mAb Developability Analytics

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Developability Analytics

in silico predictions

- Mol. wt.
- Isoelectric point (PI)
- N-Glycans
- Hydrophobicity – GRAVY
- Cysteine count
- Sequence liabilities (deamidation, isomerization, acid labile)
- Epitope analysis

Discovery/Engineering

- Identity and purity
  - SEC-HPLC
  - µCE-SDS
- Aggregation propensity
  - AC-SINS
  - DLS (Hydrodynamic radius, kD, A2)*
- Thermostability
  - Tm
  - Tagg (DLS*)
- Polyspecificity
  - BVP-ELISA

* Enquire for details

Stability

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

Readout:
- SEC-HPLC
- µCE-SDS

High-throughput ≤ 96 samples

<10 samples

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in silico predictions

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in silico Predictions

Motif Recognition in a Sequence

| *in silico* Analysis	mAb1	mAb2	mAb3
Molecular weight (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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Discovery/Engineering

Identity and Purity: SEC-HPLC
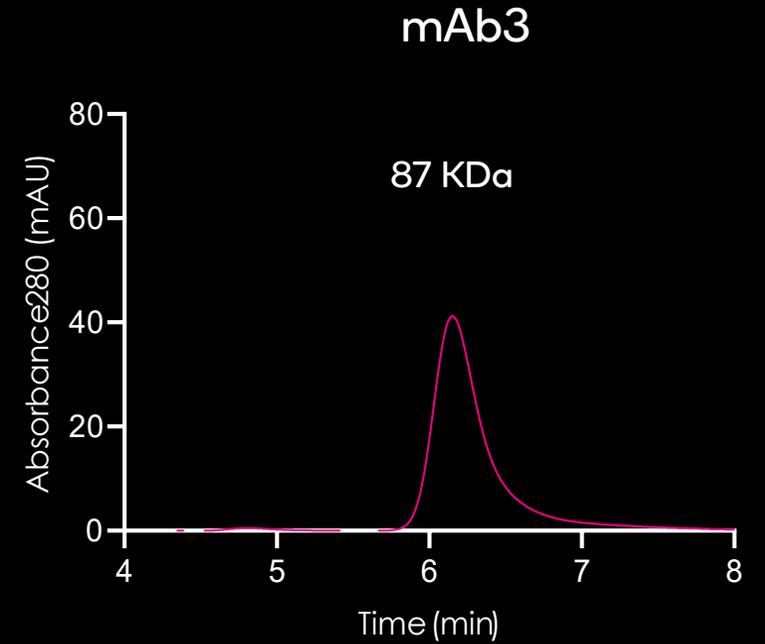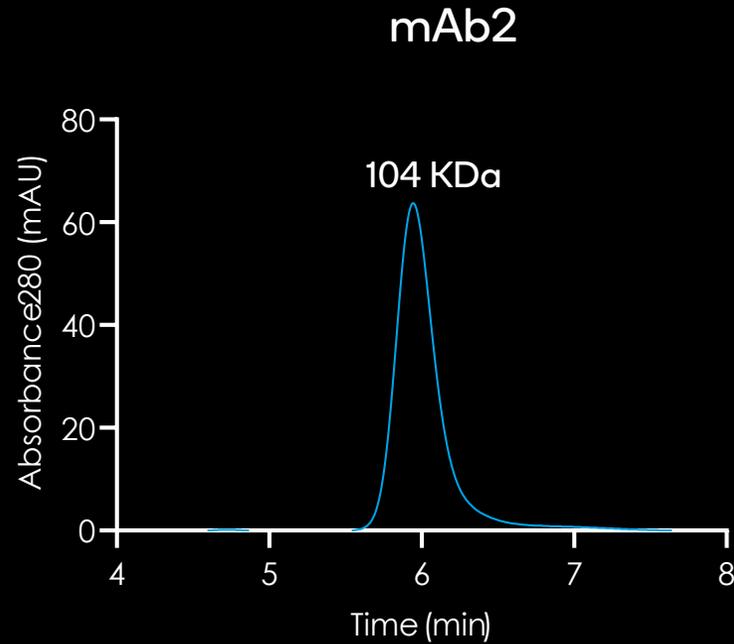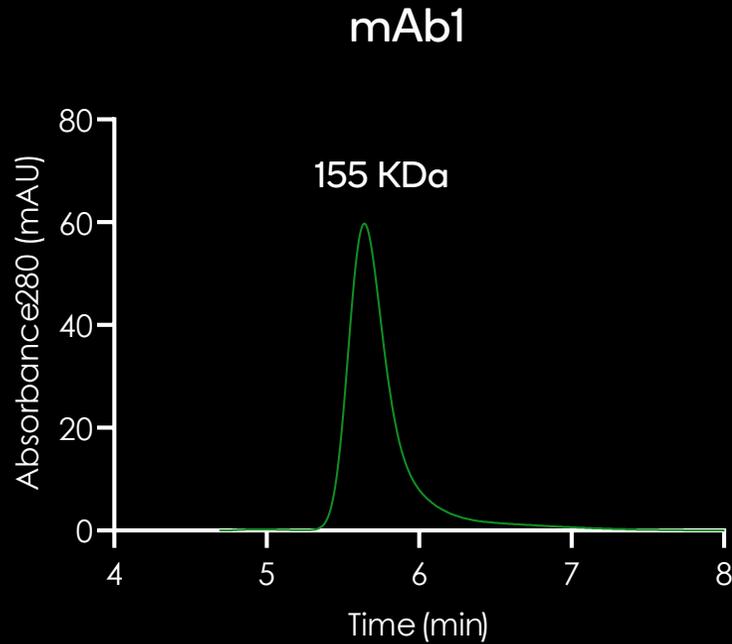
Identity and Purity: μCE-SDS

