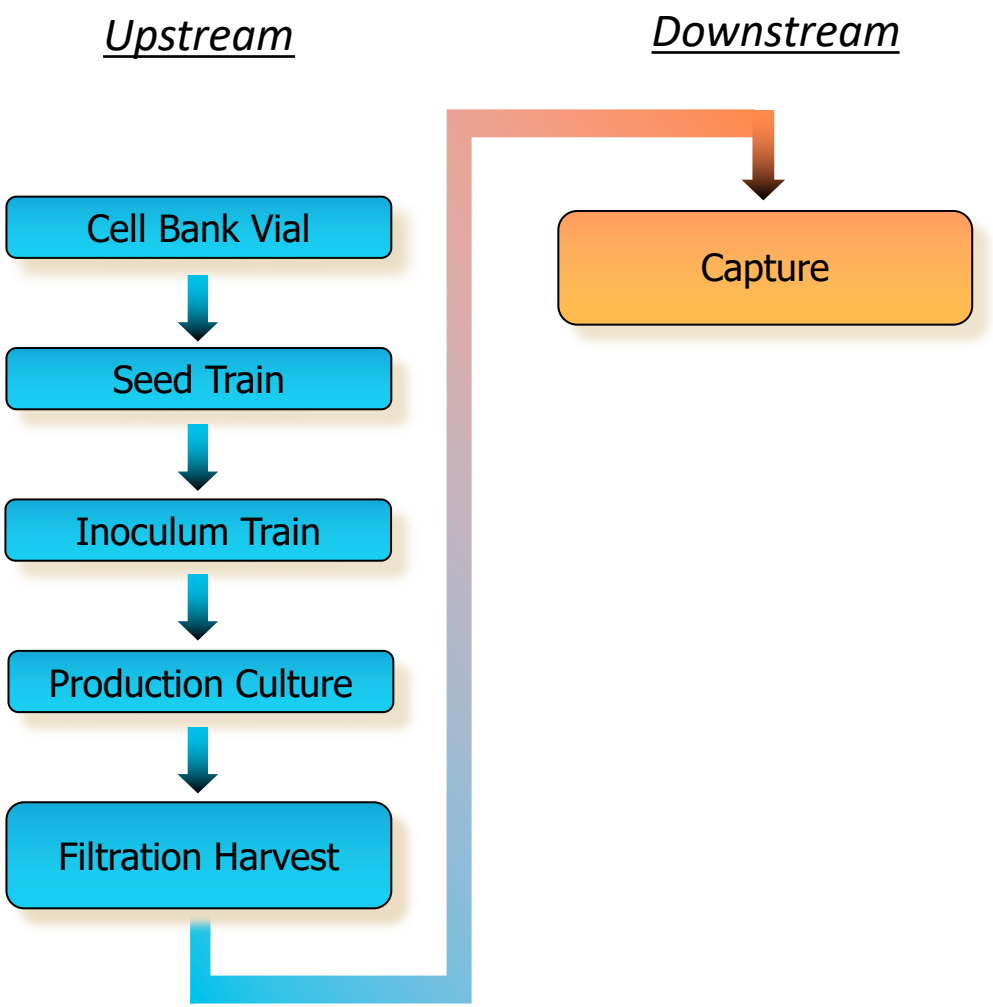
5ug/lane loaded

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

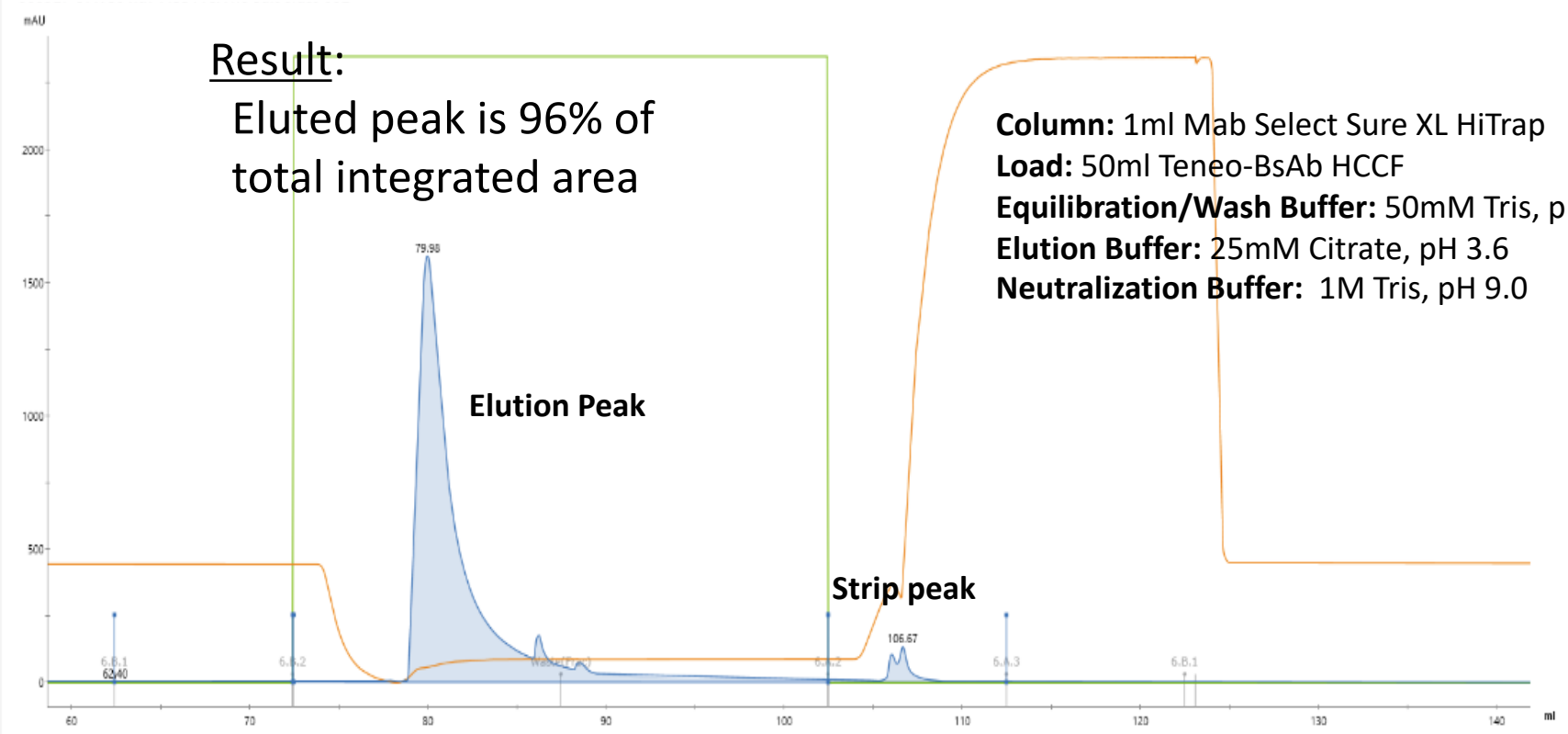


CHAMOW & Associates
Biopharmaceutical Product Development

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

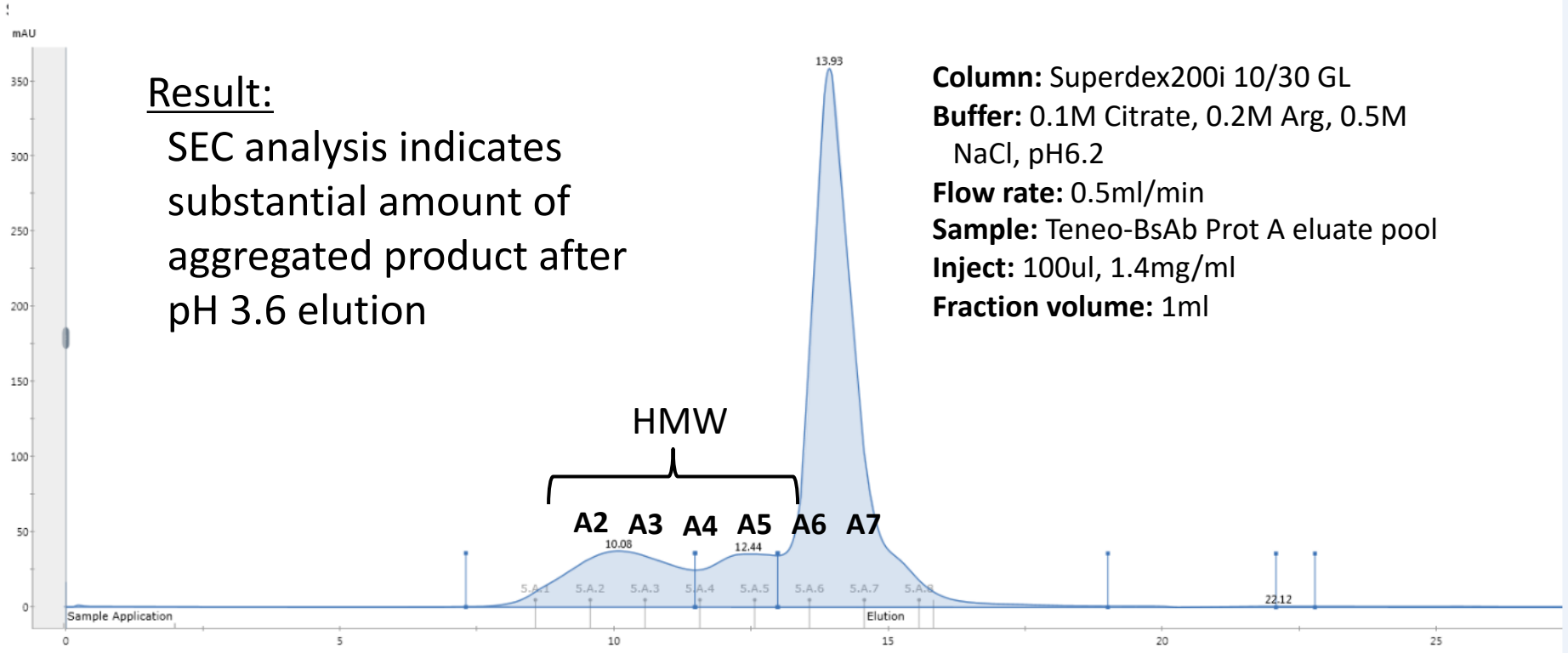
Column: 1ml Mab Select Sure XL HiTrap
Load: 50ml Teneo-BsAb HCCF
Equilibration/Wash Buffer: 50mM Tris, pH 7.0
Elution Buffer: 25mM Citrate, pH 3.6
Neutralization Buffer: 1M Tris, pH 9.0

Peak Table - UV

Peak	Retention ml	Area mAU*ml	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume ml	Conductivity mS/cm
Peak A	62.404	5.214	0.12		6.B.1	10.002	4.47
Peak B	79.983	4093	96.02		6.B.3 - 6.A.1	30.000	1.17
Peak C	106.669	164.6	3.86		6.A.2	9.997	11.73

