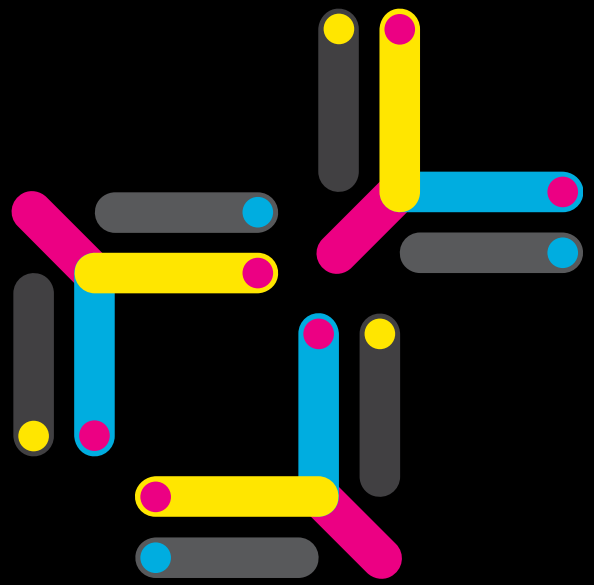

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Developability Analytics

Contact us:

info@atum.bio

+1-877-DNA-TOGO

+1-650-853-8347

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Developability Analytics

Package 1
in silico predictions

- Mol. wt.
- Isoelectric point (PI)
- N-Glycans
- Hydrophobicity
- Sequence liabilities

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

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

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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

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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

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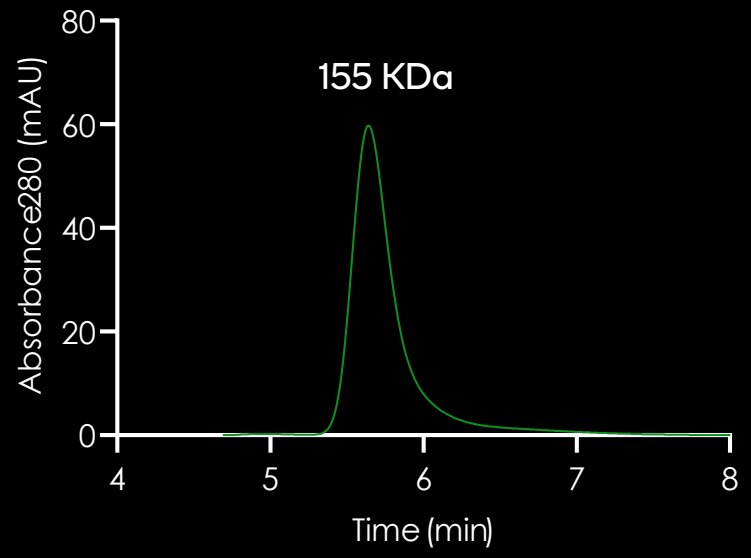
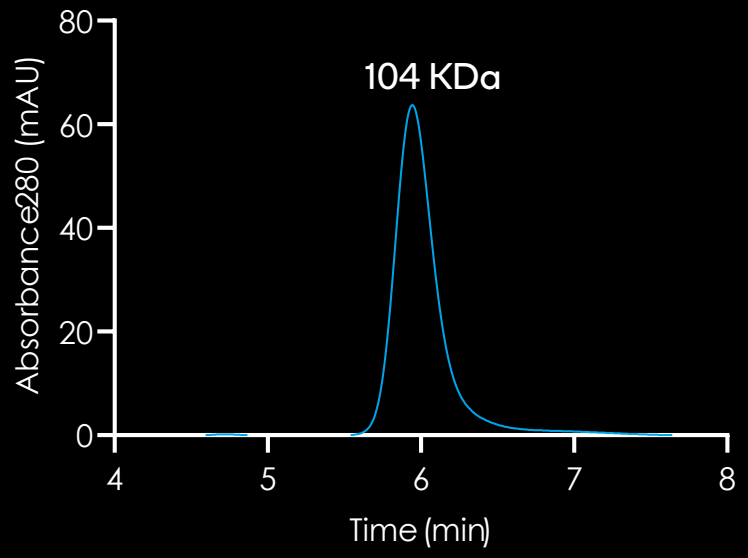
Identity and Purity: SEC-HPLC