mAb1 (Reduced)
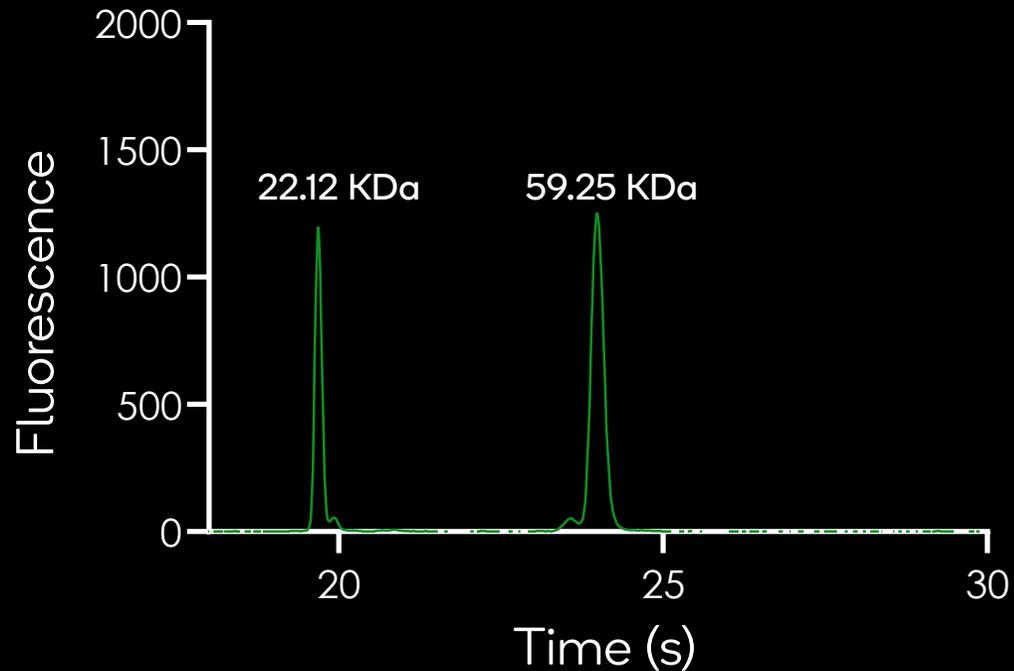
Peak Size (KDa)	Purity %
22.12	37.98
23.72	1.93
54.98	2.50
59.25	57.57

mAb1 (Non-Reduced)
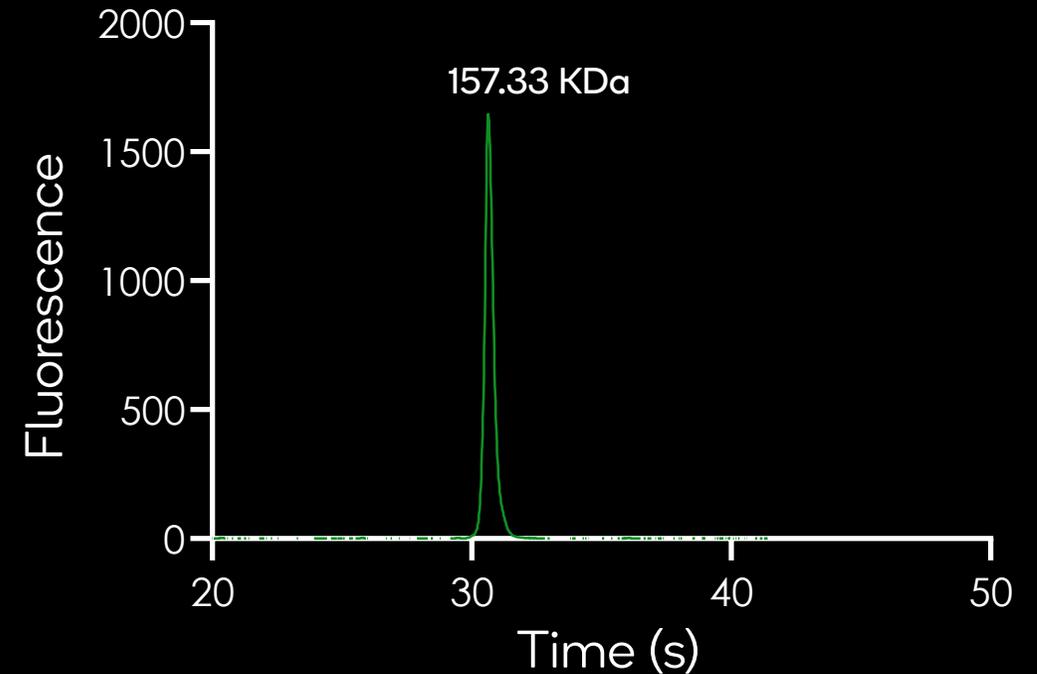
Peak Size (KDa)	Purity %
157.33	100

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Identity and Purity: µCE-SDS

mAb2 (Reduced)
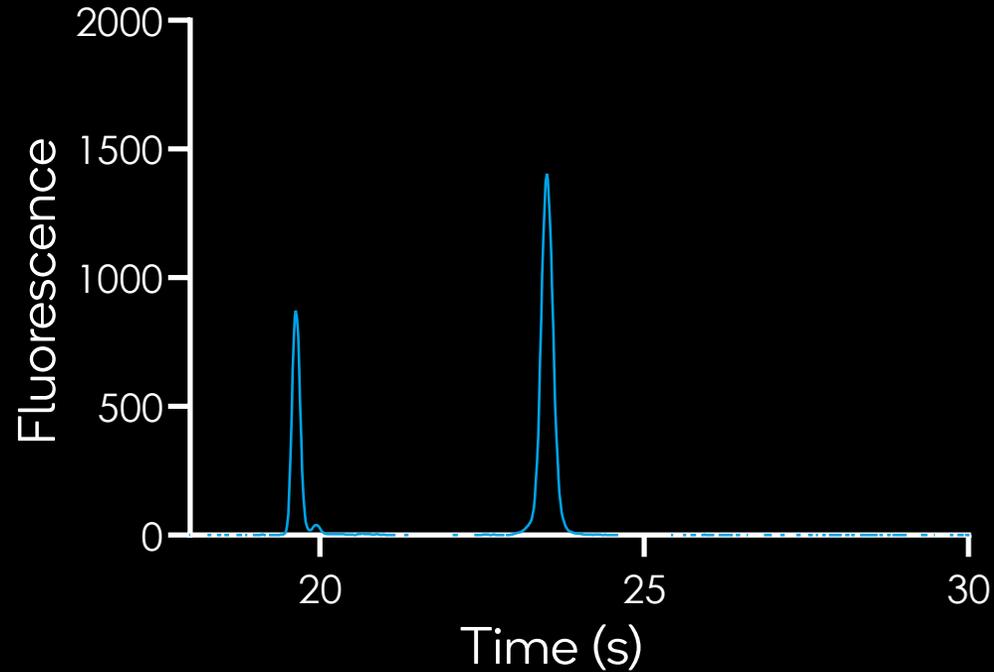
Peak Size (KDa)	Purity %
21.74	33.42
23.79	1.56
54.16	65.01