CHAMOW & Associates
Biopharmaceutical Product Development

… But causes Teneo-BsAb to aggregate



Peak Table - UV

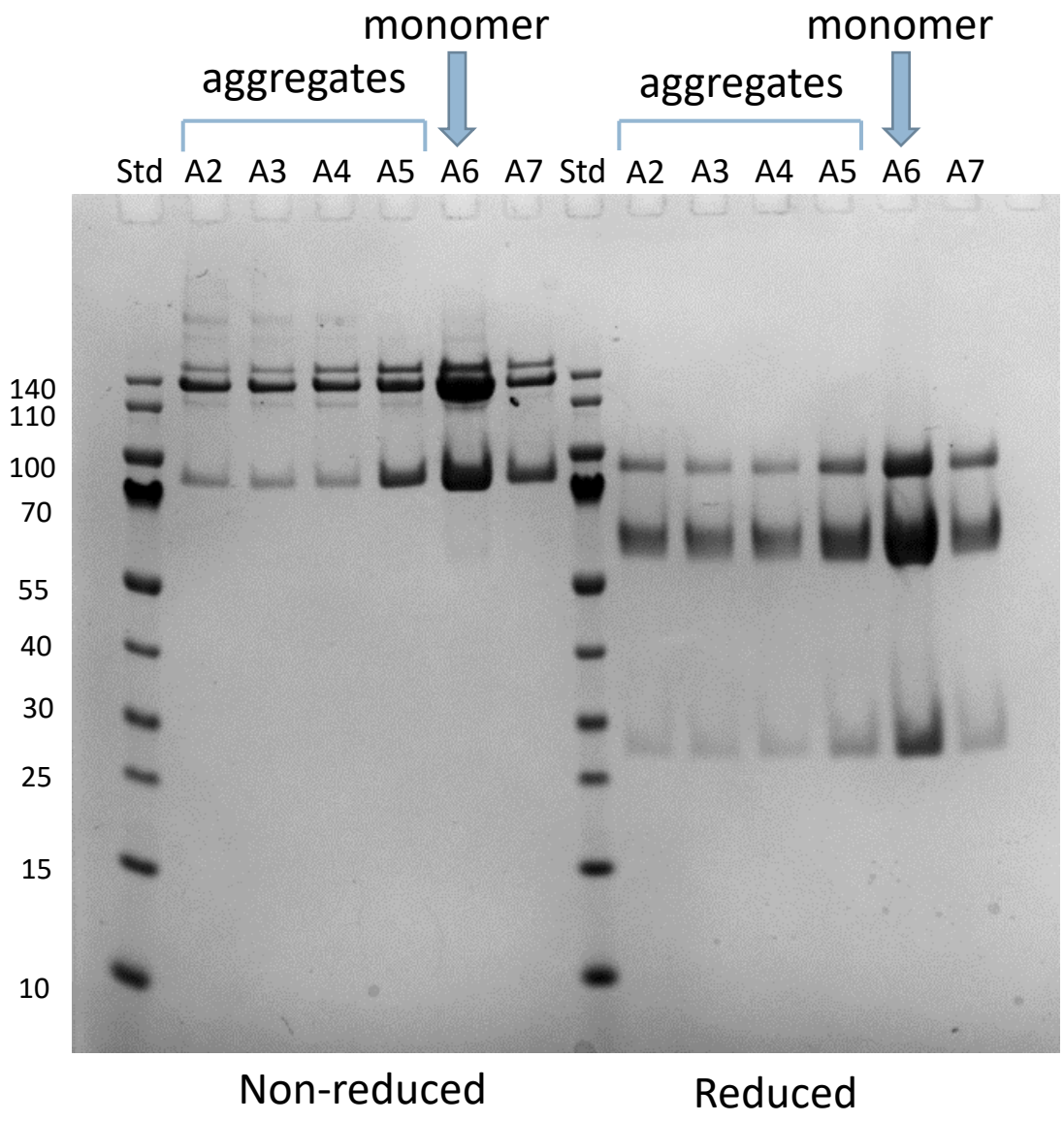
Peak	Retention ml	Area ml*mAU	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume ml	Conductivity mS/cm
Peak A	10.078	86.39	18.1		5.A.3	4.180	53.24
Peak B	12.444	48.28	10.11		5.A.3 - 5.A.5	1.508	53.23
Peak C	13.930	342.4	71.71		5.A.5 - Waste(Frac)	6.024	53.27
Peak D	22.118	0.3942	0.08		Waste(Frac)	0.713	53.20

Result:

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

Column: Superdex200i 10/30 GL
Buffer: 0.1M Citrate, 0.2M Arg, 0.5M NaCl, pH6.2
Flow rate: 0.5ml/min
Sample: Teneo-BsAb Prot A eluate pool
Inject: 100ul, 1.4mg/ml
Fraction volume: 1ml

SDS-PAGE confirms that HMW fractions are product



Protein A fractions

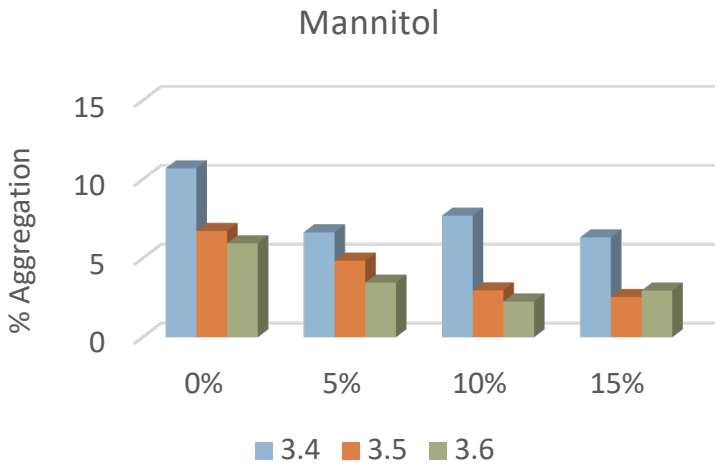
4-12% NuPAGE Gel
MES running buffer
5ug/lane load
Page Ruler Pre-----stain Markers(Thermo)
Coommasie Stained