Nivolumab



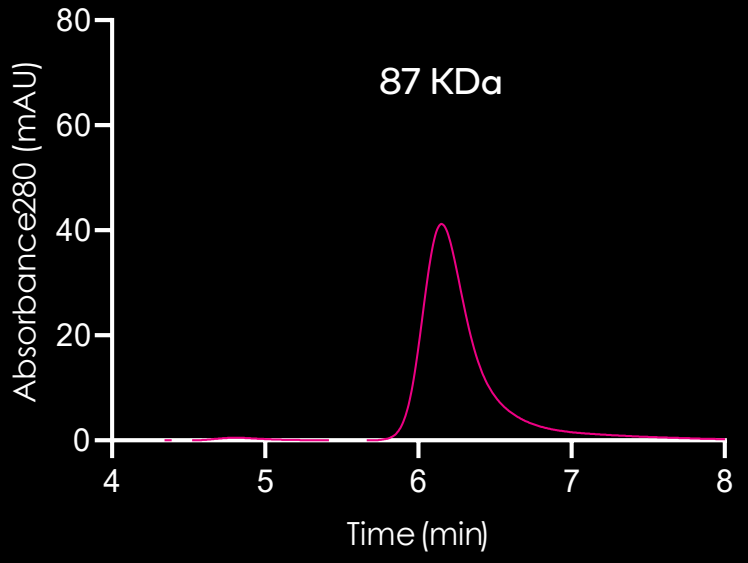
Peak Size (KDa)	Area %
155	100

Bevacizumab



Peak Size (KDa)	Purity %
104	100

Vesencumab



Peak Size (KDa)	Purity %
87	100

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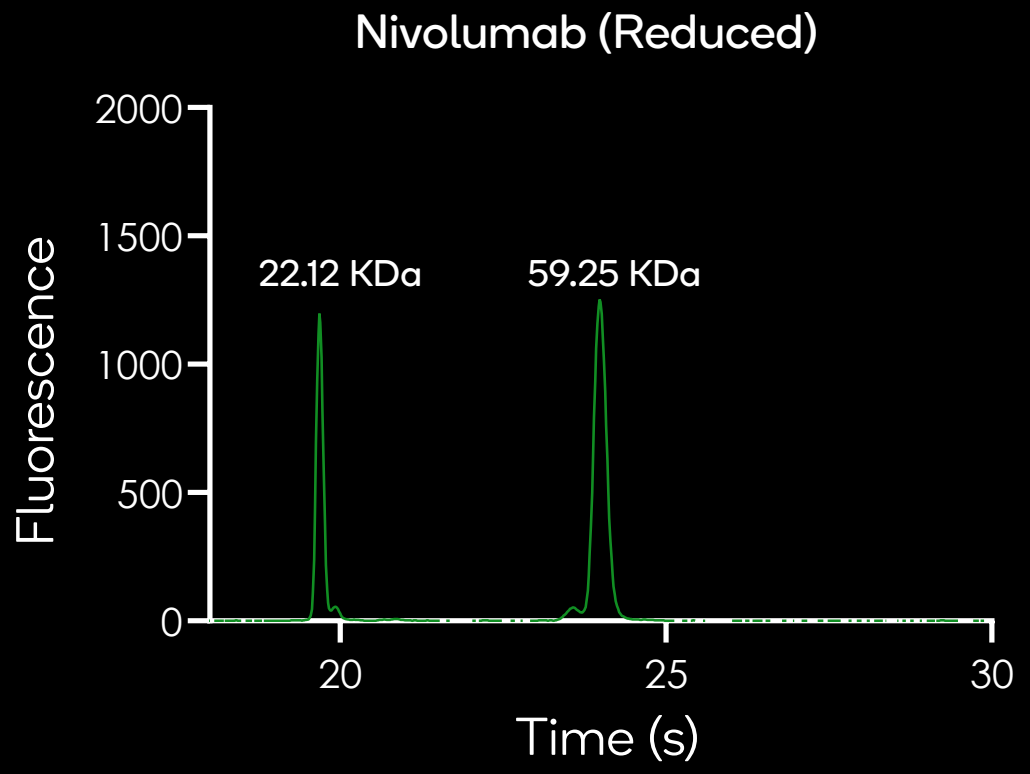
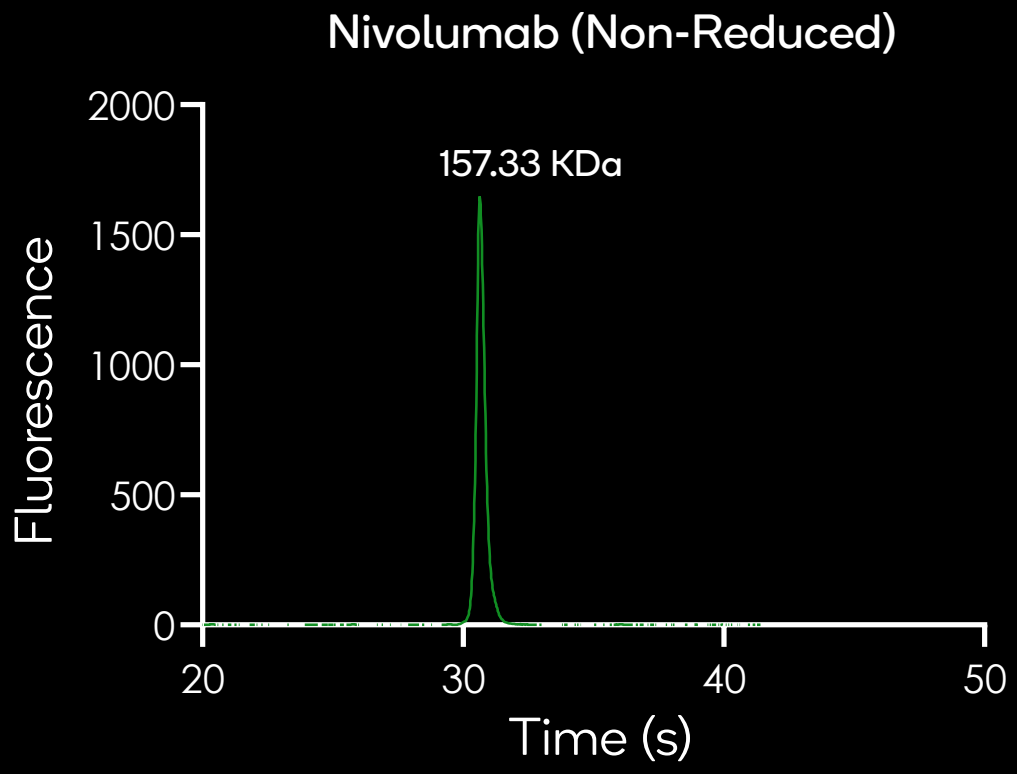
Identity and Purity: µCE-SDS



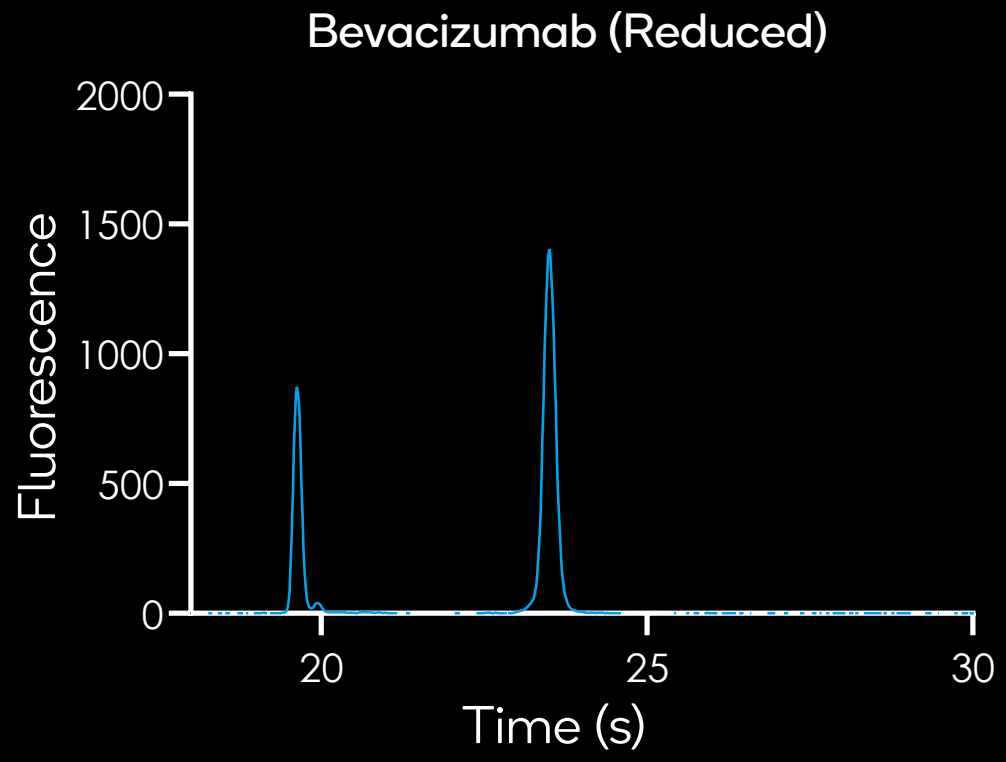
Peak Size (KDa)	Purity %
22.12	37.98
23.72	1.93
54.98	2.50
59.25	57.57



