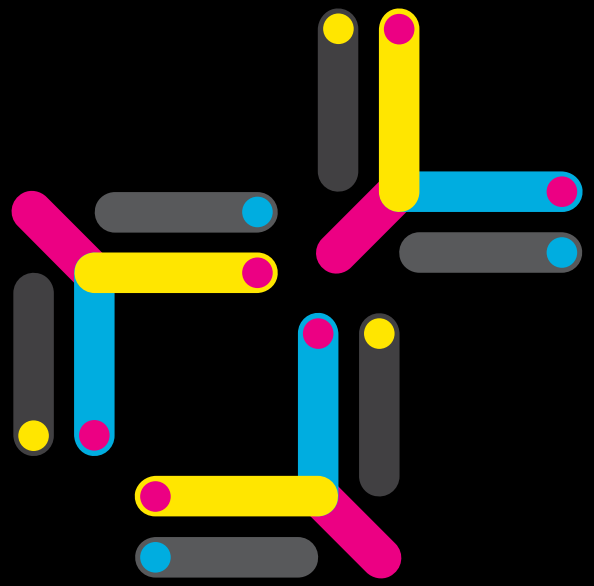


atum.bio

Developability Analytics

Contact us:

info@atum.bio

+1-877-DNA-TOGO

+1-650-853-8347

ATUM

Developability Analytics

Package 1
in silico predictions

- Mol. wt.
- Isoelectric point (PI)
- N-Glycans
- Hydrophobicity
- Sequence liabilities (free cysteines, deamidation, acid lability, isomerization)

Epitope Analyis

Germline Match

Package 2
Discovery stage

- Identity and purity
  - SEC-HPLC
  - µCE-SDS
- Aggregation propensity
  - AC-SINS
  - PIPS assay
- Thermostability
  - Tm
- Polyspecificity
  - BVP-ELISA

Package 3
Stability

- pH stress
- Thermal stress
- Freeze thaw stress
- Agitation stress

Readout:

- SEC-HPLC
- µCE-SDS

High-throughput ≤ 96 samples

<10 samples

Additional Analytics include Cell-based activity assays, FcγRI interaction assay for ADCC, FcRn interaction for mAb recycling, Binding kinetics, Formulation and Concentration

ATUM

Developability Analytics

Example

3 commercially available therapeutic antibodies were analyzed to highlight developability analytics:

- Nivolumab - human IgG4 mAb blocks PD-1; used in treatment of different cancer types
- Bevacizumab - humanized IgG1 mAb blocks VEGF-A; used in treatment of different cancer types
- Vesencumab - human IgG1 mAb blocks NRP-1; used in treatment of solid tumors

ATUM

in silico Predictions

Motif Recognition in a Sequence

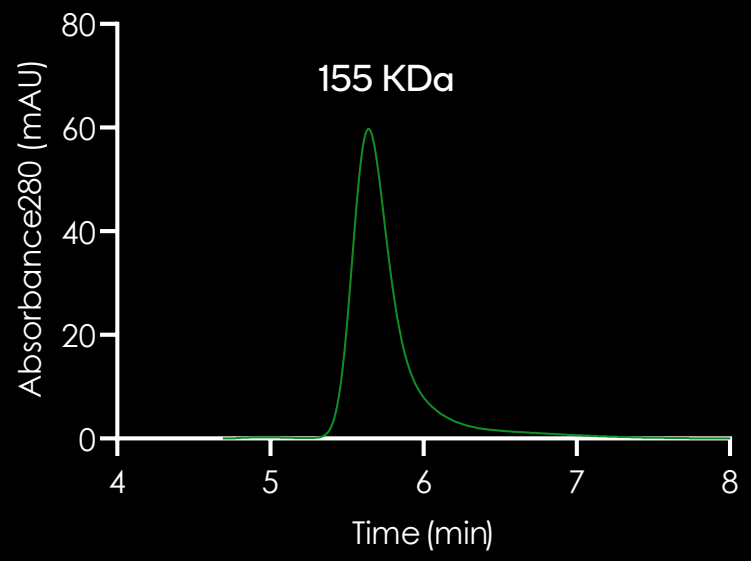
in silico Analysis	Nivolumab	Bevacizumab	Vesencumab
Mol. wt. (MW)	143653	146597	145263
Isoelectric point (PI)	7.92	8.09	8.53
N-Glycans*	289, N/A	302, N/A	302, N/A
GRAVY - Hydrophobicity*	-0.41, -0.44	-0.40, -0.44	-0.36, -0.44
Number of Cysteines	16	16	16

**Heavy chain, Light chain (H,L)*
N-Glycans - N-X-S/T motif (X is any amino acid except proline)
Positive GRAVY values indicate hydrophobic, negative values indicate hydrophilic
Cysteines - Could be a potential issue to folding and cause aggregation