mAb2 (Non-Reduced)
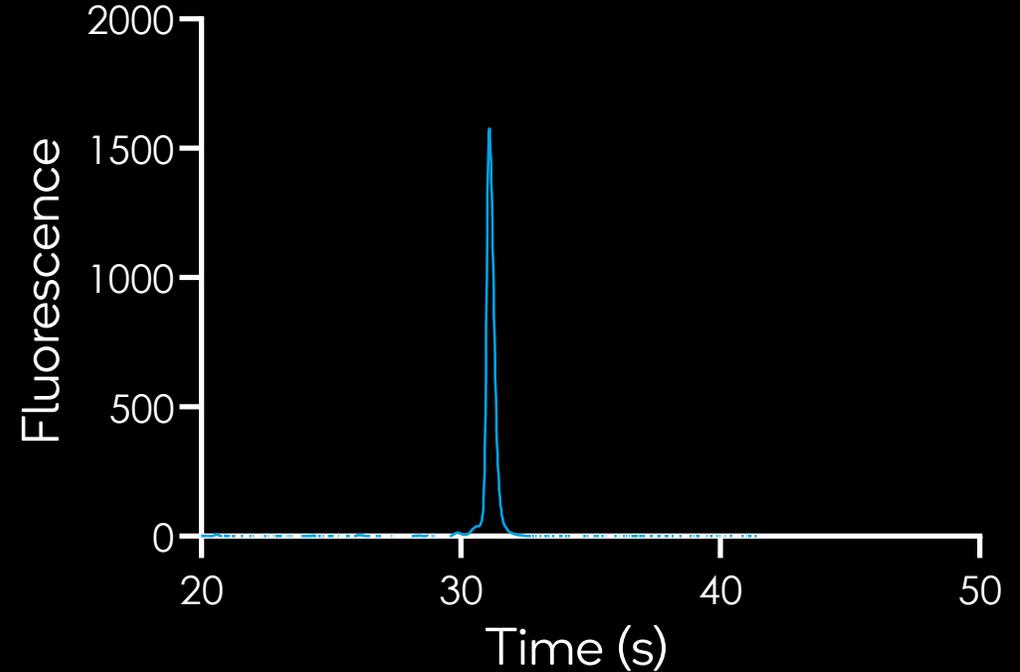
Peak Size (KDa)	Purity %
145.81	0.79
164.83	99.20

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Identity and Purity: μCE-SDS

mAb3 (Reduced)
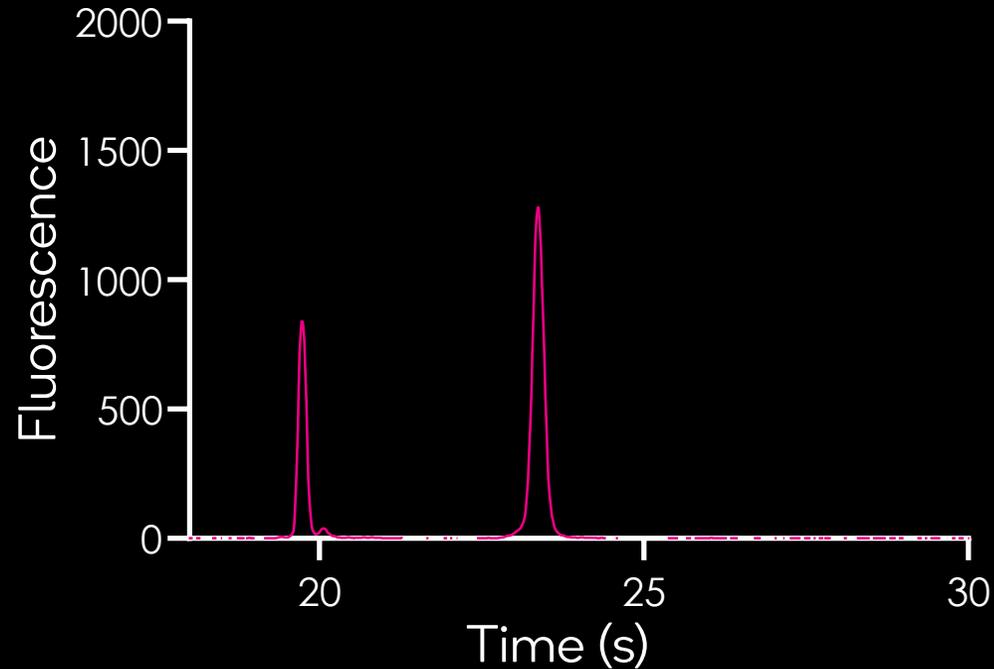
Peak Size (KDa)	Purity %
22.51	34.69
24.69	1.91
52.83	63.38

mAb3 (Non-Reduced)
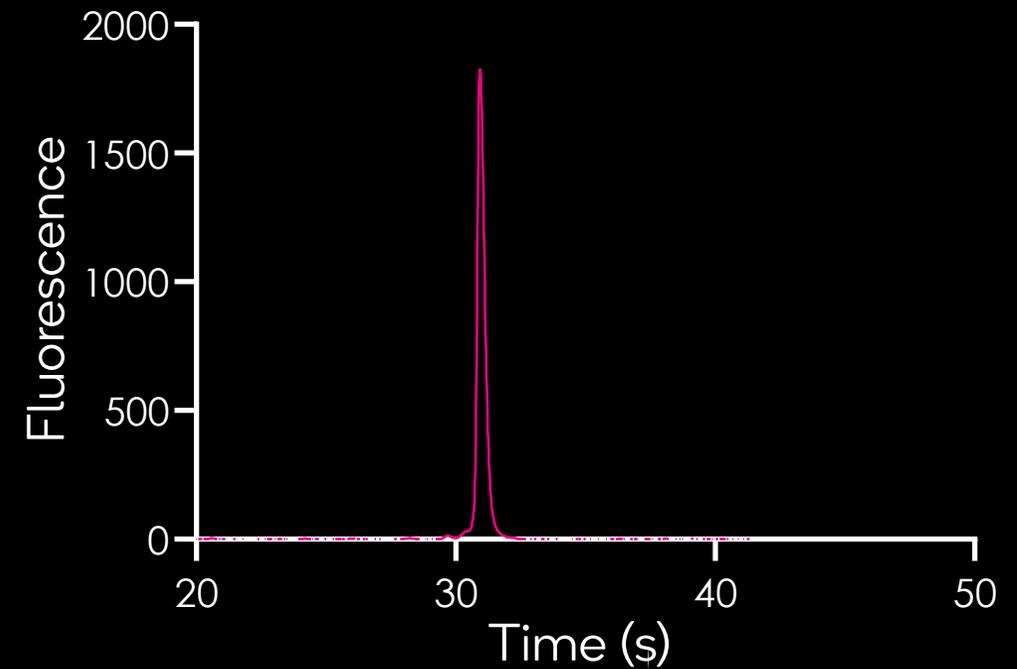
| Peak Size (KDa)	Purity %
142.77	0.63
154.08	1.24
162.18	98.11