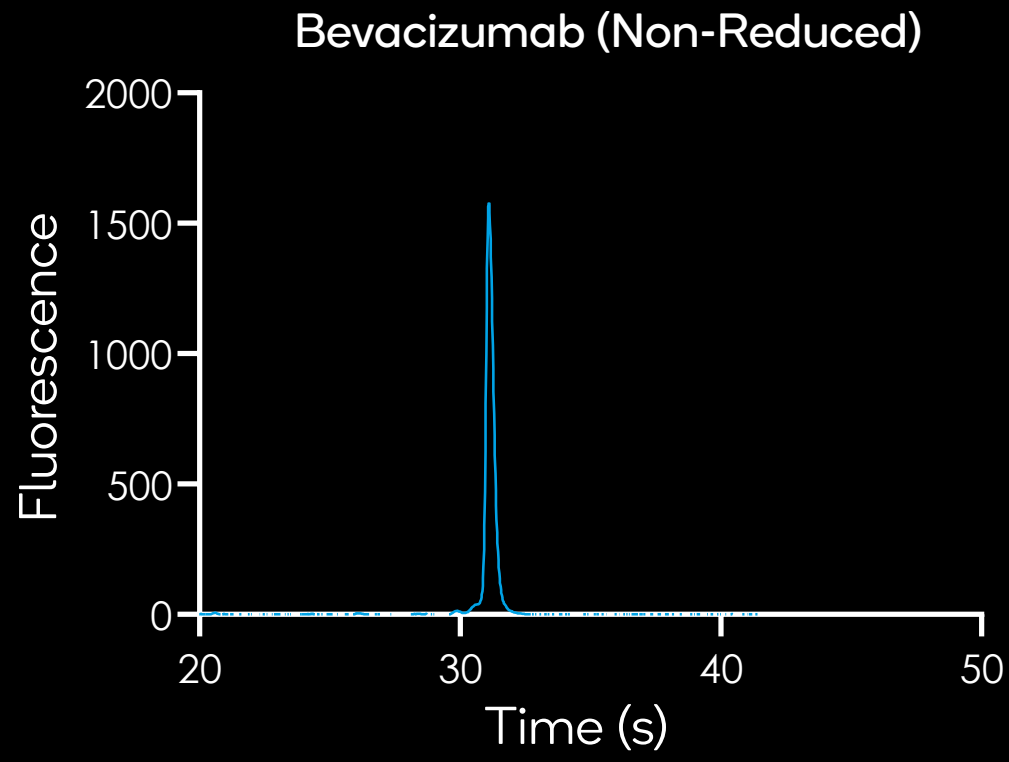
Peak Size (KDa)	Purity %
157.33	100

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

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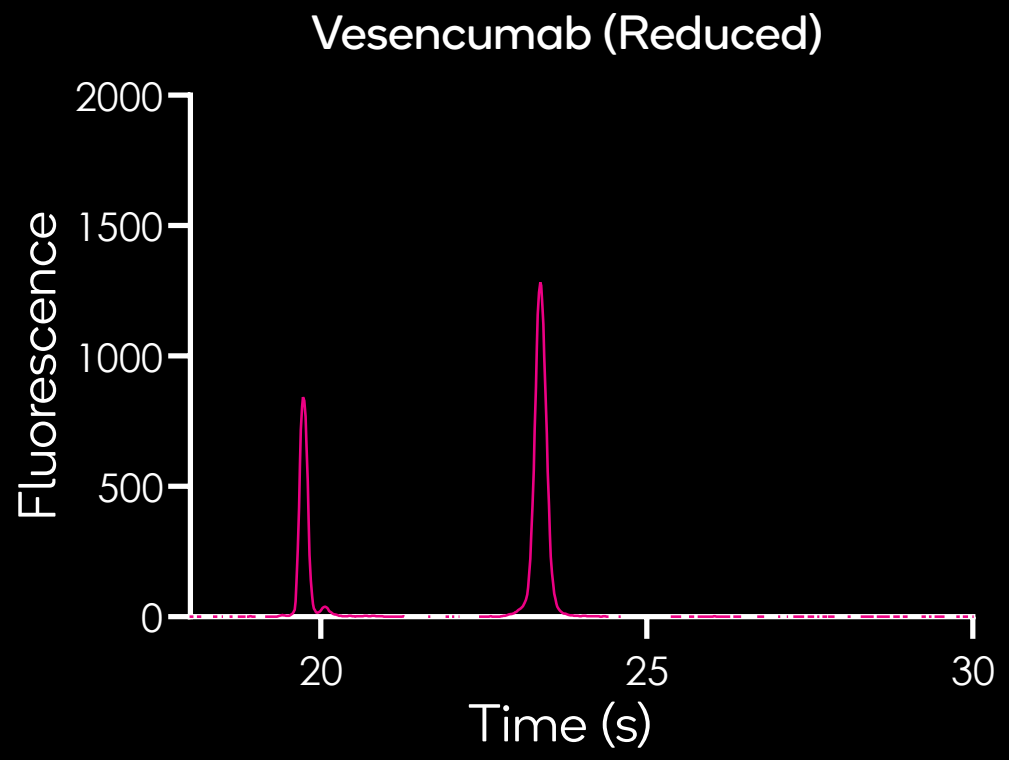
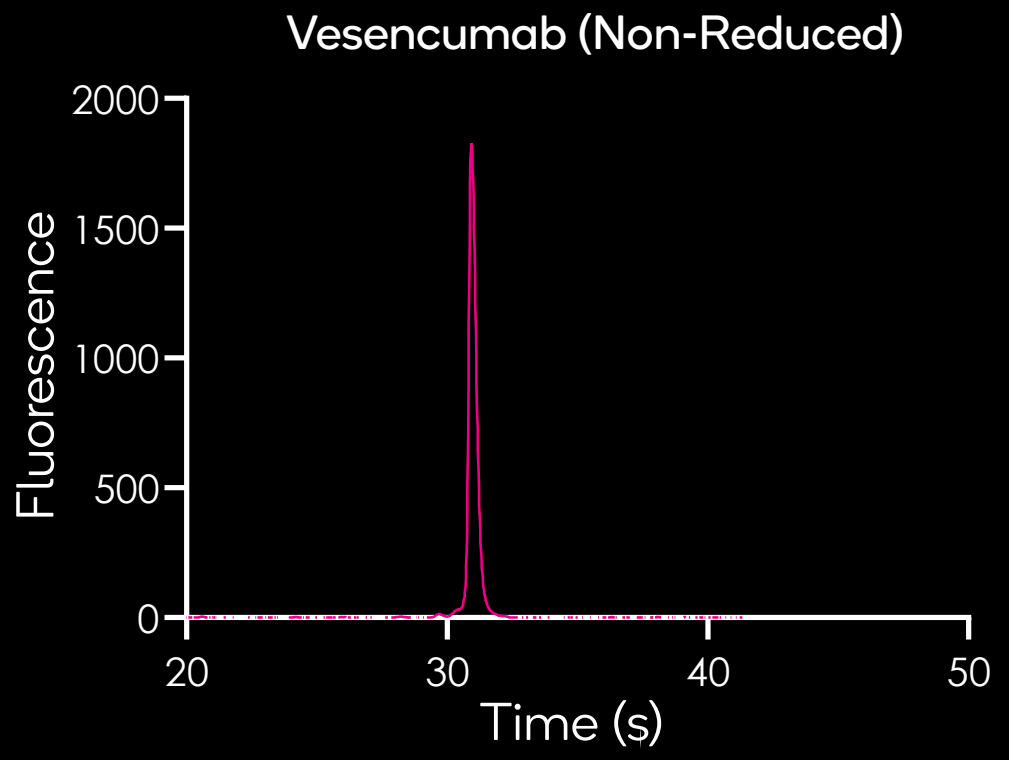
Identity and Purity: µCE-SDS