ATUM

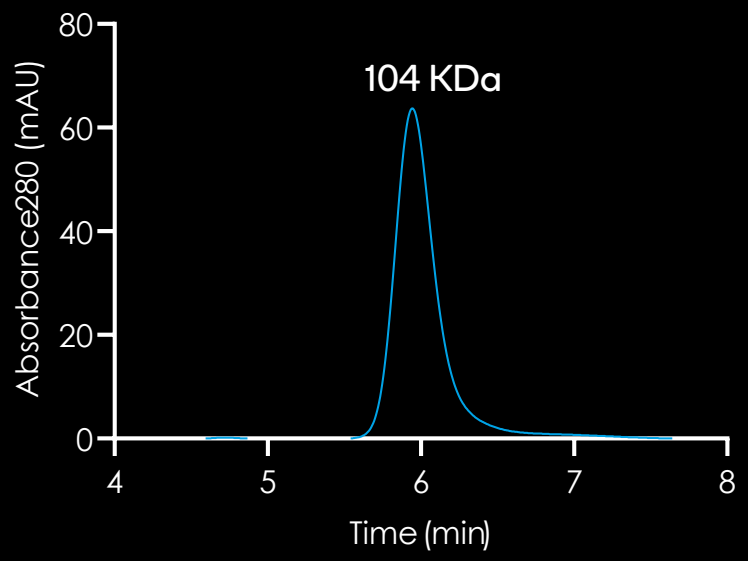
Identity and Purity: SEC-HPLC

Nivolumab



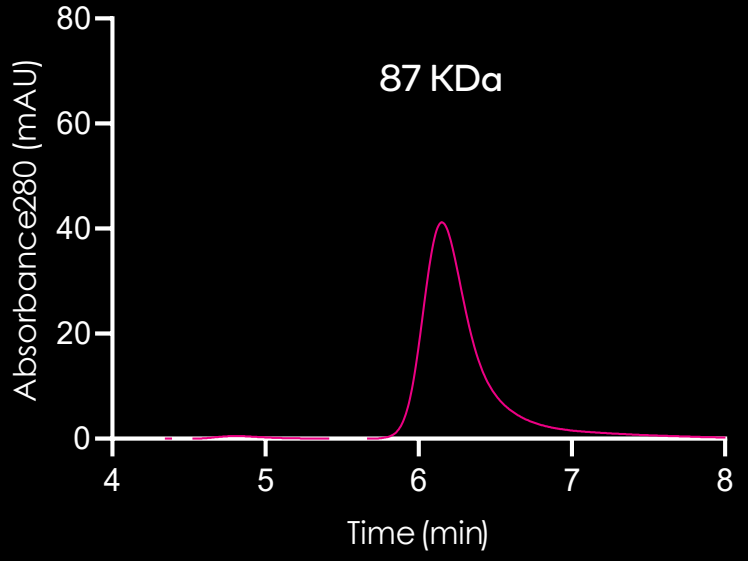
Peak Size (KDa)	Area %
155	100

Bevacizumab



Peak Size (KDa)	Purity %
104	100

Vesencumab

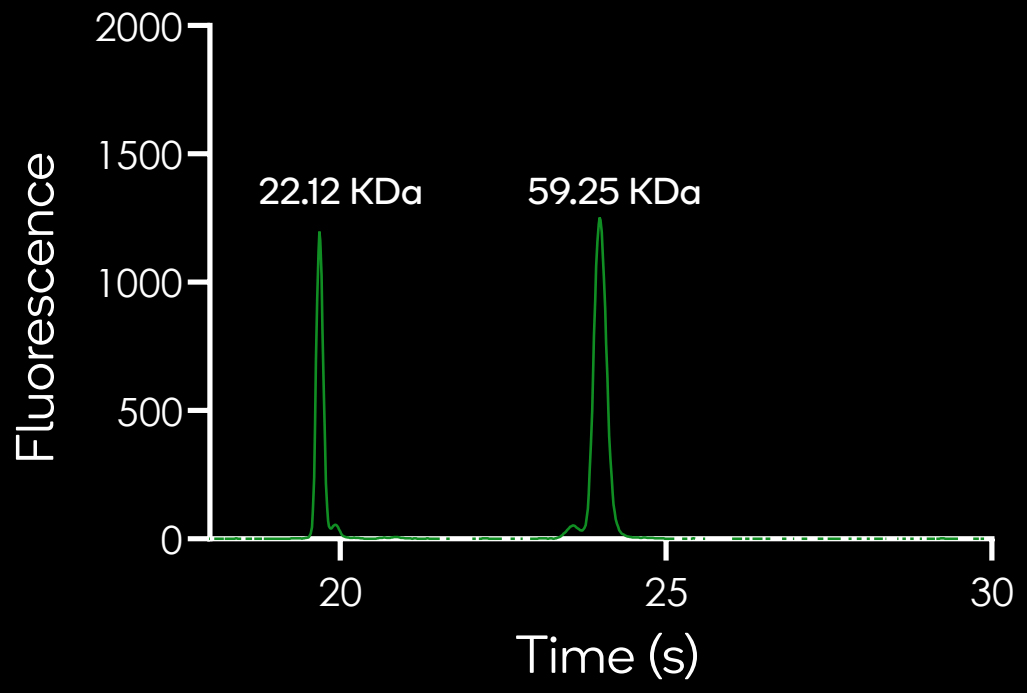


Peak Size (KDa)	Purity %
87	100

ATUM

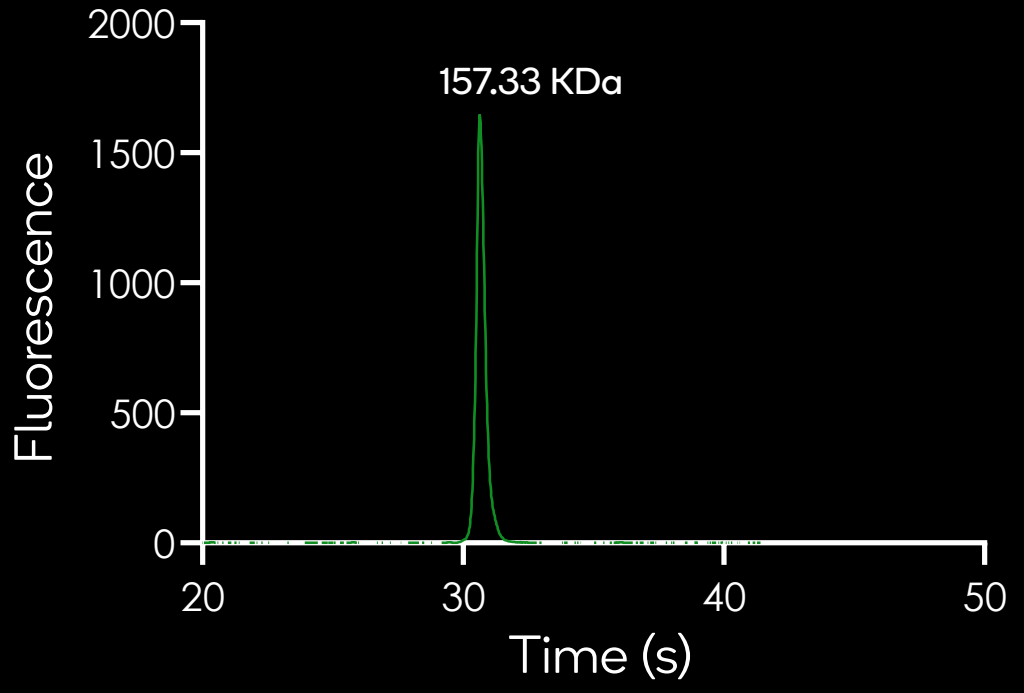
Identity and Purity: µCE-SDS

Nivolumab (Reduced)



Peak Size (KDa)	Purity %
22.12	37.98
23.72	1.93
54.98	2.50
59.25	57.57

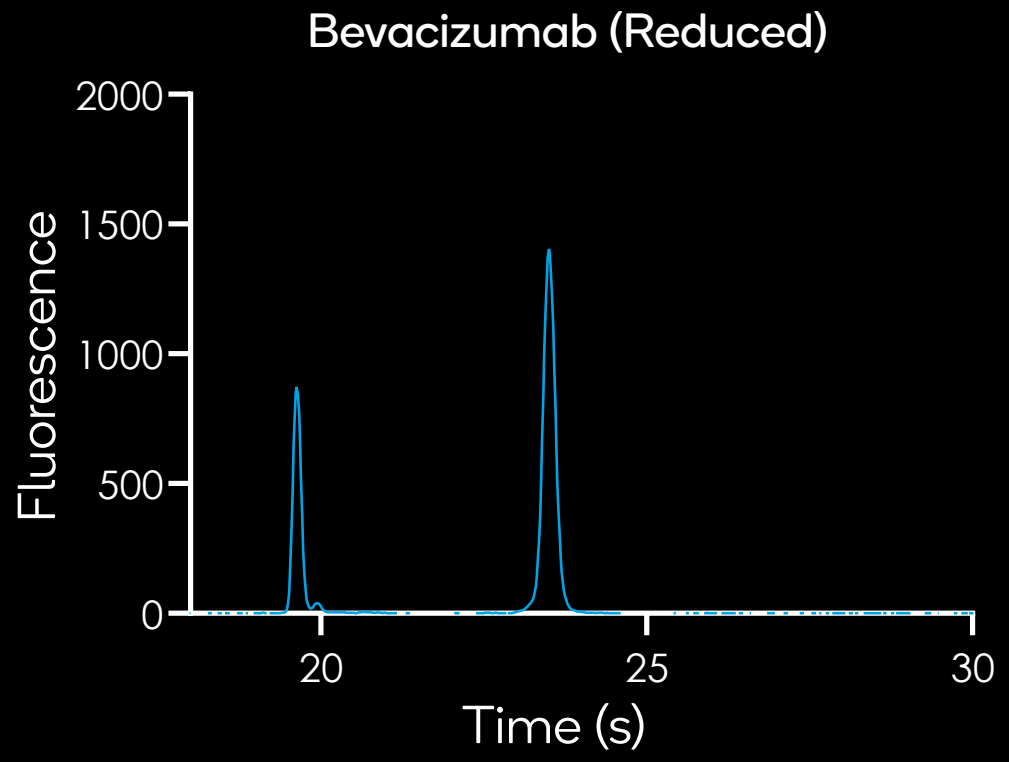
Nivolumab (Non-Reduced)