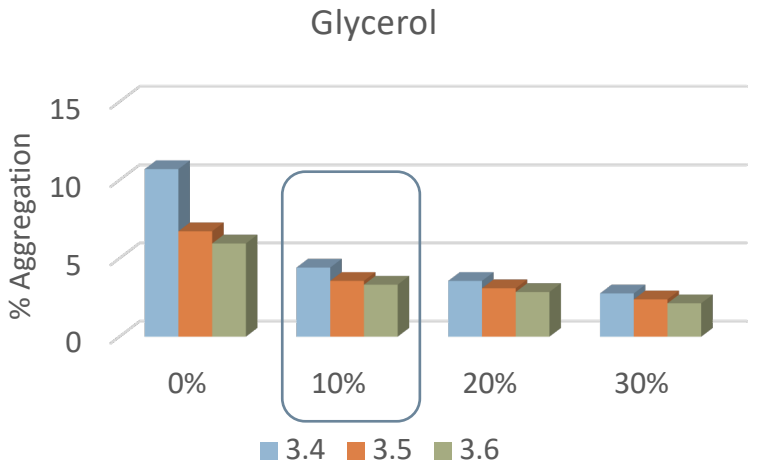
CHAMOW & Associates
Biopharmaceutical Product Development

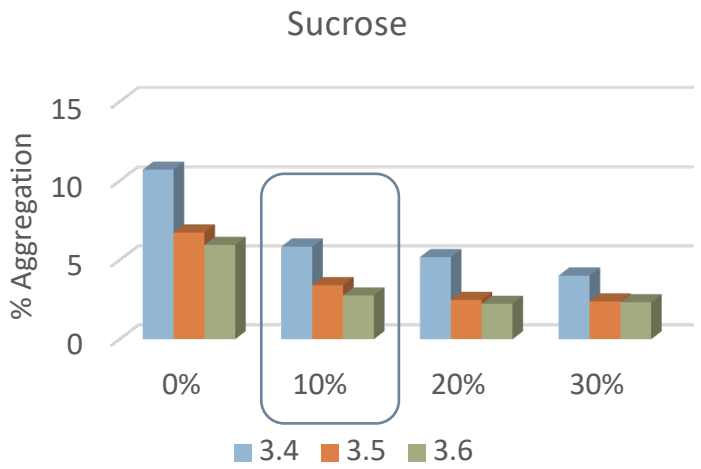
Can additives protect Teneo-BsAb at low pH?

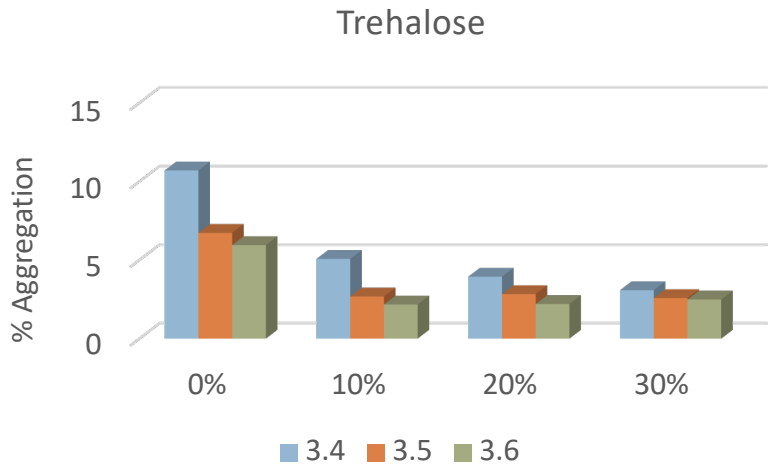
- DOE in GE Predictor Plates
- Elution buffer supplemented with polyols
- Test of three factors
  - Mannitol, glycerol, sucrose, trehalose
  - 5% - 30%
  - pH 3.4, 3.5, 3.6