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

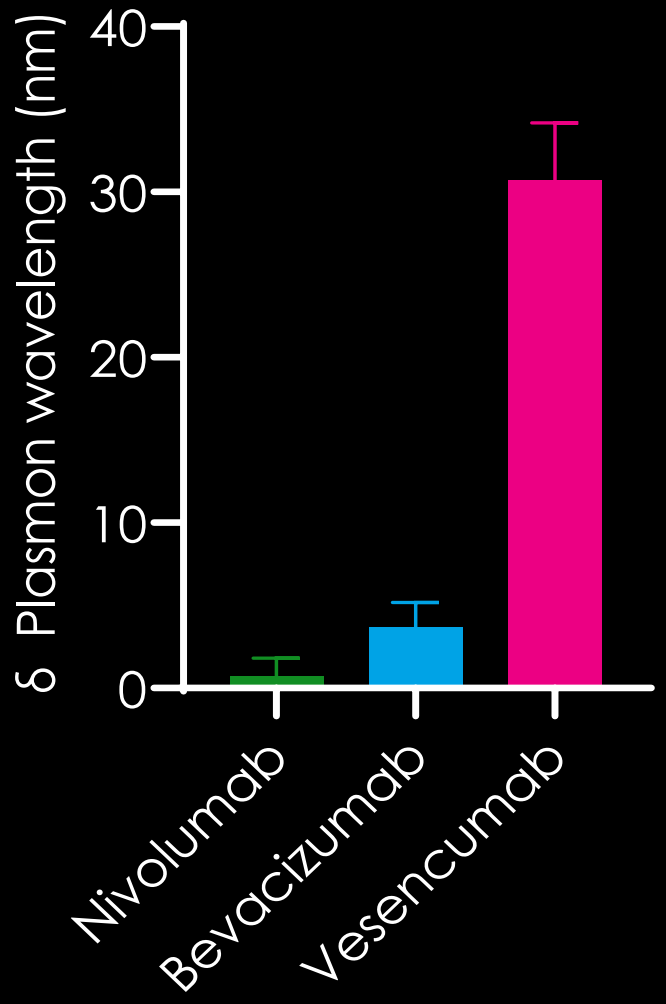


Peak Size (KDa)	Purity %
142.77	0.63
154.08	1.24
162.18	98.11

ATUM

Aggregation Propensity: AC-SINS

(Affinity Capture - Self Interaction Nanoparticle Spectroscopy)



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

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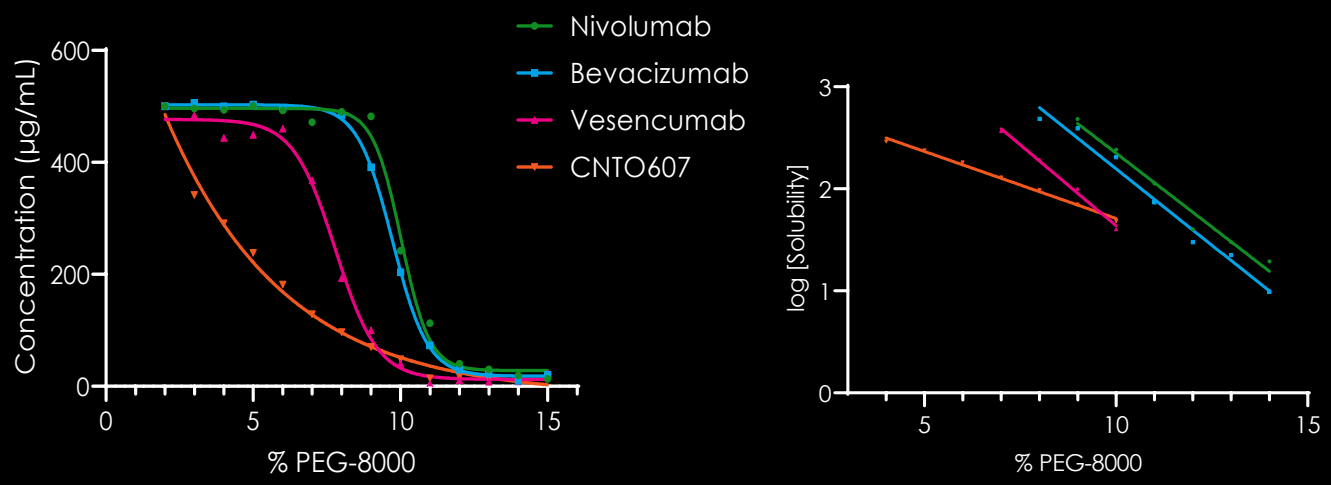
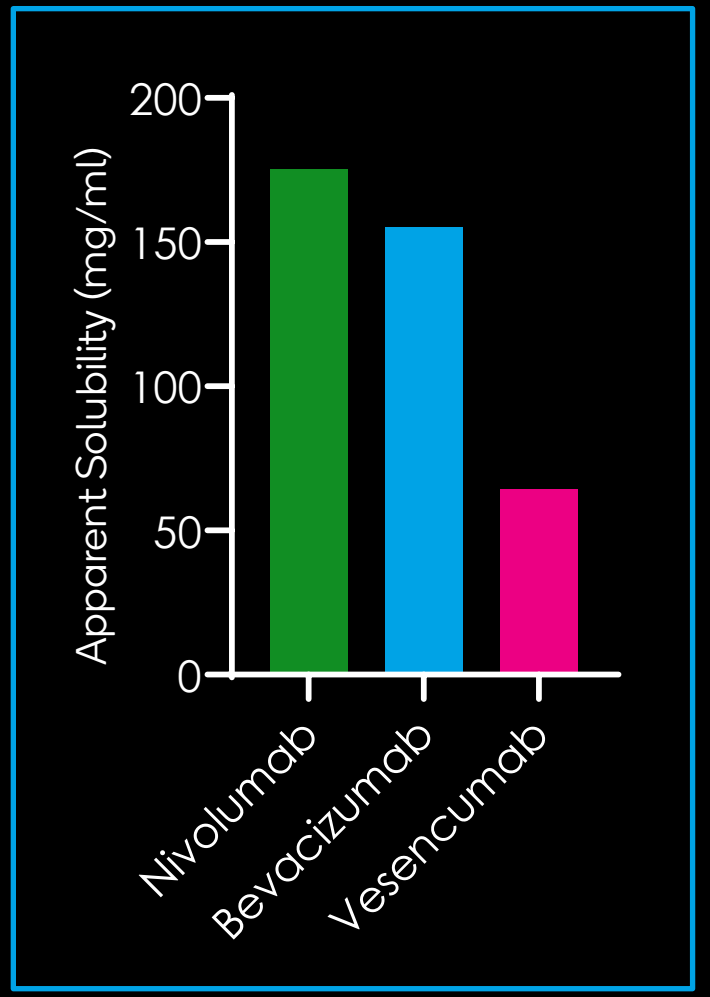
Aggregation Propensity: PIPS

(*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.



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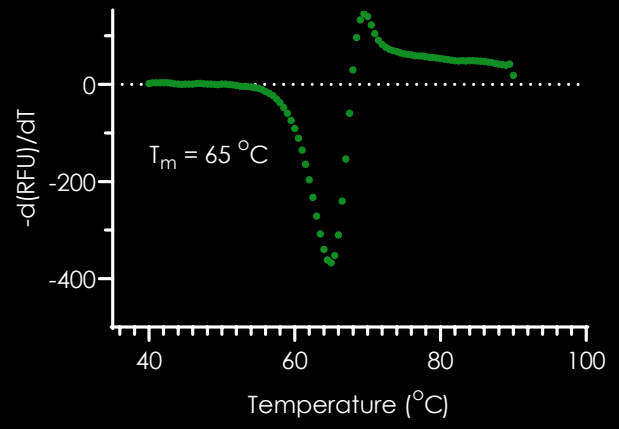
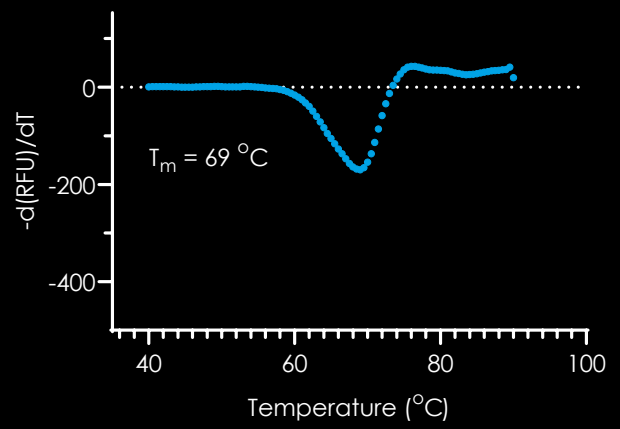
Thermo Stability: Tm