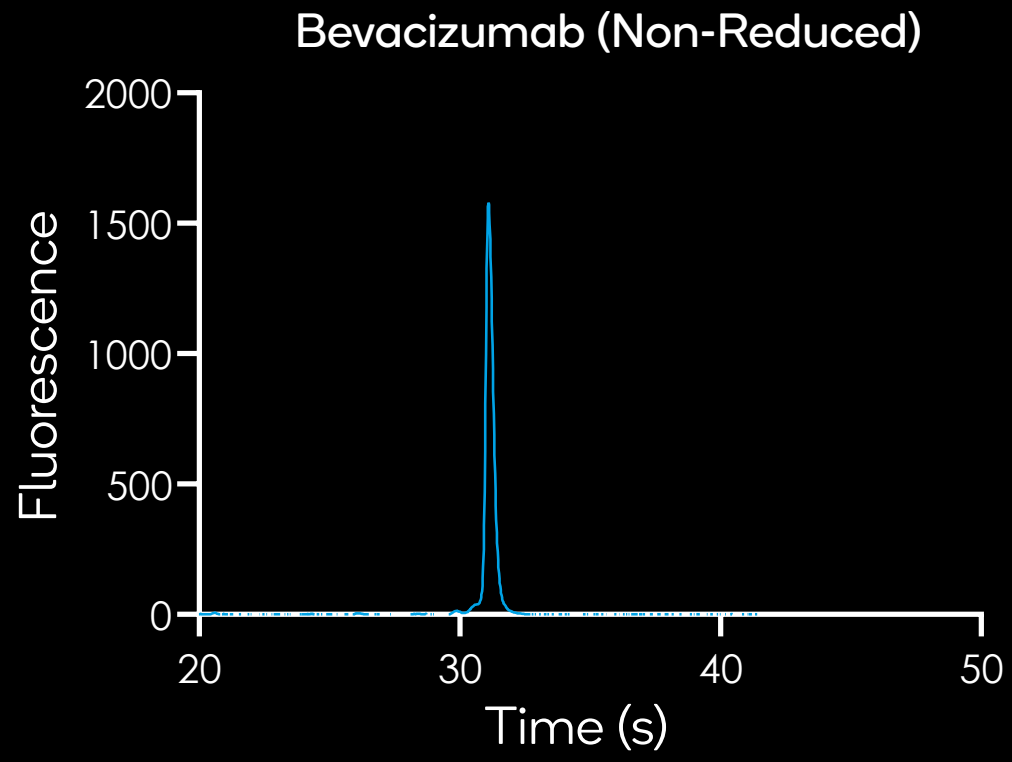
Peak Size (KDa)	Purity %
157.33	100

ATUM

Identity and Purity: µCE-SDS



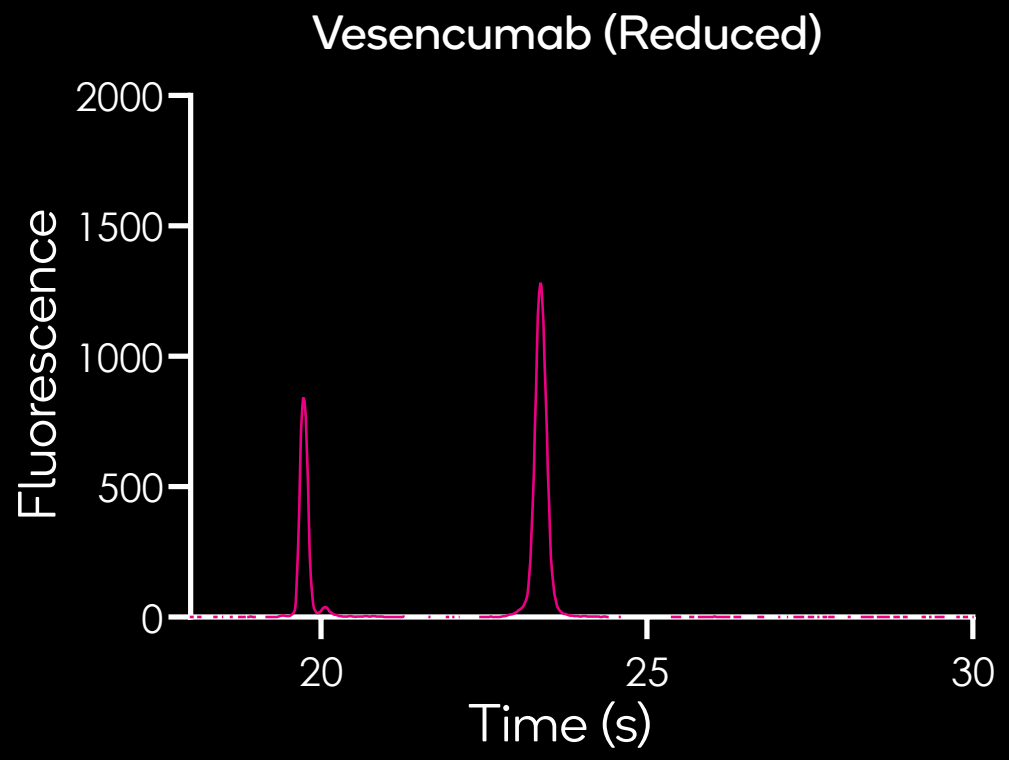
Peak Size (KDa)	Purity %
21.74	33.42
23.79	1.56
54.16	65.01



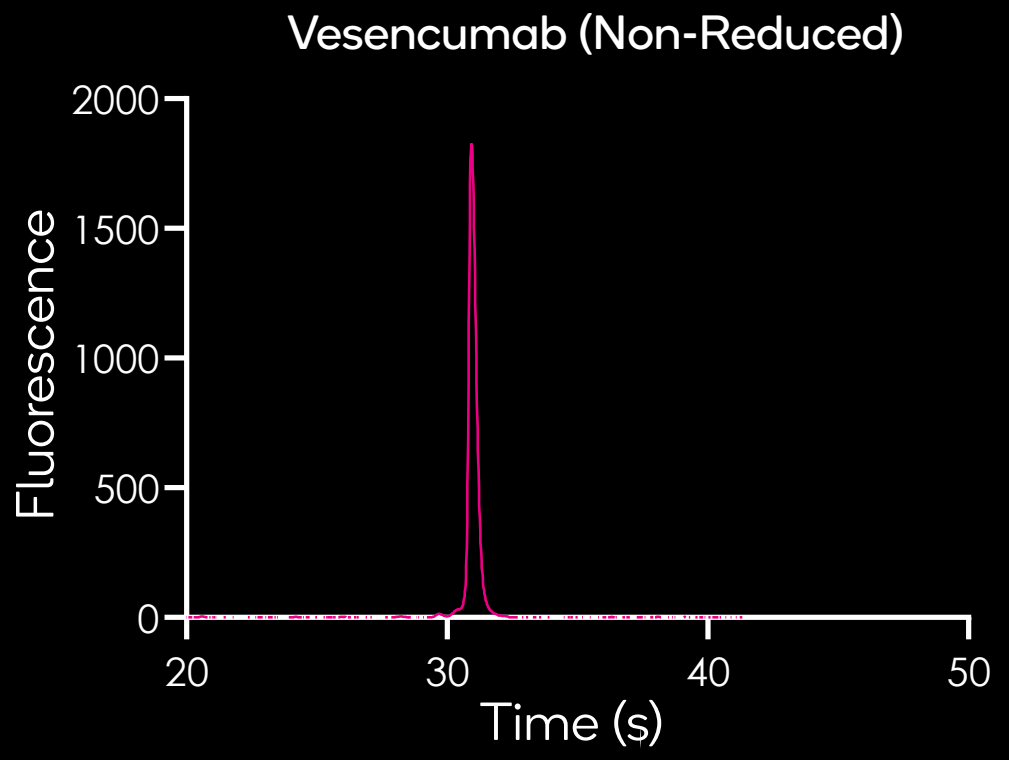
Peak Size (KDa)	Purity %
145.81	0.79
164.83	99.20

ATUM

Identity and Purity: µCE-SDS



Peak Size (KDa)	Purity %
22.51	34.69
24.69	1.91
52.83	63.38

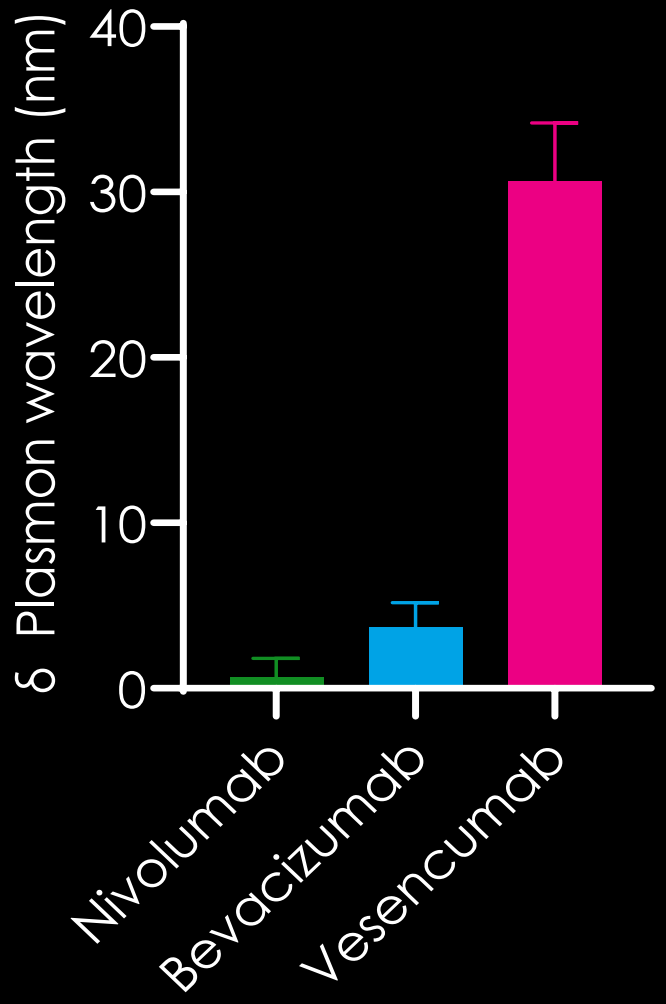


Peak Size (KDa)	Purity %
142.77	0.63
154.08	1.24
162.18	98.11

ATUM

Aggregation Propensity: AC-SINS

(Affinity Capture - Self Interaction Nanoparticle Spectroscopy)