Aggregation Propensity: AC-SINS
(Affinity Capture - Self Interaction Nanoparticle Spectroscopy)
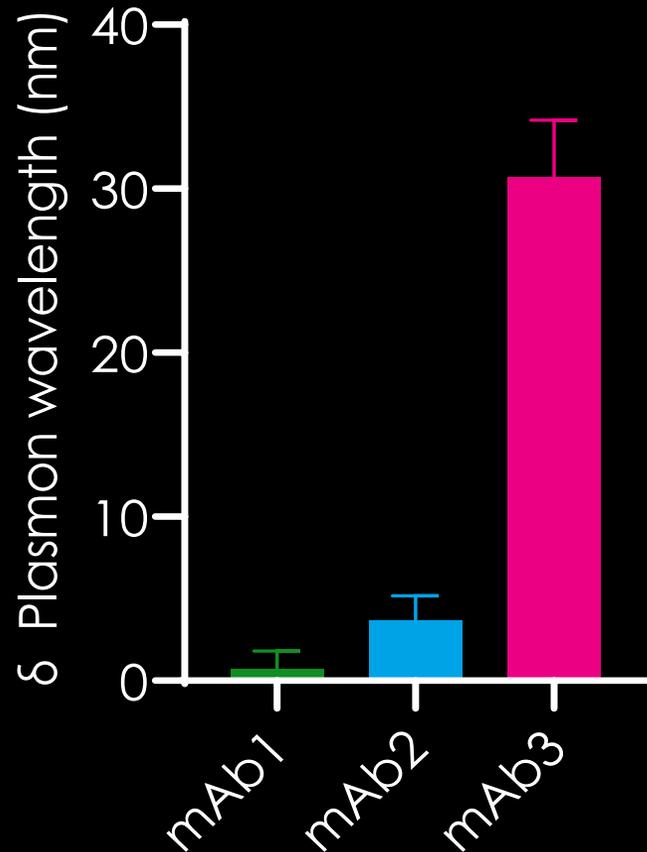
- A high-throughput method to detect antibody self interaction.

- Higher signal = Higher aggregation

Aggregation Propensity: DLS (k_D and A_2)
(Dynamic Light Scattering)

Antibody	k_D (x 10^-9)
mAb1	-3.17
mAb2	-6.93
mAb3	-8.81

| Antibody	A_2 (x 10^-8)
mAb1	-0.48
mAb2	-8.51
mAb3	-15.48
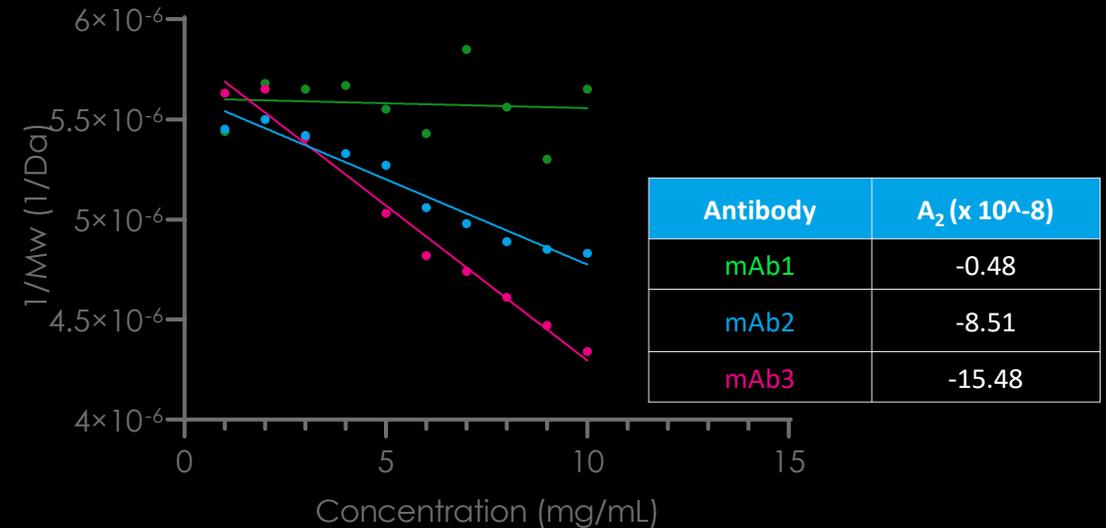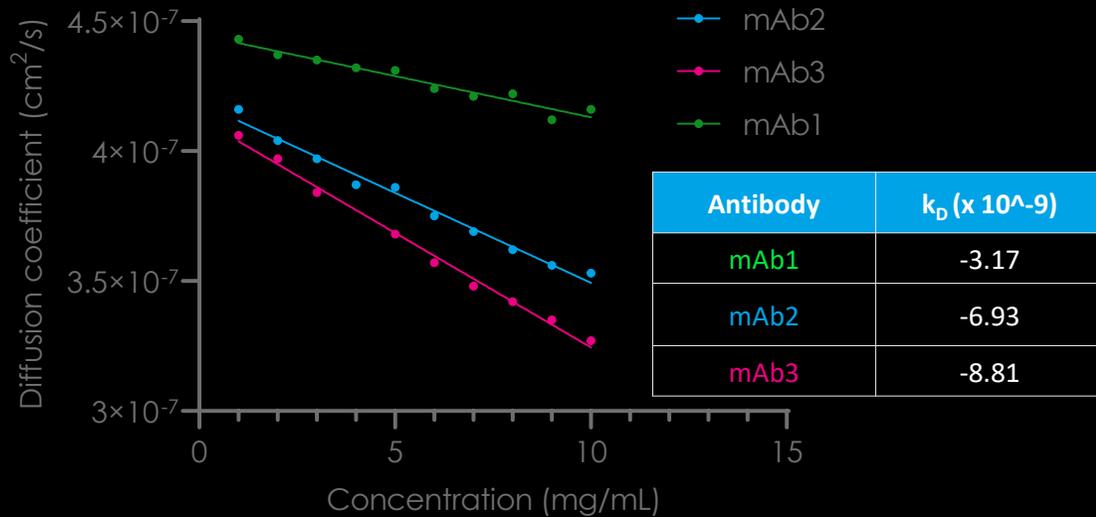
DLS can be used to rank molecules for their propensity to self-aggregate.

Two parameters (kD and A2) can be negative (self-association), neutral (no interaction) or positive (repulsive forces).