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

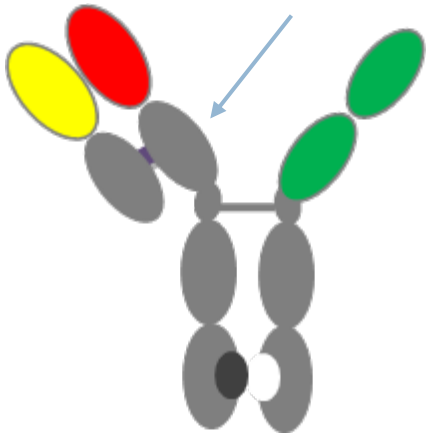
CaptureSelect CH1-XL

- 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

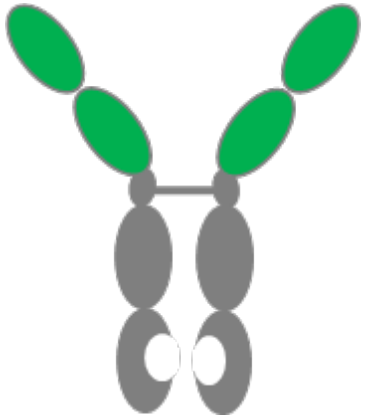
Active

CH1 domain



Heterodimer

Inactive

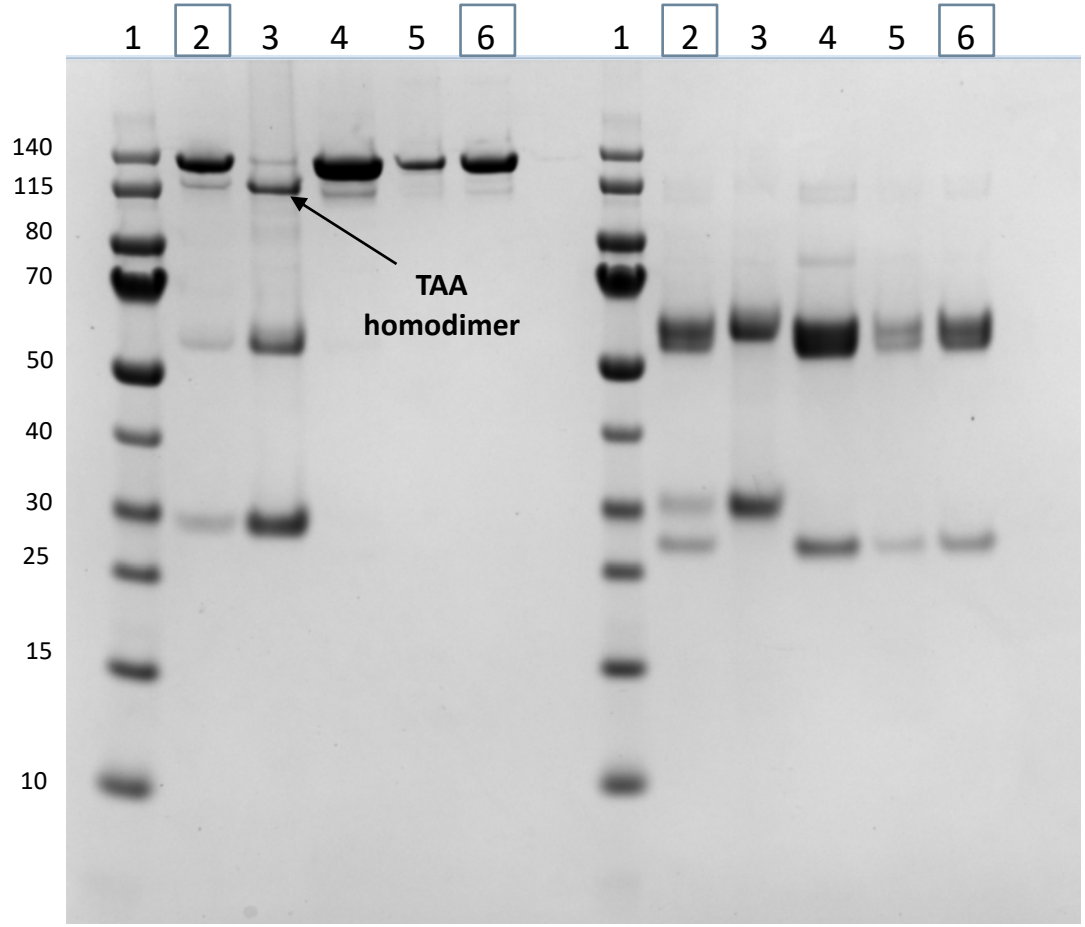


TAA Homodimer

CHAMOW & Associates

Biopharmaceutical Product Development

Teneo-BsAb Protein A and CaptureSelect CH1 pools compared



Lanes:

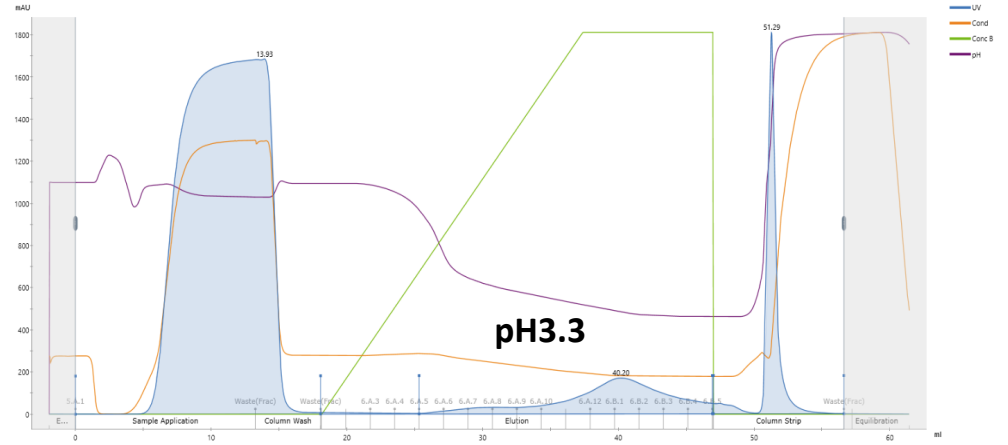
1) MW stds:
2) Bispec.IgG ProA pool
3) Bispec.IgG CH1 **Flow trough**
4) CH1 salt wash:
5) CH1 NaOH strip:
6) CH1 pool:

Protein load: 2ug/lane
NuPAGE 4-12% Bis-Tris gel
MES Running Buffer
InstantBlue Stain (Expedeon)
PageRuler Prestained Protein Ladder
Run Conditions:
35min.
200V
120mA
25watts

<u>Result</u>: TAA homodimer is present in the CH1-XL flow through

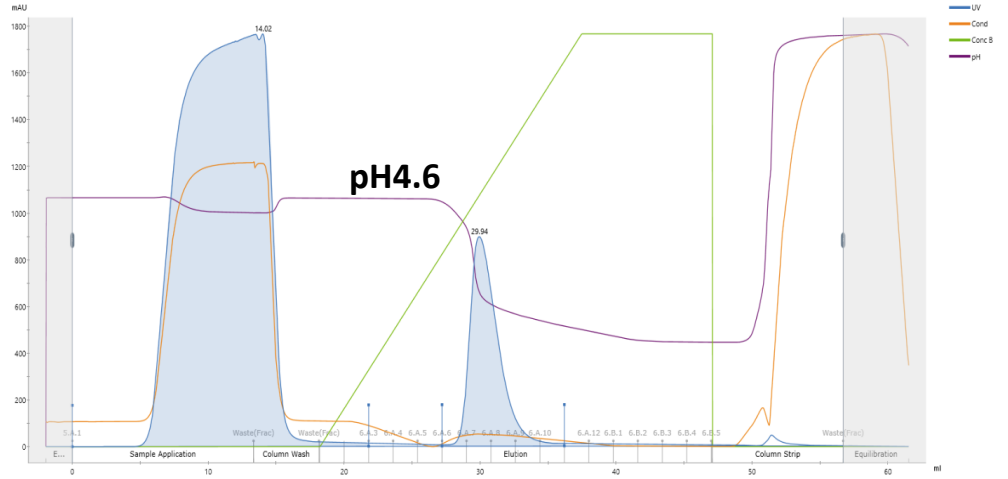
CHAMOW & Associates
Biopharmaceutical Product Development