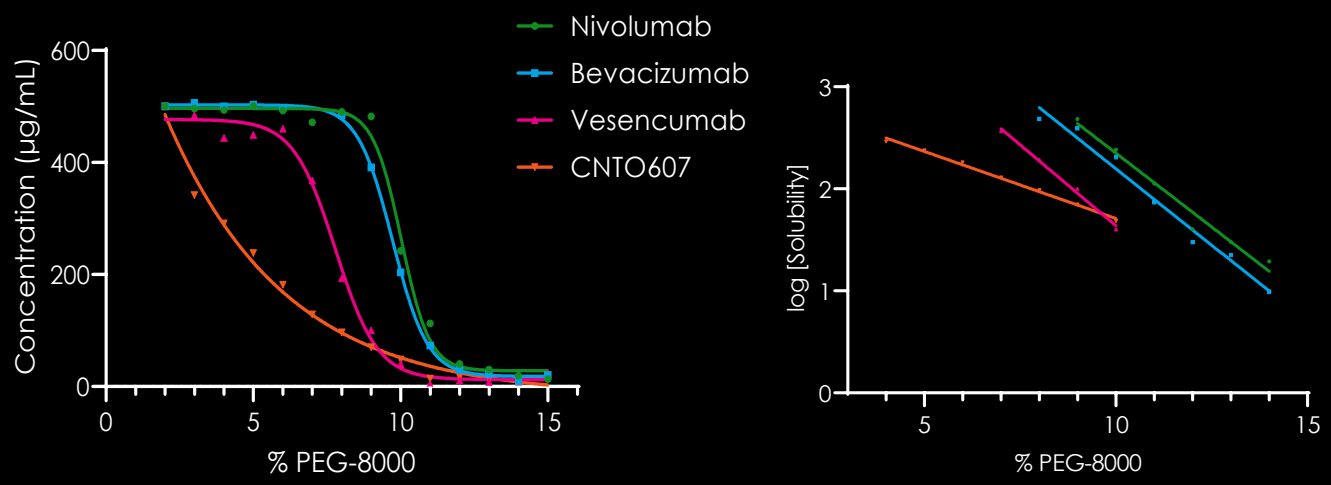


- A high-throughput method to detect antibody self interaction.
- Higher signal = Higher aggregation

ATUM

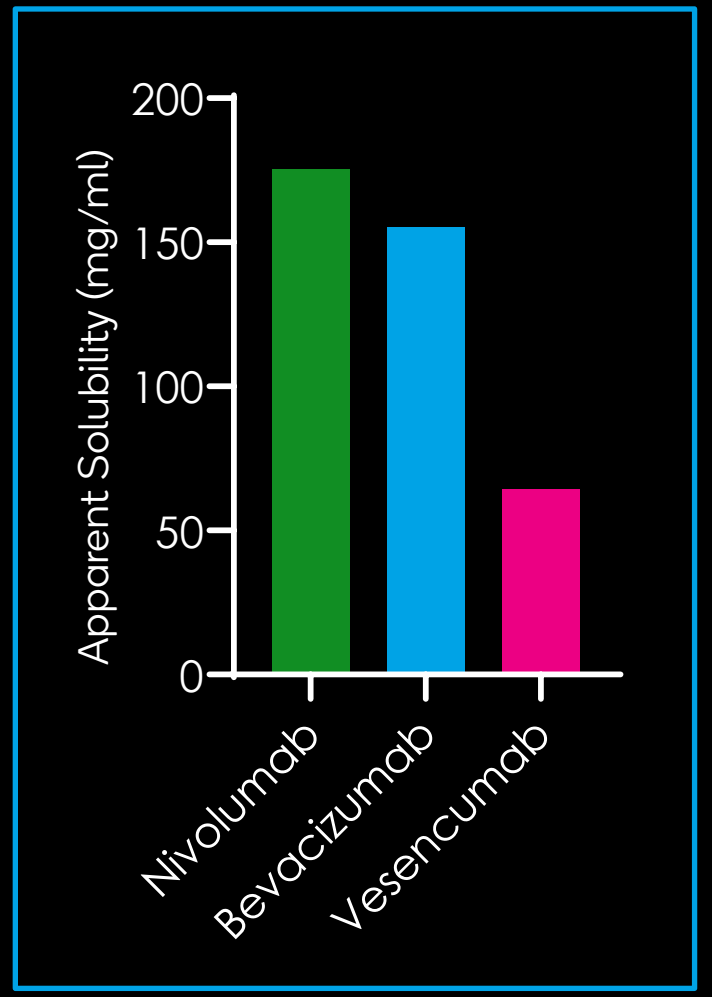
Aggregation Propensity: PIPS

(*PEG Induced Precipitation Solubility Assay*)



Antibody	PEG_{midpt} (% w/v)	Log S_o	Apparent solubility (mg/mL)
Nivolumab	10.0	5.2	175.4
Bevacizumab	9.7	5.2	155.2
Vesencumab	7.8	4.8	64.4
CNTO607	NA	3.0	1.1

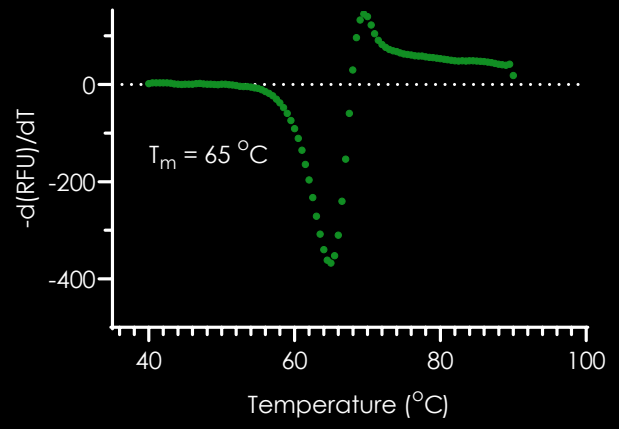
CNTO607 represents a low solubility mAb control.