Thermo Stability: Tm
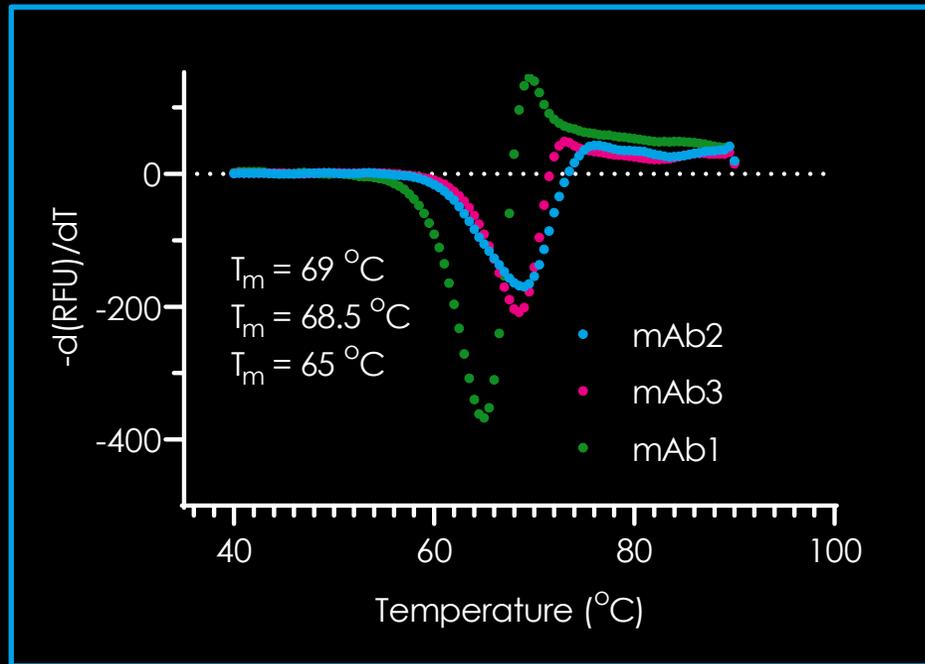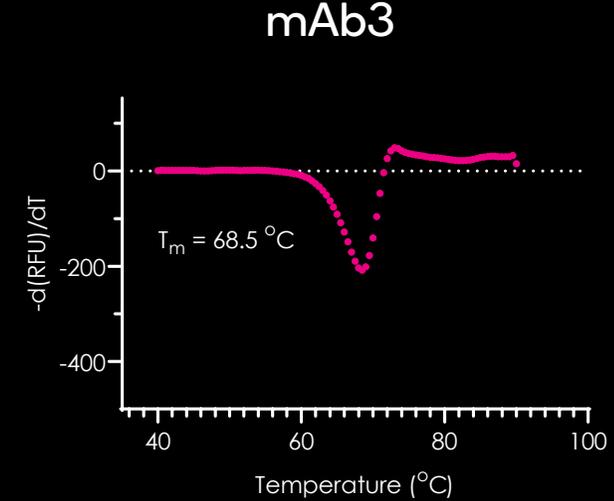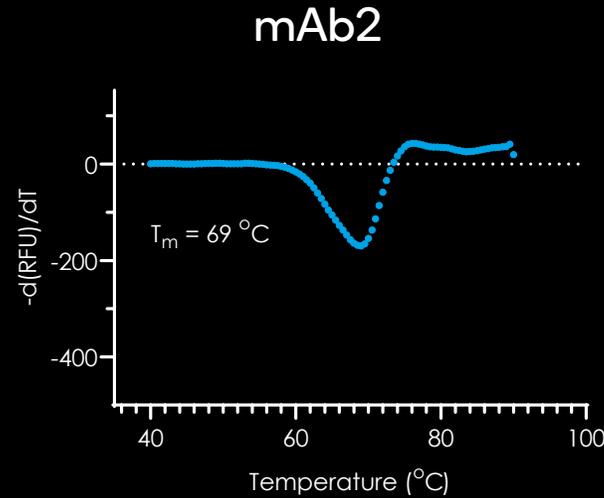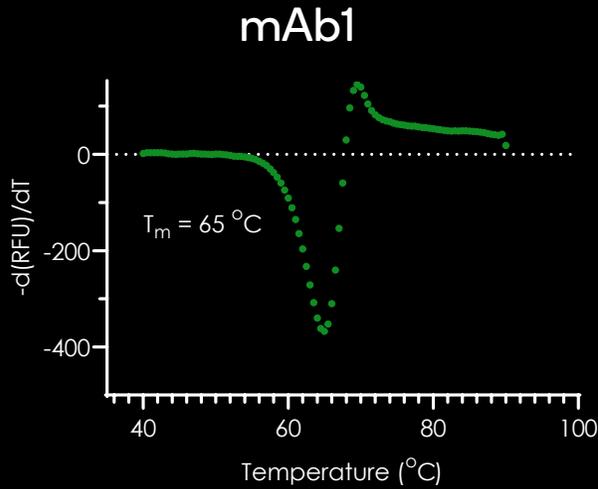
Higher Tm = Higher conformational stability and favorable developability.

Polyspecificity: BVP-ELISA
## *(BaculoViral Particle – ELISA)*
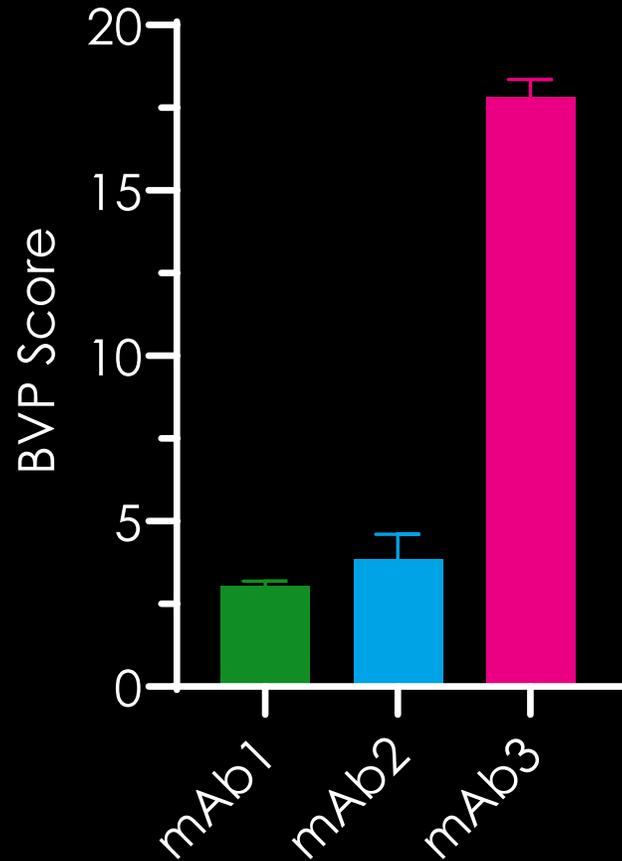
- A high-throughput method to detect polyspecificity of antibody candidates.