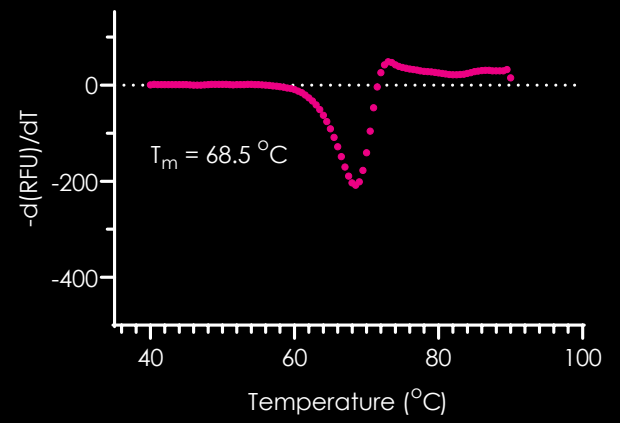
Nivolumab

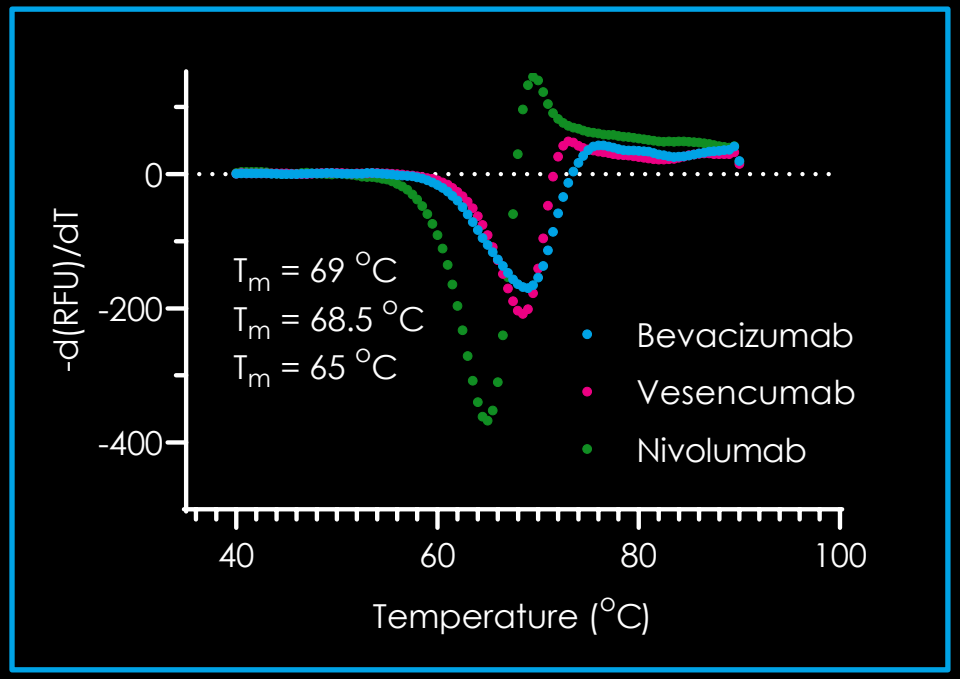


Bevacizumab



Vesencumab



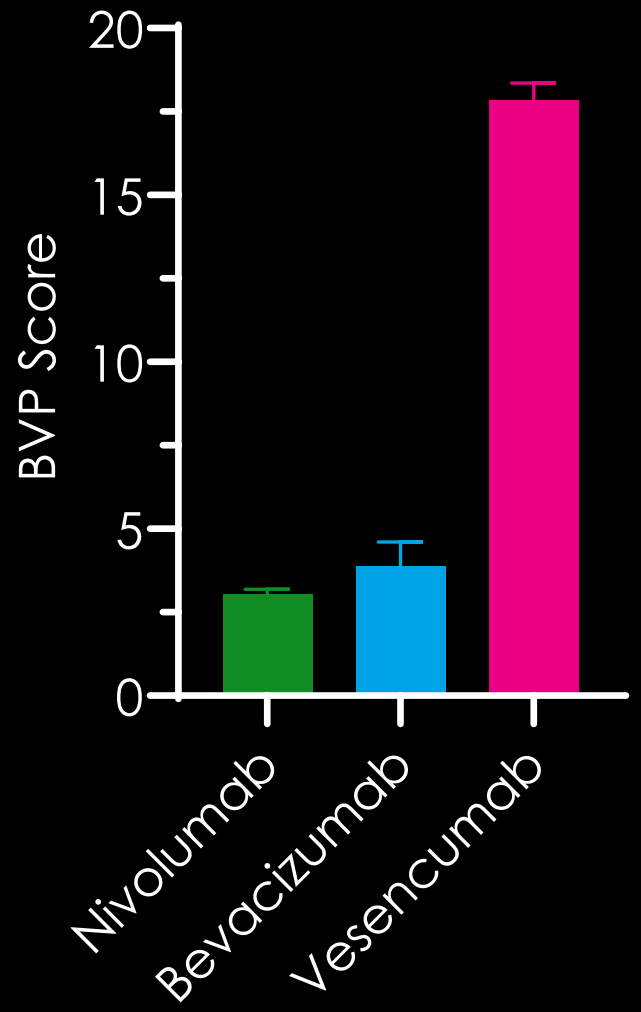


Higher Tm = Higher conformational stability and favorable developability.

ATUM

Polyspecificity: BVP-ELISA

(BaculoViral Particle – ELISA)



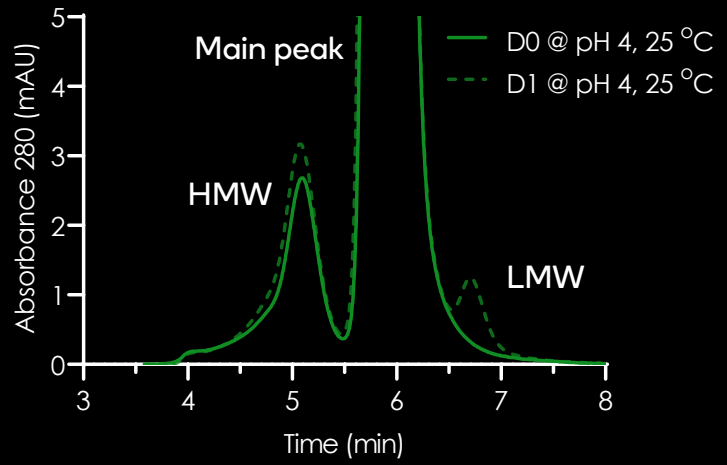
- A high-throughput method to detect polyspecificity of antibody candidates.
- Higher BVP score = Poorer *in vivo* PK

ATUM

Forced Degradation: pH Stress (pH 4)