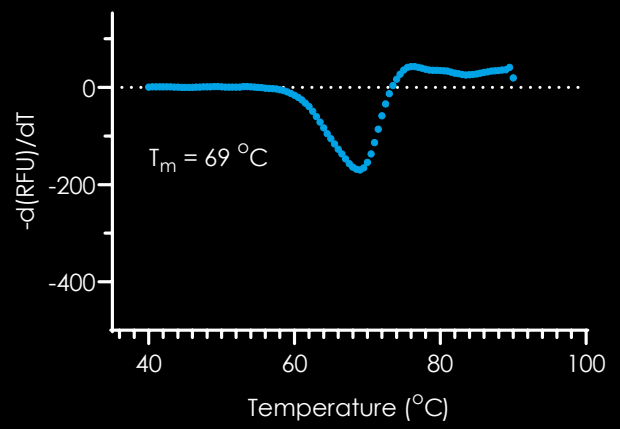
ATUM

Thermo Stability: Tm

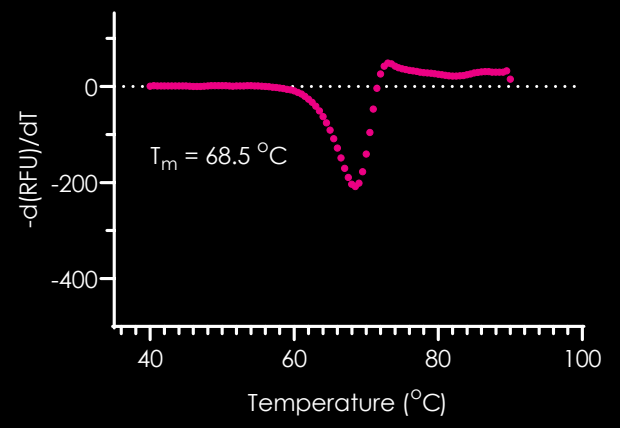
Nivolumab

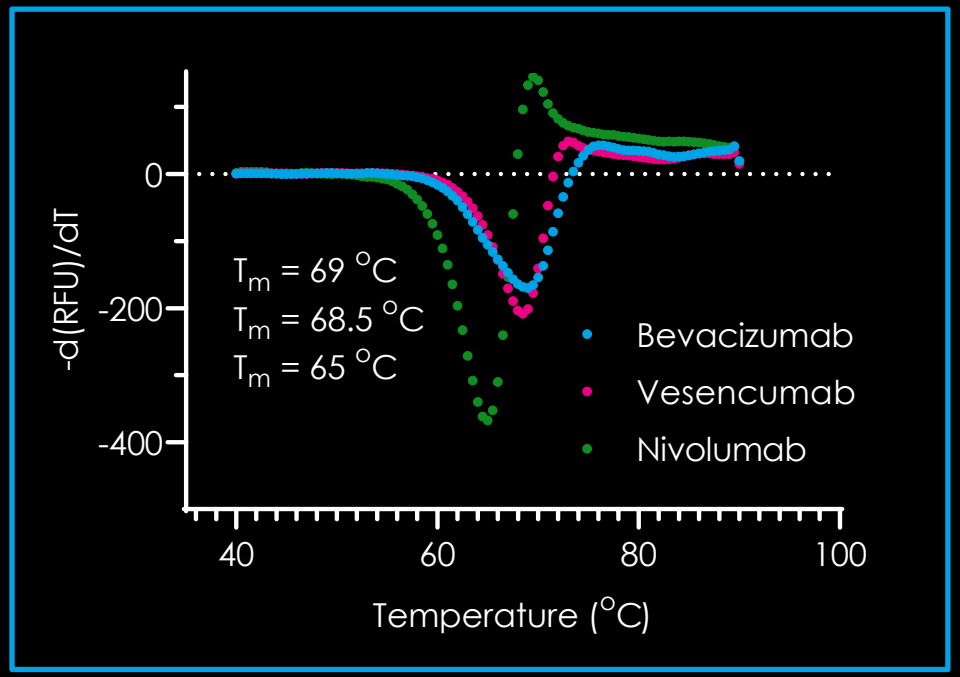


Bevacizumab



Vesencumab



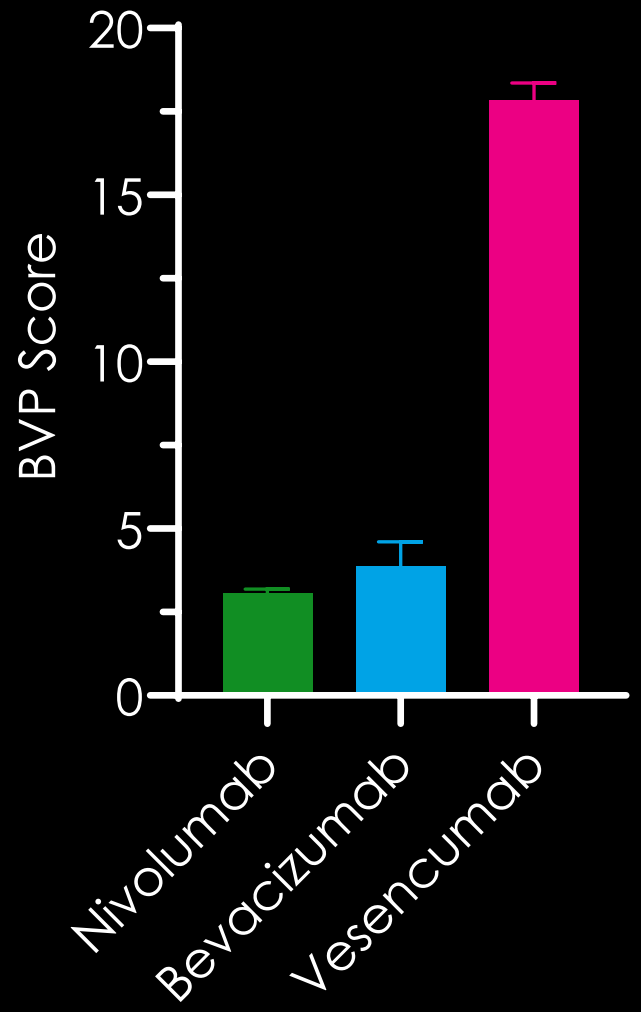


Higher Tm = Higher conformational stability and favorable developability.

ATUM

Polyspecificity: BVP-ELISA

(BaculoViral Particle – ELISA)



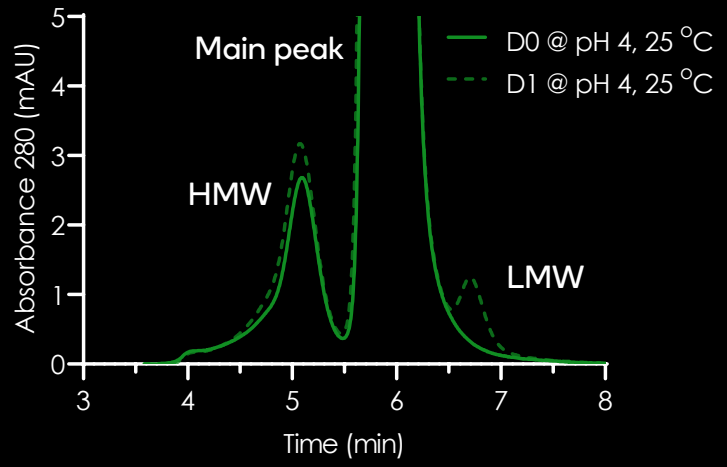
- A high-throughput method to detect polyspecificity of antibody candidates.
- Higher BVP score = Poorer *in vivo* PK

ATUM

Forced Degradation: pH Stress (pH 4)

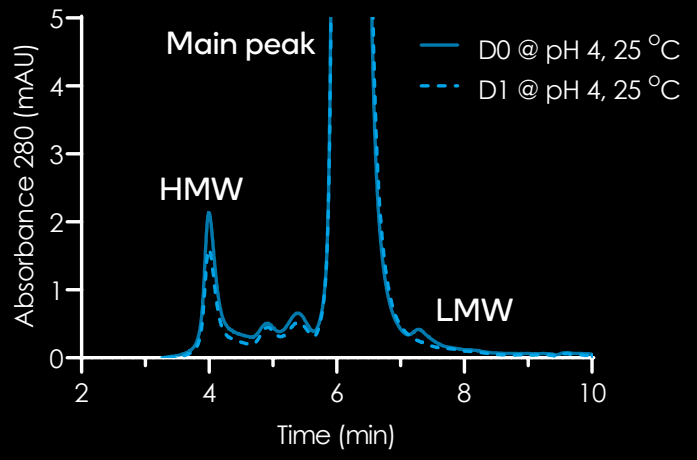
SEC-HPLC

Nivolumab



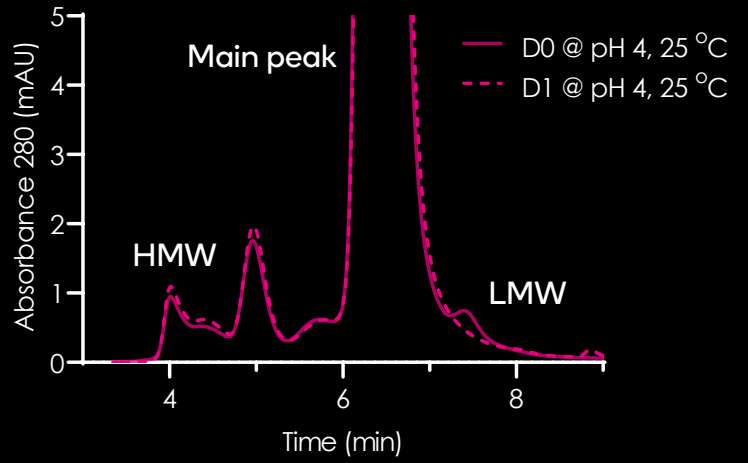
Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% HMW	2.5	2.8
% Main Peak	97.5	96.8
% LMW	0.0	0.4

Bevacizumab



Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% HMW	0.8	1.3
% Main Peak	99.2	98.6
% LMW	0.0	0.1

Vesencumab



Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% HMW	2.8	2.8
% Main Peak	97.2	97.0
% LMW	0.0	0.2

Nivolumab, Bevacizumab and Vesencumab show a slight increase in aggregation and fragmentation upon induction of low pH stress.