- Higher BVP score = Poorer *in vivo* PK

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Stability

Forced Degradation: pH Stress (pH 4)
SEC-HPLC

mAb1
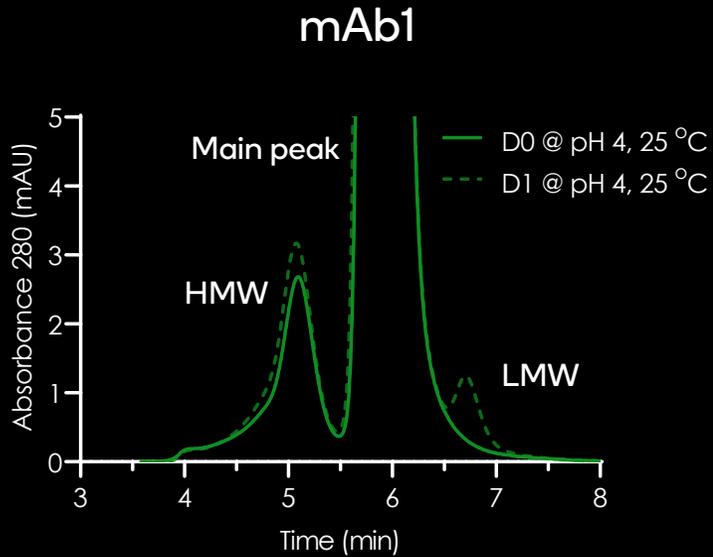
Peak Assignment	D0 @ pH 4, 25 °C	D1 @ pH 4, 25 °C
% HMW	2.5	2.8
% Main Peak	97.5	96.8
% LMW	0.0	0.4
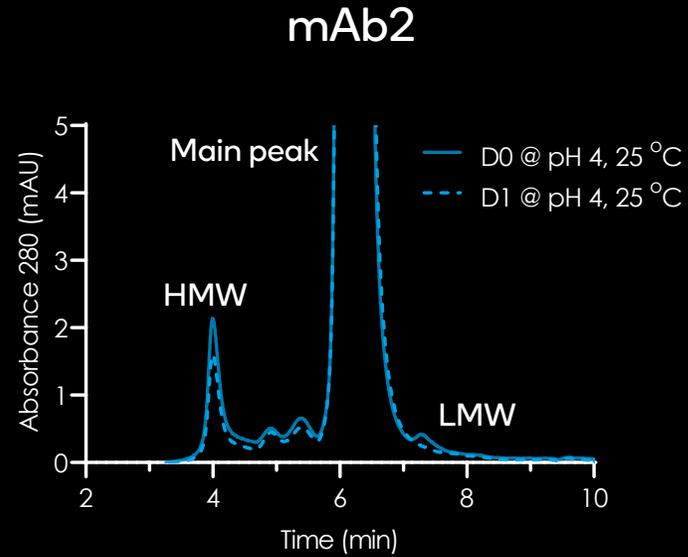
mAb2

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
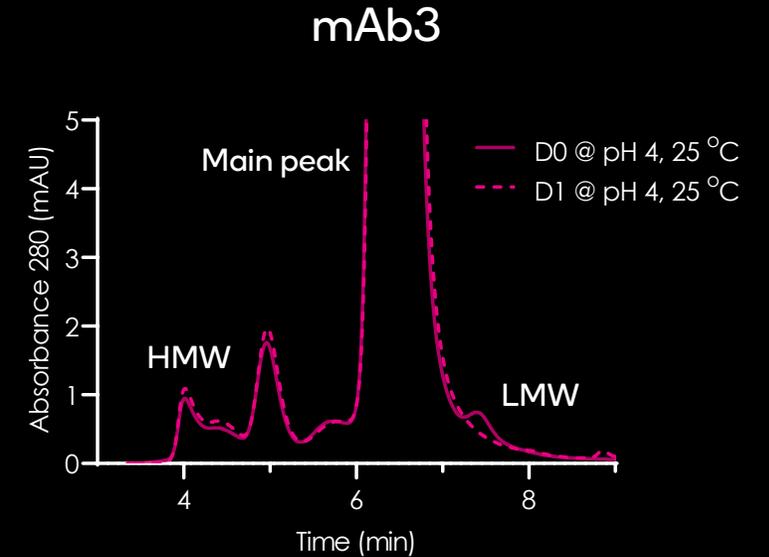
mAb3

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

mAb1, mAb2 and mAb3 show a slight increase in aggregation and fragmentation upon induction of low pH stress.

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Forced Degradation: pH Stress (pH 4)
μCE-SDS

mAb1

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

mAb2

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

mAb3

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

mAb1, mAb2 show a small but detectable increase in fragmentation upon induction of low pH stress.

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

mAb1
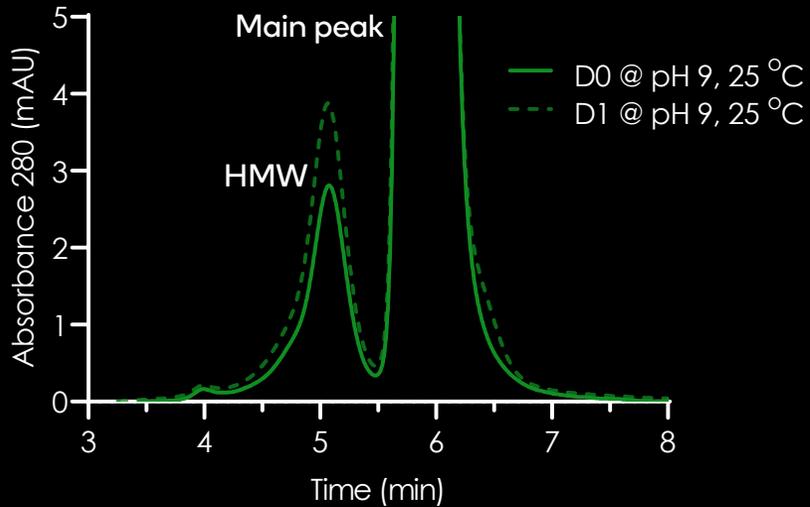
Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.2	4.6
% Main Peak	96.8	95.4
% LMW	0.0	0.0

mAb2
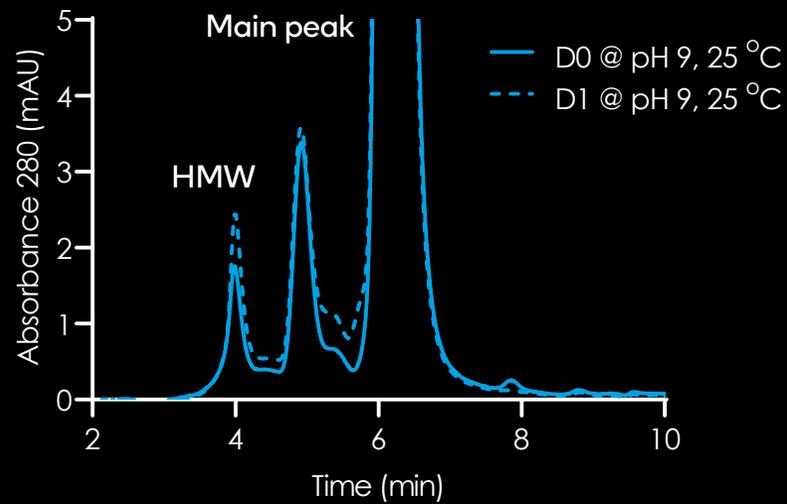
Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.0	3.8
% Main Peak	97.0	96.2
% LMW	0.0	0.0