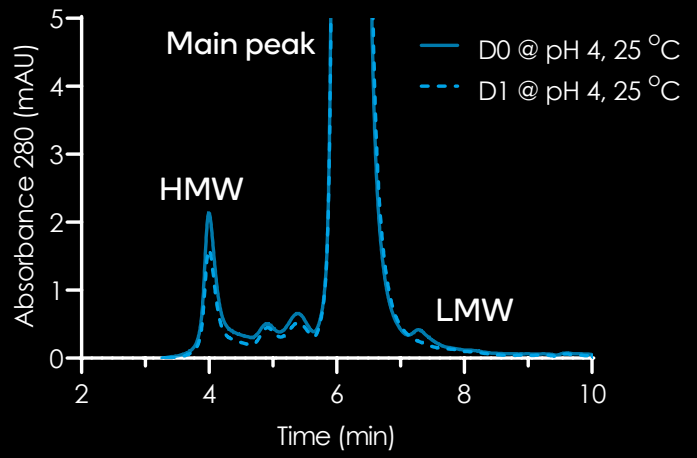
SEC-HPLC

Nivolumab



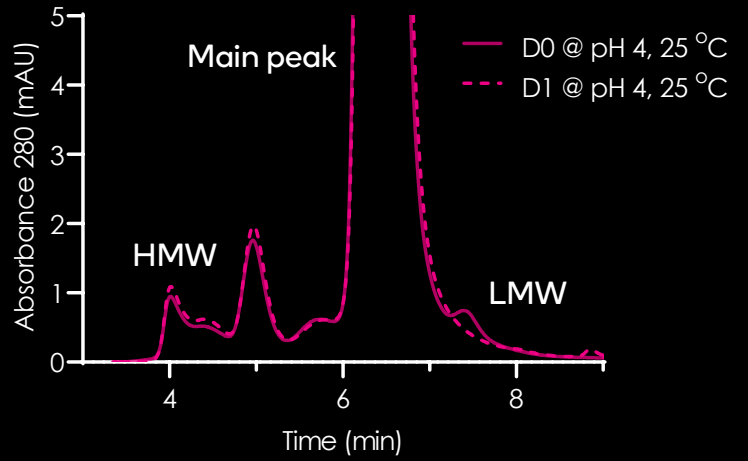
Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% HMW	2.5	2.8
% Main Peak	97.5	96.8
% LMW	0.0	0.4

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

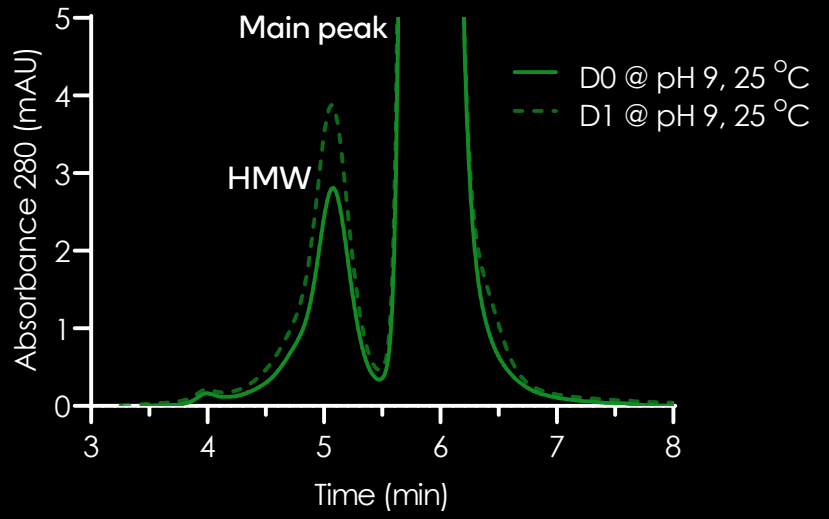
Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% LMW	1.8	1.9
% Main Peak	98.2	98.3

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

ATUM

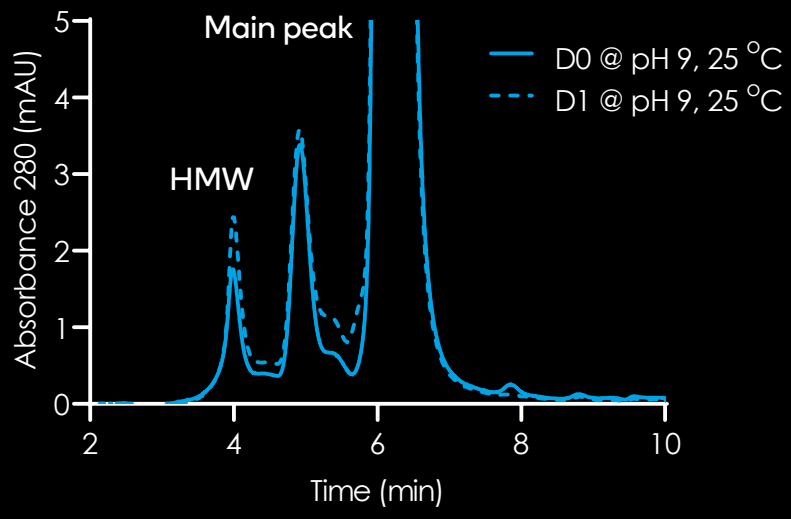
Forced Degradation: pH Stress (pH 9)
SEC-HPLC

Nivolumab



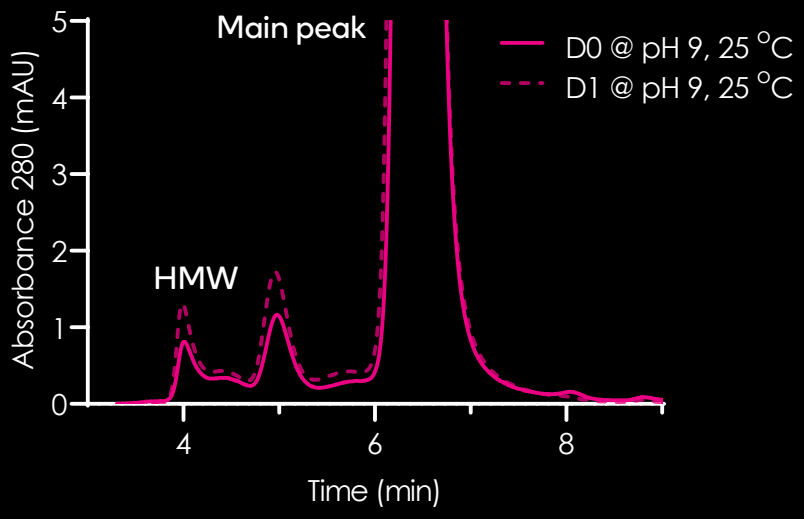
Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.2	4.6
% Main Peak	96.8	95.4
% LMW	0.0	0.0

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

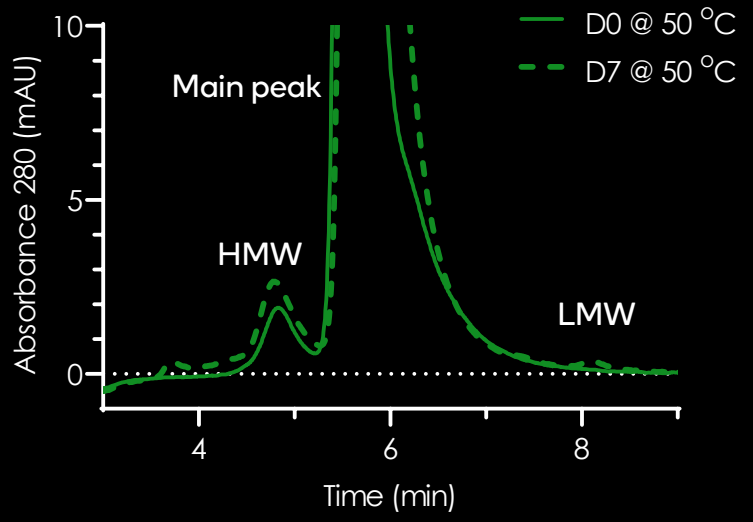
- Bevacizumab showed a detectable increase in aggregation upon induction of high pH stress.
- Nivolumab and Vesencumab were resistant to high pH stress.