ATUM

Forced Degradation: pH Stress (pH 4)

µCE-SDS

Nivolumab

Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% LMW	2.5	3.1
% Main Peak	97.5	96.9

Bevacizumab

Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% LMW	1.9	3.6
% Main Peak	98.1	96.4

Vesencumab

Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% LMW	1.8	1.9
% Main Peak	98.2	98.3

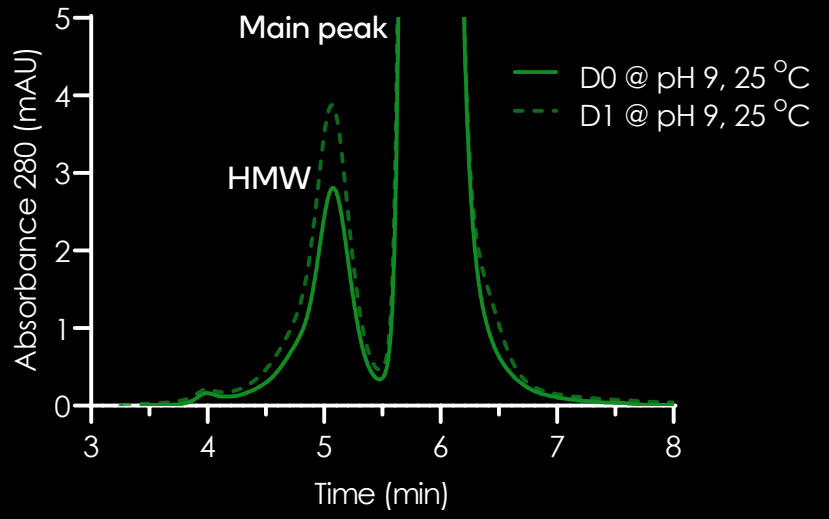
Nivolumab, Bevacizumab show a small but detectable increase in fragmentation upon induction of low pH stress.

ATUM

Forced Degradation: pH Stress (pH 9)

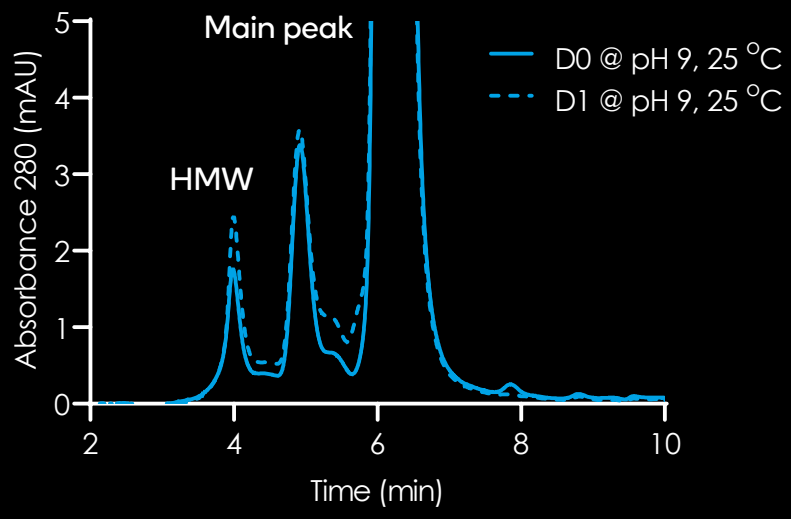
SEC-HPLC

Nivolumab



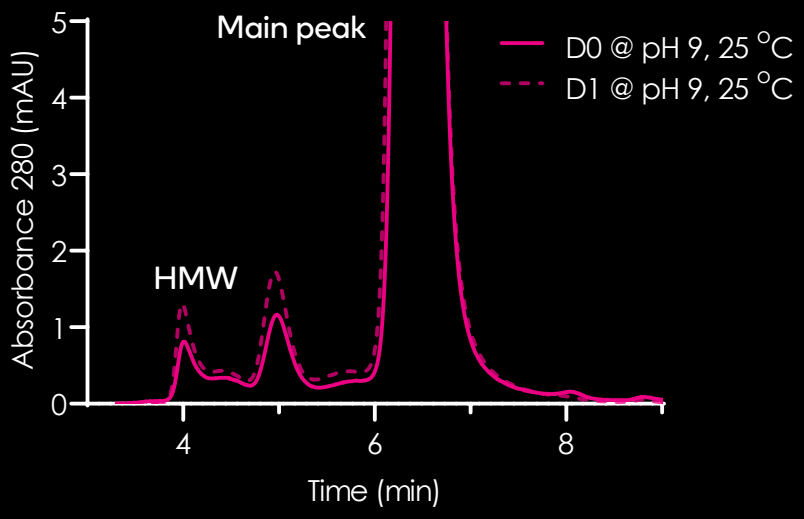
Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.2	4.6
% Main Peak	96.8	95.4
% LMW	0.0	0.0

Bevacizumab



Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.0	3.8
% Main Peak	97.0	96.2
% LMW	0.0	0.0

Vesencumab



Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.0	3.4
% Main Peak	97.0	96.6
% LMW	0.0	0.0

Nivolumab, Bevacizumab and Vesencumab showed a slight increase in aggregation upon induction of high pH stress

ATUM

Forced Degradation: pH Stress (pH 9)

µCE-SDS

Nivolumab

Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% LMW	2.5	2.5
% Main Peak	97.5	97.5

Bevacizumab

Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% LMW	1.8	4.8
% Main Peak	98.2	95.2

Vesencumab

Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% LMW	1.9	1.8
% Main Peak	98.3	98.2

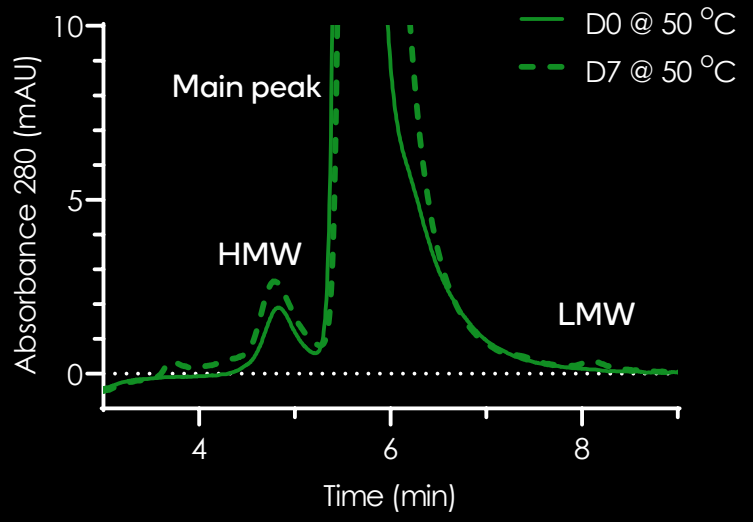
- Bevacizumab showed a detectable increase in aggregation upon induction of high pH stress.
- Nivolumab and Vesencumab were resistant to high pH stress.