mAb3
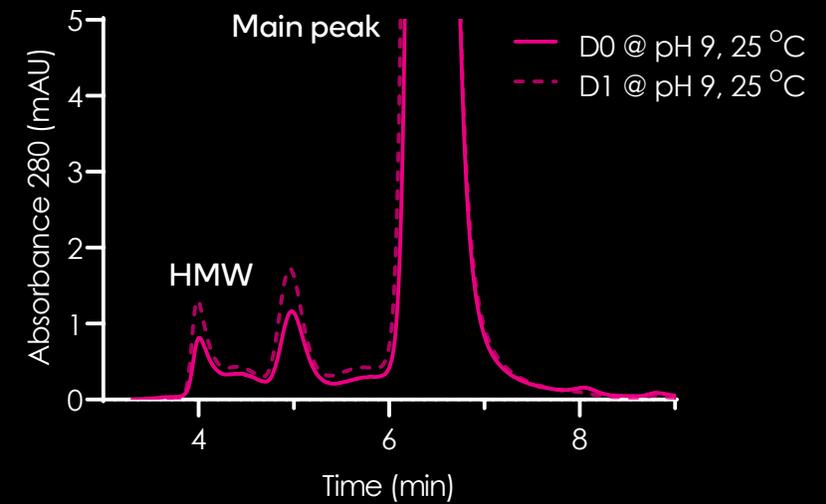
Peak Assignment	D0 @ pH 9, 25 °C	D1 @ pH 9, 25 °C
% HMW	3.0	3.4
% Main Peak	97.0	96.6
% LMW	0.0	0.0

mAb1, mAb2 and mAb3 showed a slight increase in aggregation upon induction of high pH stress

Forced Degradation: pH Stress (pH 9)
μCE-SDS

mAb1

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

mAb2

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

mAb3

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

- mAb2 showed a detectable increase in aggregation upon induction of high pH stress.

- mAb1 and mAb3 were resistant to high pH stress.

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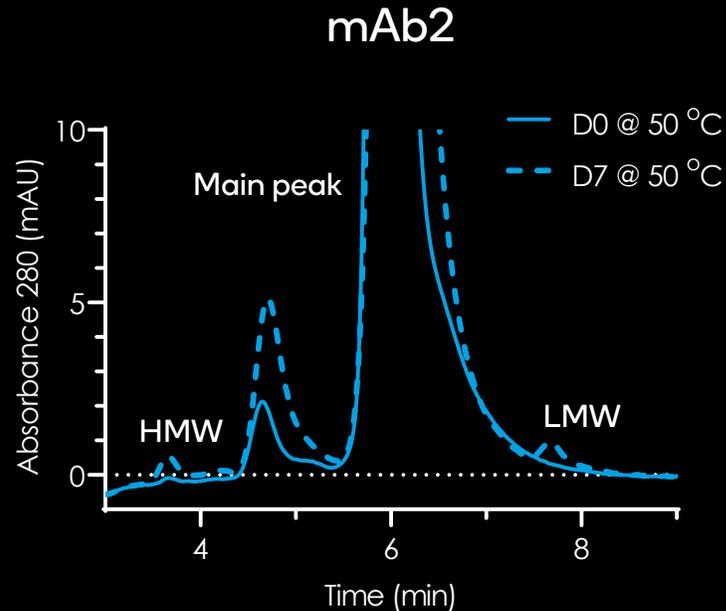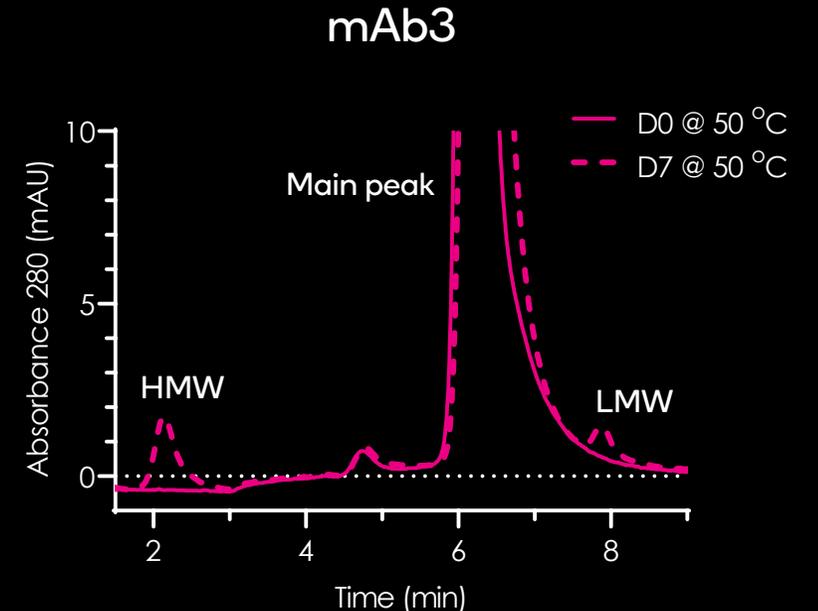
Forced Degradation: Thermal Stress
SEC-HPLC

mAb1, mAb2 and mAb3 show increased aggregation and fragmentation upon induction of thermal stress.

Forced Degradation: Thermal Stress
SEC-HPLC

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

mAb1, mAb2 and mAb3 show increased aggregation and fragmentation upon induction of thermal stress.