ATUM

Forced Degradation: Thermal Stress

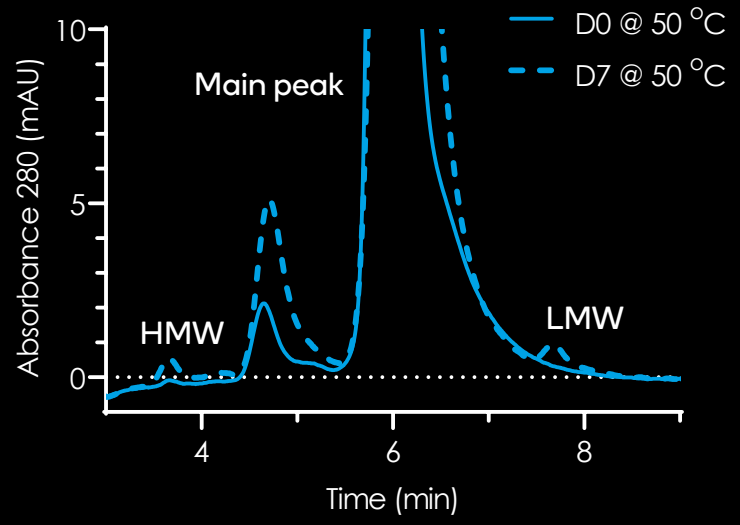
SEC-HPLC

Nivolumab



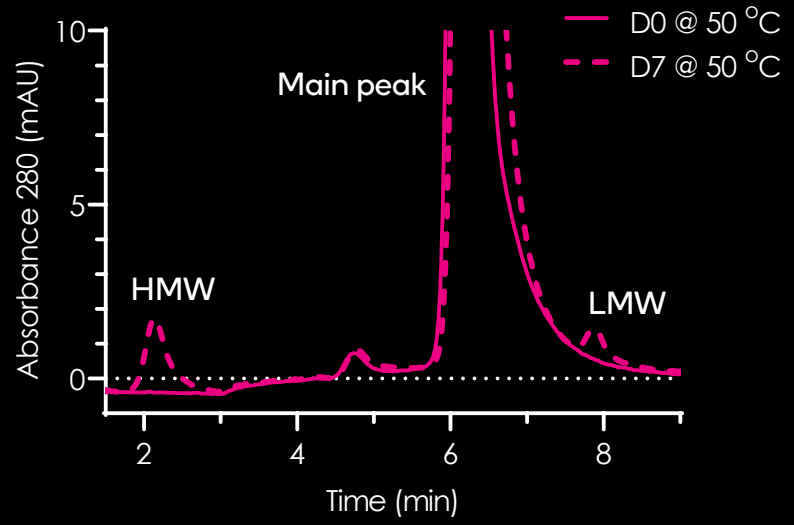
Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% HMW	2.0	2.9
% Main Peak	98.0	97.0
% LMW	0.0	0.1

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



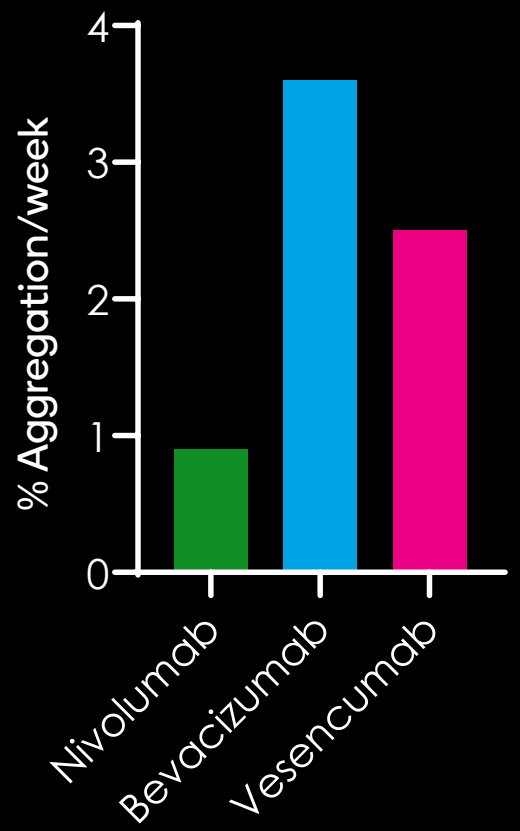
Peak Assignment	D0 @ 50 °C	D7 @ 50 °C
% HMW	0.6	3.1
% Main Peak	99.4	95.8
% LMW	0.0	1.1

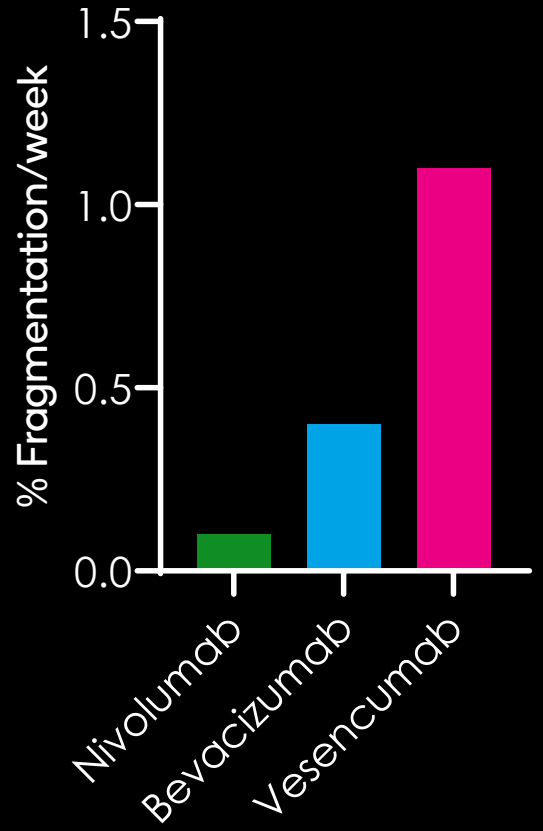
Nivolumab, Bevacizumab and Vesencumab show increased aggregation and fragmentation upon induction of thermal stress.

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

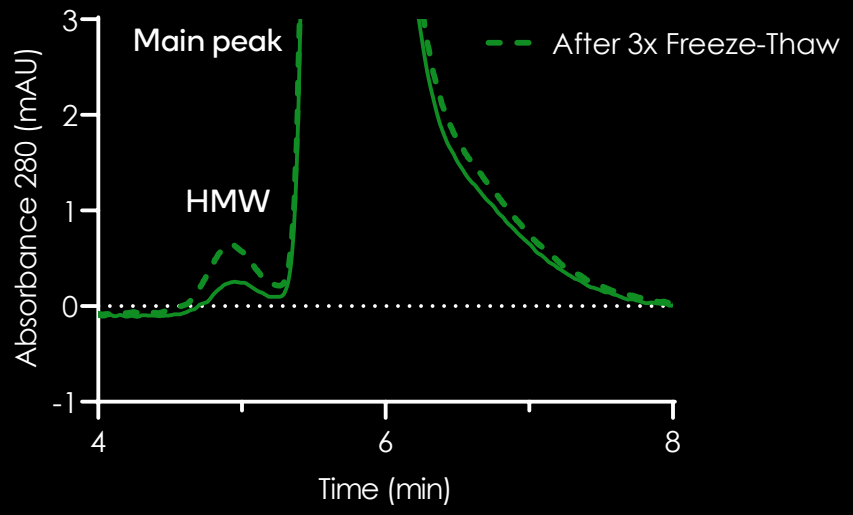
Slight fragmentation of Bevacizumab and Vesencumab was seen upon induction of thermal stress.