ATUM

Forced Degradation: Thermal Stress

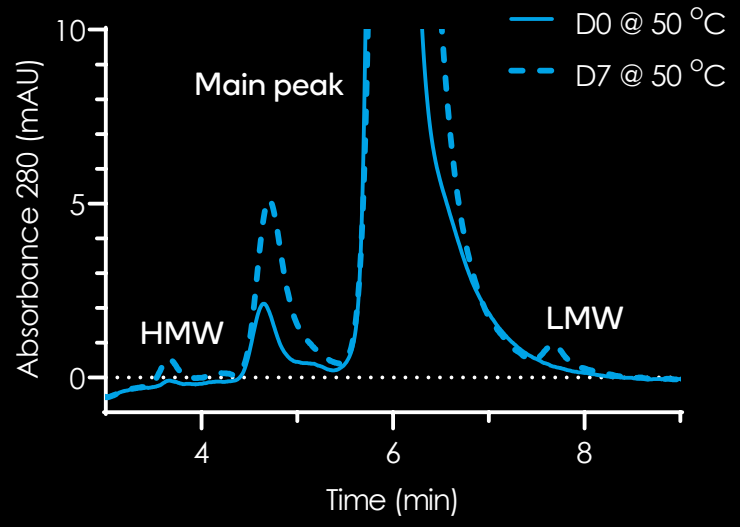
SEC-HPLC

Nivolumab



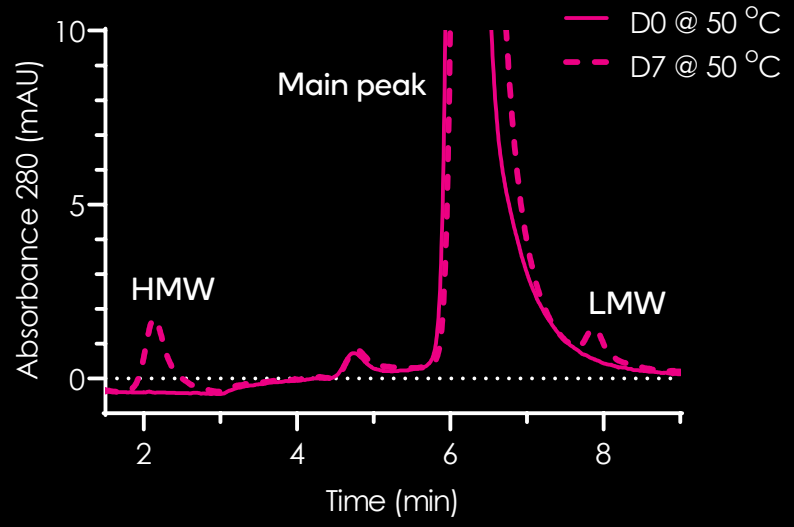
Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% HMW	2.0	2.9
% Main Peak	98.0	97.0
% LMW	0.0	0.1

Bevacizumab



Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% HMW	1.9	5.5
% Main Peak	98.1	94.1
% LMW	0.0	0.4

Vesencumab



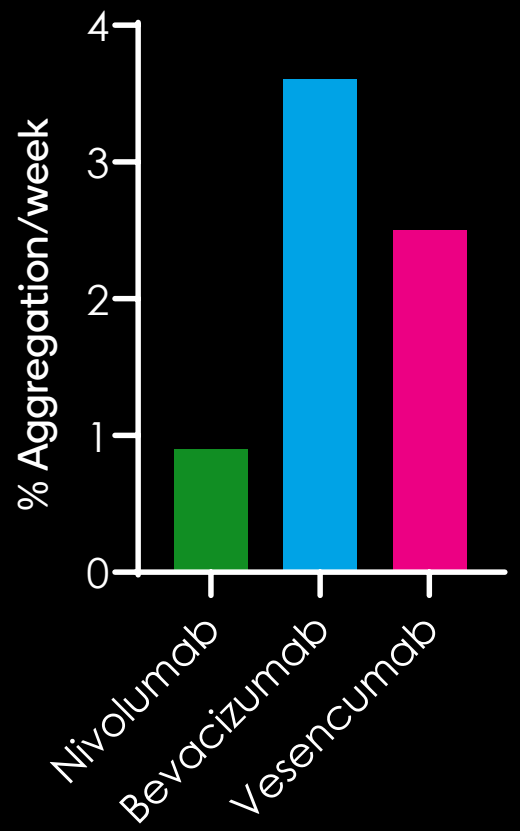
Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% HMW	0.6	3.1
% Main Peak	99.4	95.8
% LMW	0.0	1.1

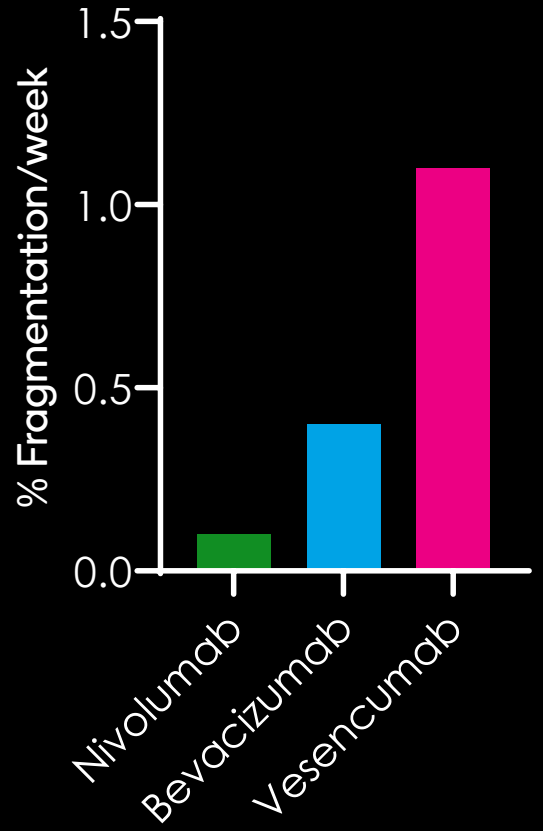
Nivolumab, Bevacizumab and Vesencumab show increased aggregation and fragmentation upon induction of thermal stress.

ATUM

Forced Degradation: Thermal Stress

SEC-HPLC





Low solubility control (CNTO607) showed visible precipitation within one day of incubation at 50°C

Nivolumab, Bevacizumab and Vesencumab show increased aggregation and fragmentation upon induction of thermal stress.

ATUM

Forced Degradation: Thermal Stress

µCE-SDS

Nivolumab

Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% LMW	2.5	2.6
% Main Peak	97.5	97.4

Bevacizumab

Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% LMW	1.9	2.3
% Main Peak	98.1	97.7

Vesencumab

Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% LMW	0.0	0.8
% Main Peak	100.0	99.2

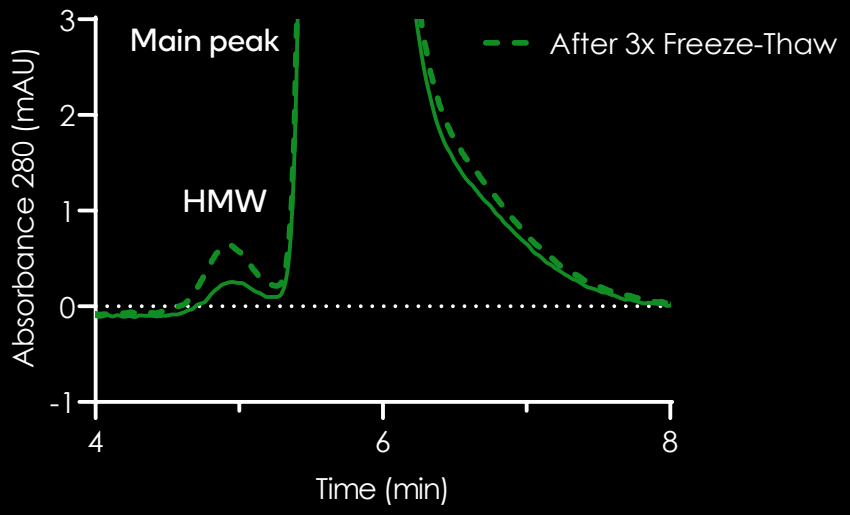
Slight fragmentation of Bevacizumab and Vesencumab was seen upon induction of thermal stress.