Comparison of the elution pH of capture media



Peak Table - UV

Peak	Retention ml	Area mAU*ml	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume ml	Conductivity mS/cm
Peak A	13.929	12856	82.14		5.A.1 - 5.A.2	18.066	8.14
Peak B	40.197	1439	9.19		6.A.5 - 6.B.4	21.601	2.72
Peak C	51.288	1357	8.67		5.A.3	9.624	11.36



Peak Table - UV

Peak	Retention ml	Area mAU*ml	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume ml	Conductivity mS/cm
Peak A	14.016	13516	86.33		5.A.1 - 6.A.2	21.805	8.03
Peak B	29.938	2140	13.67		6.A.6 - 6.A.10	9.000	2.75

Column: 1ml Mab Select SuRe column
Buffers:
A- 50mM Tris, pH7.0
B-50mM Acetate, pH3.0
C- Strip Buffer: 0.1M NaOH
Elution: Linear grad. 10CV – 100%B
10ml HCCF load

Column: 1ml CaptureSelect CH1-XL
Buffers:
A- 50mM Tris, pH7.0
B-50mM Acetate, pH3.0
C- Strip Buffer: 0.1M NaOH
Elution: Linear grad. 10CV – 100%B
10ml HCCF load

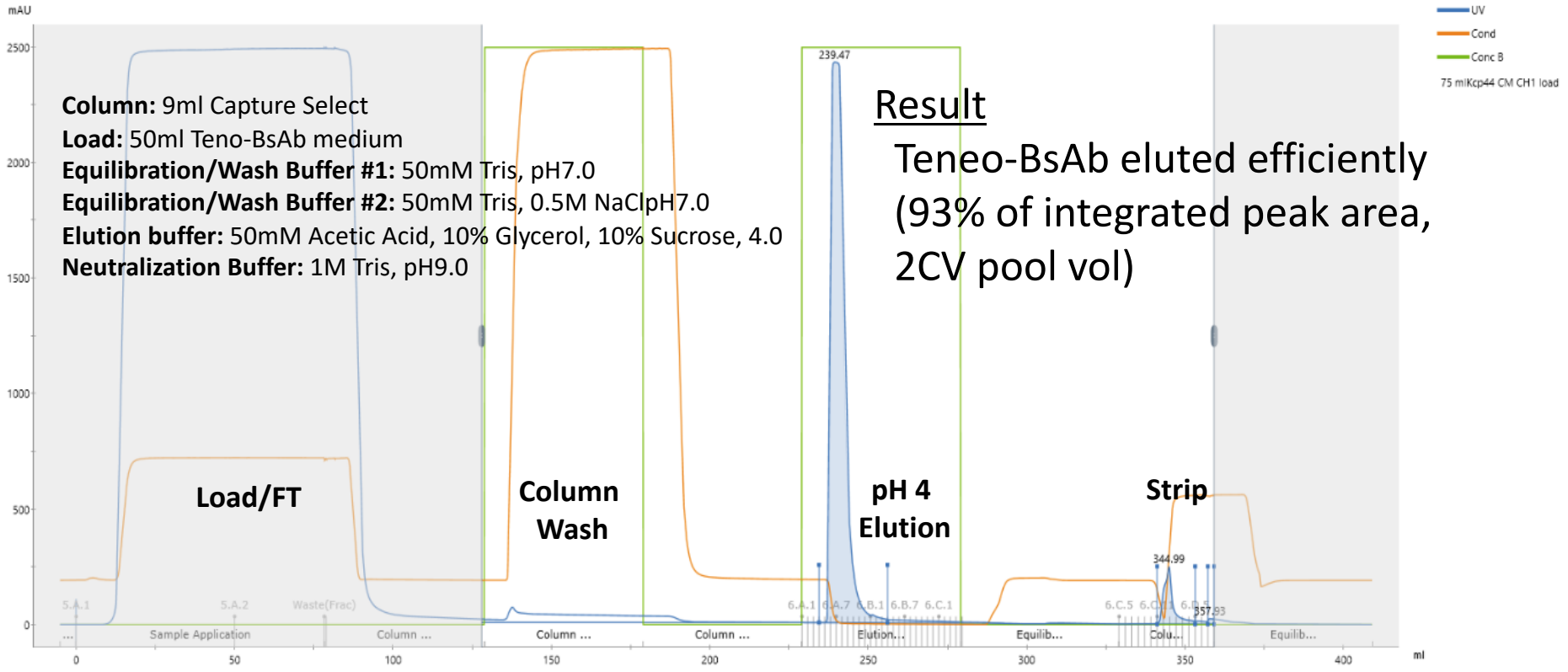
CHAMOW & Associates
Biopharmaceutical Product Development

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

Column: 9ml Capture Select
Load: 50ml Teno-BsAb medium
Equilibration/Wash Buffer #1: 50mM Tris, pH7.0
Equilibration/Wash Buffer #2: 50mM Tris, 0.5M NaClpH7.0
Elution buffer: 50mM Acetic Acid, 10% Glycerol, 10% Sucrose, 4.0
Neutralization Buffer: 1M Tris, pH9.0