ATUM

Forced Degradation: Freeze-Thaw

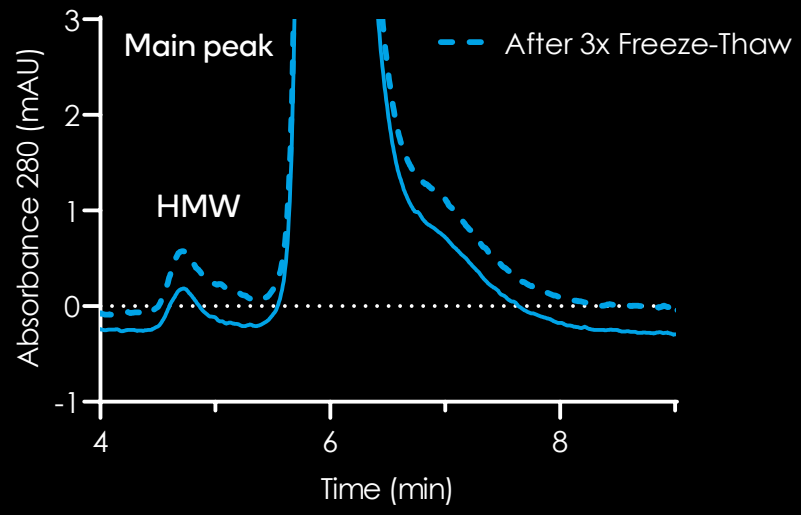
SEC-HPLC

Nivolumab



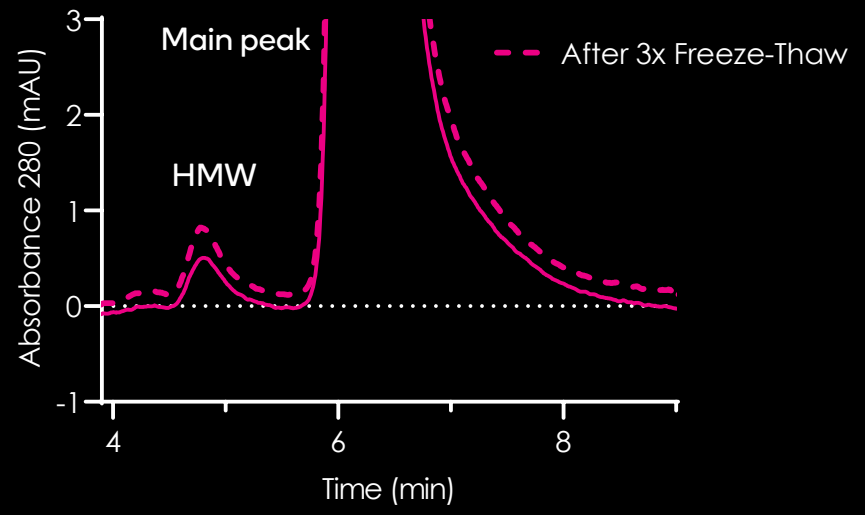
Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	0.5	0.8
% Main Peak	99.5	99.2

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

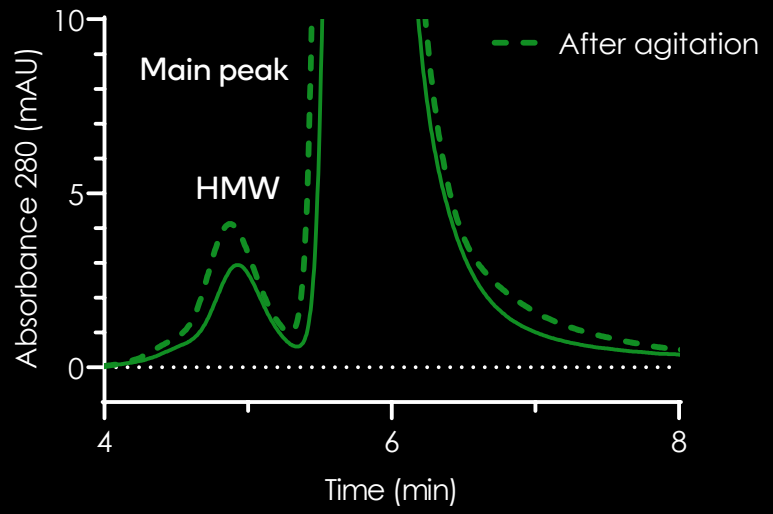
No differences after 3x freeze-thaw cycles.

ATUM

Forced Degradation: Agitation Stress

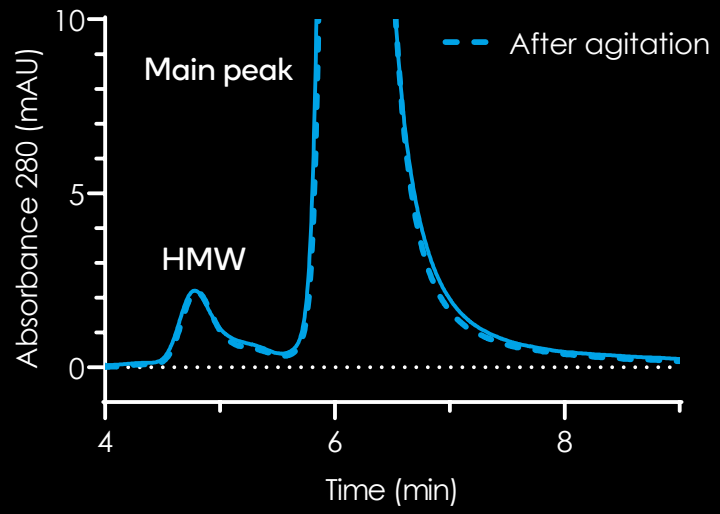
SEC-HPLC

Nivolumab



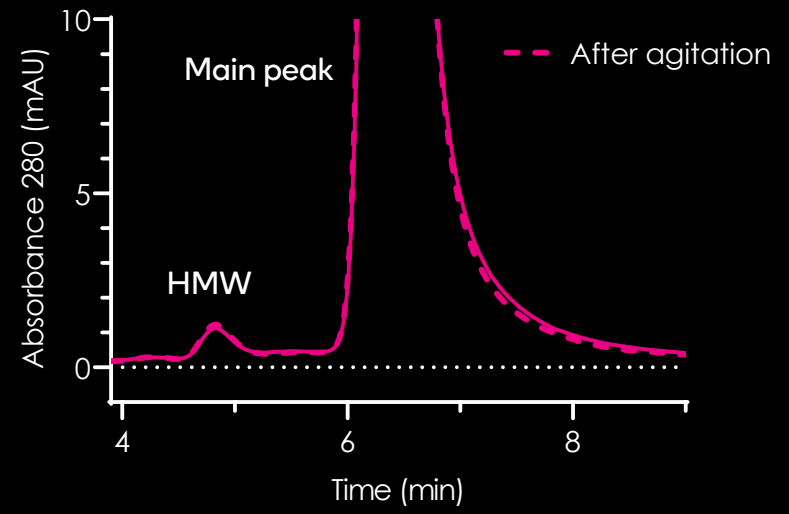
Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% HMW	2.3	3.6
% Main Peak	97.7	96.4

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.

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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

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