ATUM

Forced Degradation: Freeze-Thaw

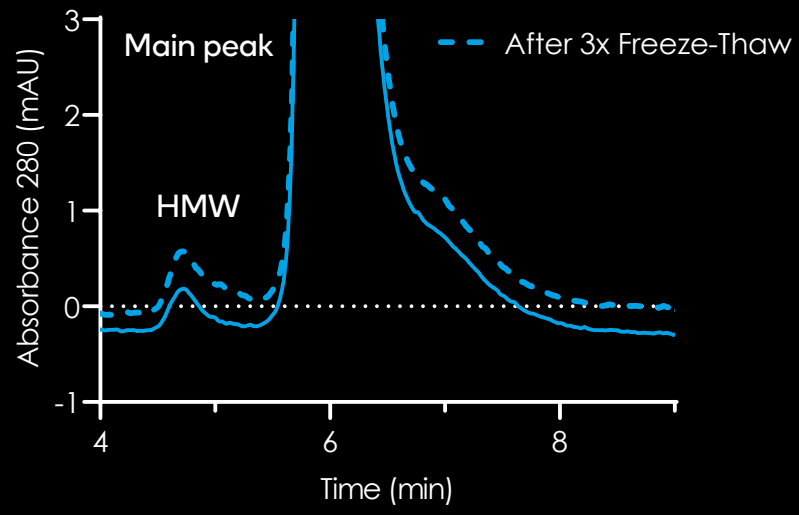
SEC-HPLC

Nivolumab



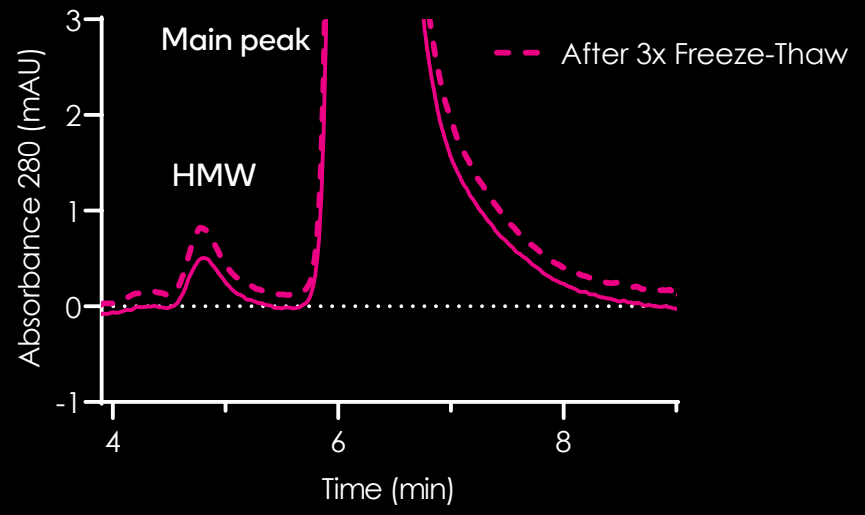
Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	0.5	0.8
% Main Peak	99.5	99.2

Bevacizumab



Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	0.4	0.5
% Main Peak	99.6	99.5

Vesencumab



Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	1.2	1.6
% Main Peak	98.8	98.4

Nivolumab, Bevacizumab and Vesencumab show slight but detectable aggregation upon multiple (3x) freeze-thaw cycles.

ATUM

Forced Degradation: Freeze-Thaw

µCE-SDS

Nivolumab

Peak Assignment	Before	After 3x Freeze-Thaw
% LMW	2.5	2.4
% Main Peak	97.5	97.6

Bevacizumab

Peak Assignment	Before	After 3x Freeze-Thaw
% LMW	1.9	2.0
% Main Peak	98.1	98.0

Vesencumab

Peak Assignment	Before	After 3x Freeze-Thaw
% LMW	0.0	0.0
% Main Peak	100.0	100.0

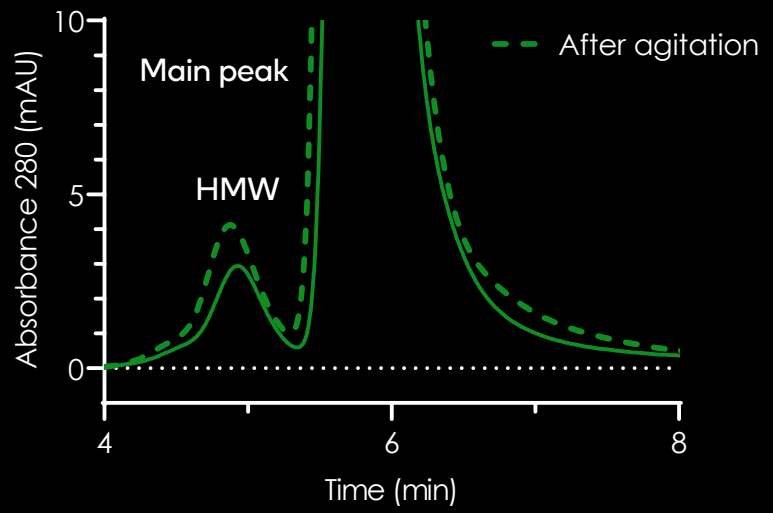
No differences after 3x freeze-thaw cycles.

ATUM

Forced Degradation: Agitation Stress

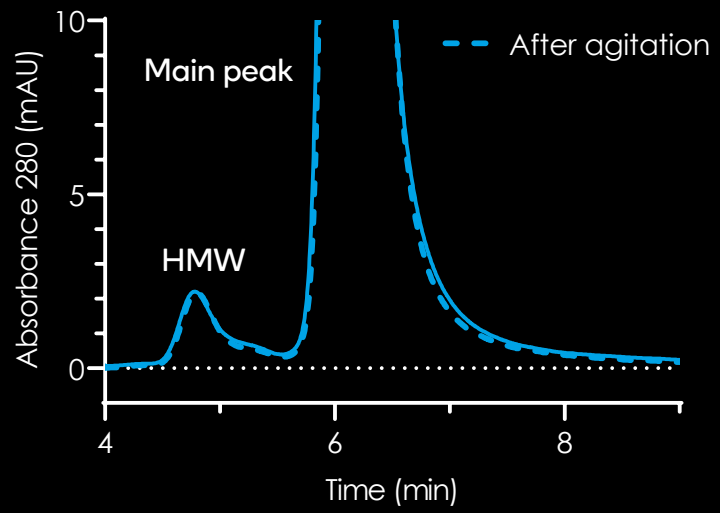
SEC-HPLC

Nivolumab



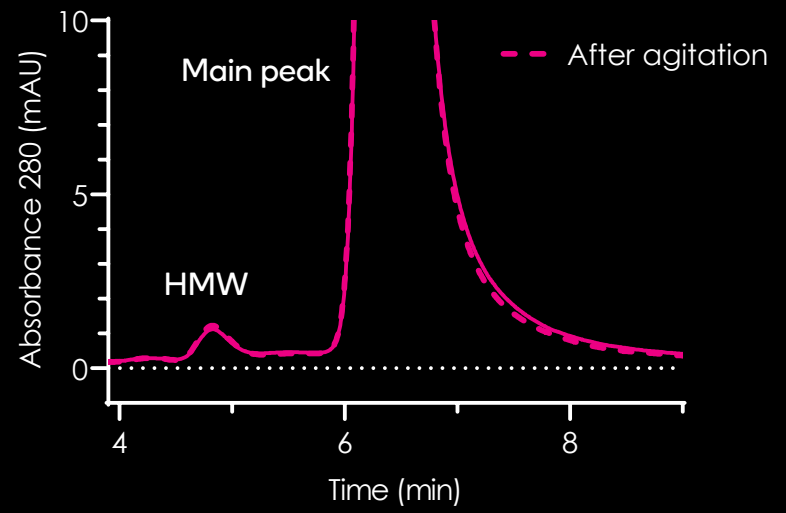
Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% HMW	2.3	3.6
% Main Peak	97.7	96.4

Bevacizumab



Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% HMW	2.2	2.1
% Main Peak	97.8	97.9

Vesencumab



Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% HMW	1.0	1.1
% Main Peak	99.0	98.9

- Nivolumab shows increase in aggregation upon agitation
- Bevacizumab and Vesencumab were resistant to agitation.

ATUM

Forced Degradation Study: Agitation Stress

µCE-SDS

Nivolumab

Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% LMW	2.5	2.3
% Main Peak	97.5	97.7

Bevacizumab

Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% LMW	1.9	2.0
% Main Peak	98.1	98.0

Vesencumab

Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% LMW	0.0	0.0
% Main Peak	100.0	100.0

No differences upon induction of agitation stress.

ATUM

Express, Purify and Analyze your protein with us, or send us your protein for Analytics assessment

For questions and additional information

Contact us:

E-mail: info@atum.bio

Call us: +1-877-DNA-TOGO

+1-650-853-8347

ATUM