Result

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

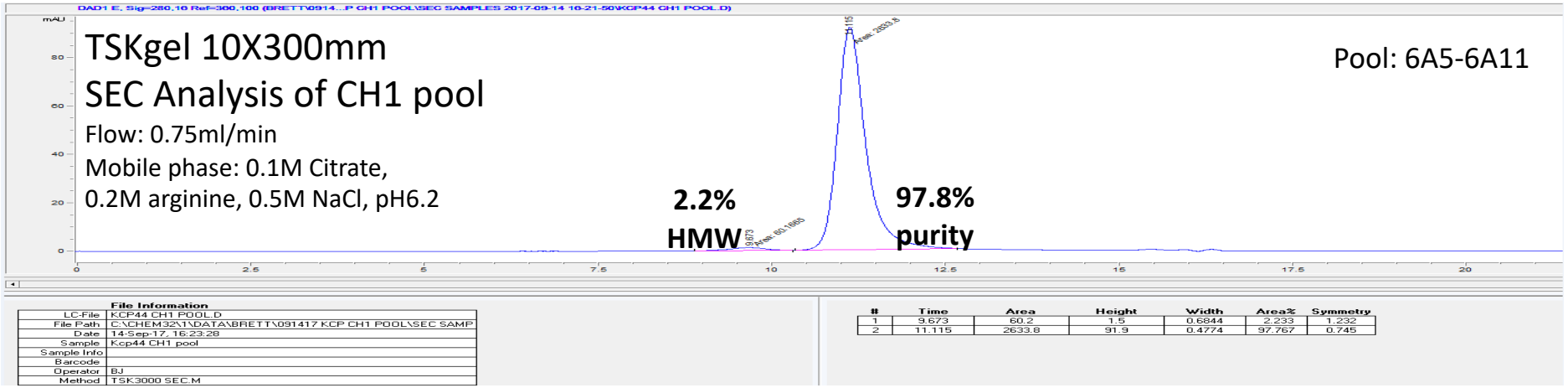


Peak Table - UV

Peak	Retention ml	Area mAU*ml	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume ml	Conductivity mS/cm
Peak A	239.465	12113	93.21		6.A.4 - 6.B.3	21.602	1.31
Peak B	344.987	839.3	6.46		6.C.11 - 6.D.4	12.000	8.67
Peak C	357.934	43.83	0.34		6.D.7 - 6.D.8	2.009	11.86

CHAMOW & Associates
Biopharmaceutical Product Development

Teneo-BsAb eluted from CH1 XL contains minimal aggregates



Result

CaptureSelect CH1-XL pool has low HMW content at 2.2% with efficient binding of product out of HCCF.

CHAMOW & Associates
Biopharmaceutical Product Development

Preliminary dynamic binding capacity of CaptureSelect CH1-XL for Teneo-BsAb

Residence Time (min)	Dynamic Binding Capacity (mg/mL)
1	3.6
2	8.4
4	9.3
8	9.4

1ml CH1-XL column (0.7 X 2.5cm)
Load: Purified Teneo-BsAb 5mg/ml
Res. Times: 1,2,4,8 min
10% Breakout before elution
P.C.: by 280nm of pool

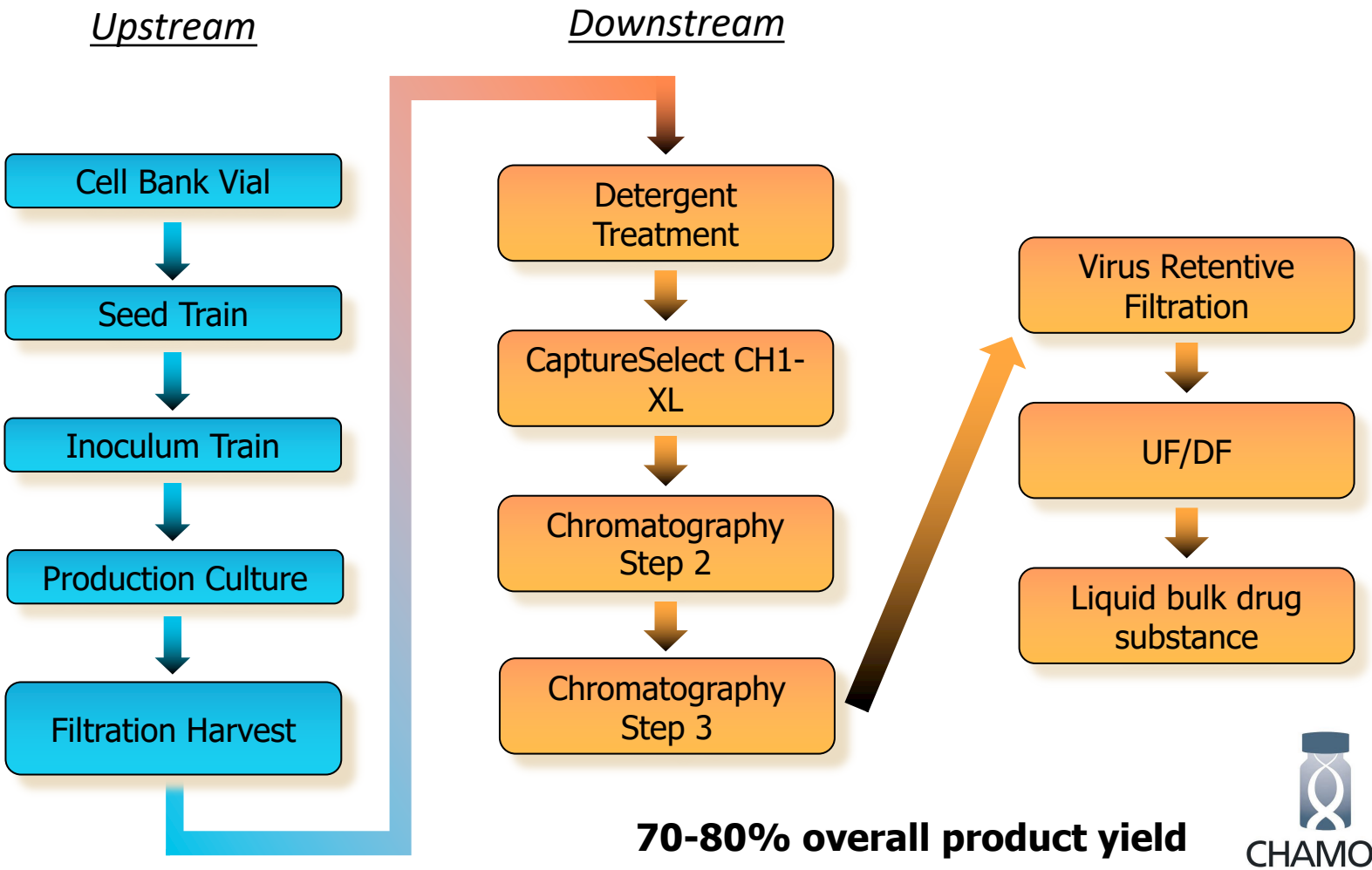
Result

DBC plateaus at 4 min (9.3 mg/mL). Set minimum 4 minute residence time during loading of CaptureSelect CH1-XL.

NOTE:

Subsequent pilot-scale work using HCCF as load demonstrated the potential to increase load density (<19 mg/mL).

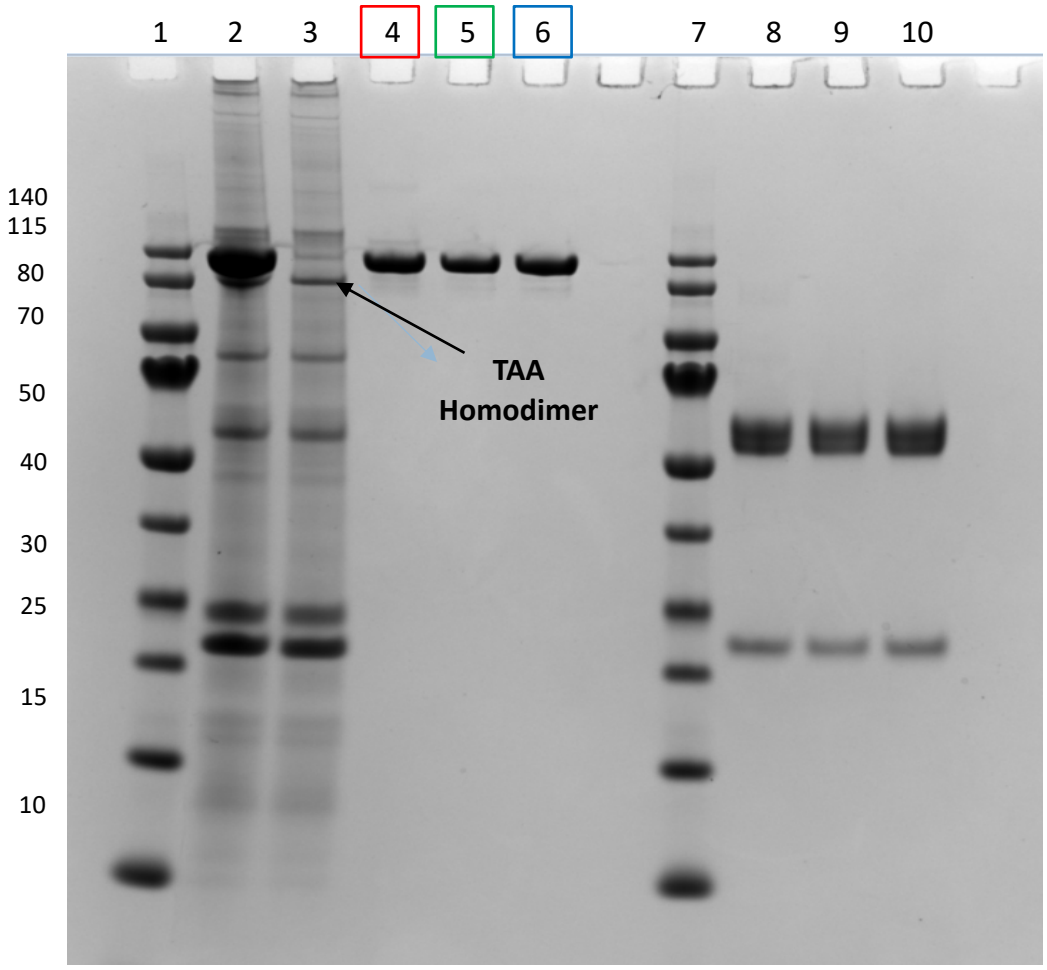
Process for clinical production of Teneo-BsAb drug substance



70-80% overall product yield

CHAMOW & Associates
Biopharmaceutical Product Development

Summary of Teneo-BsAb purification through downstream process



Lanes:

1) MWS
2) HCCF 5ul
3) CH1 Flow through 5ul
4) CH1-XL1 pool 2ug
5) Purification step 2- pool 2 ug
6) Purification step 3- pool 2 ug
7) MWS
8) CH1 pool 2ug reduced
9) Purification step 2- pool 2 ug
10) Purification step 3- pool 2 ug

NuPAGE 4-12% Bis-Tris gel
MES Running Buffer
InstantBlue Stain(Expedeon)
PageRuler Prestained Protein Ladder
Protein load: 2ug/lane
Run Conditions:

35min.
200V
120mA
25watts

CHAMOW & Associates
Biopharmaceutical Product Development

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

Summary (cont'd.)

- 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

Thank you!

Steven Chamow, PhD
steve@chamowassociates.com
(650) 274-3753

CHAMOW
& Associates
Biopharmaceutical Product Development