Forced Degradation: Thermal Stress
μCE-SDS

mAb1

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

mAb2

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

mAb3

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

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Forced Degradation: Freeze-Thaw
SEC-HPLC

mAb1
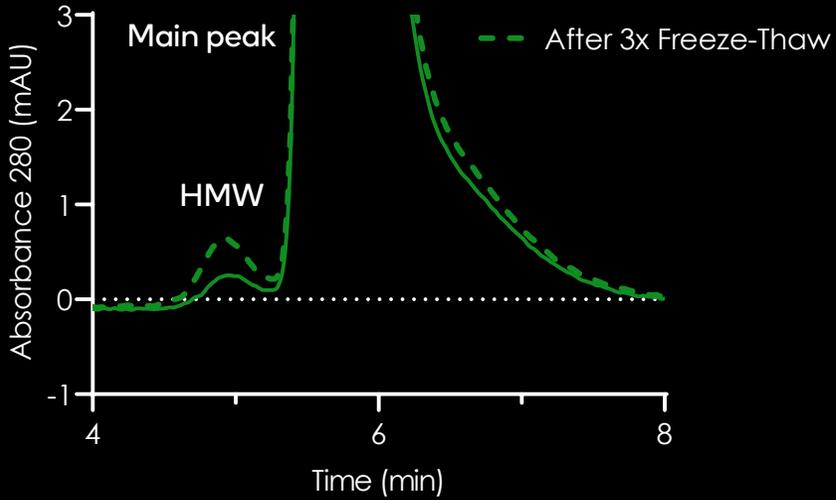
Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	0.5	0.8
% Main Peak	99.5	99.2

mAb2
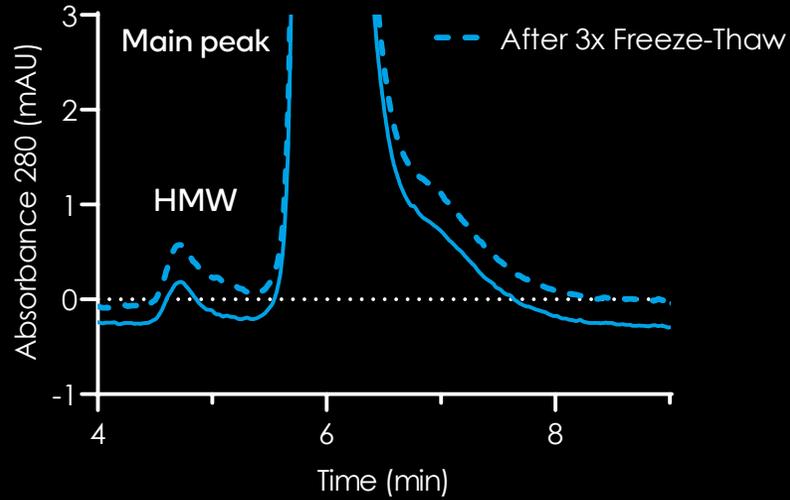
Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	0.4	0.5
% Main Peak	99.6	99.5

mAb3
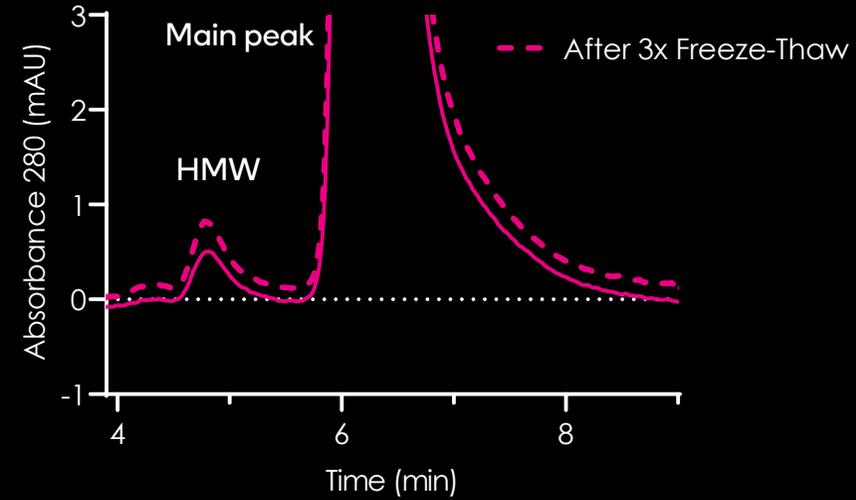
Peak Assignment	Before	After 3x Freeze-Thaw
% HMW	1.2	1.6
% Main Peak	98.8	98.4

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Forced Degradation: Freeze-Thaw
μCE-SDS

mAb1

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

mAb2

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

mAb3

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

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Forced Degradation: Agitation Stress
SEC-HPLC
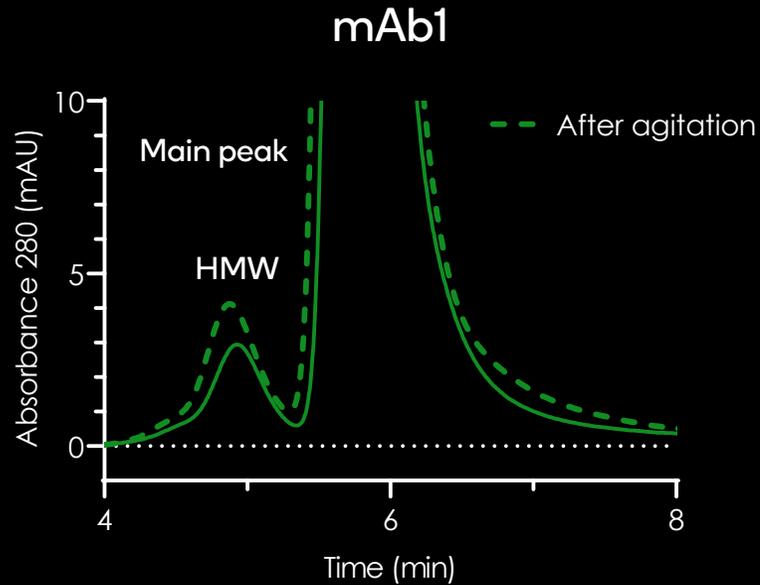
mAb1

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

mAb2
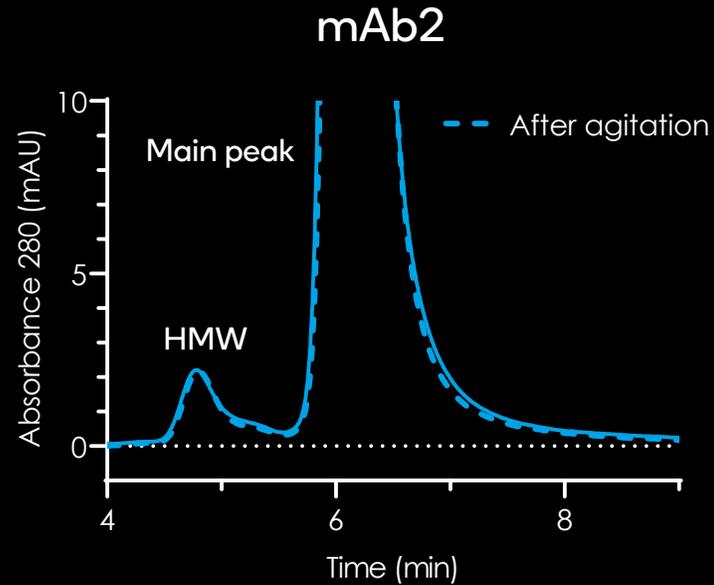
Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% HMW	2.2	2.1
% Main Peak	97.8	97.9

mAb3
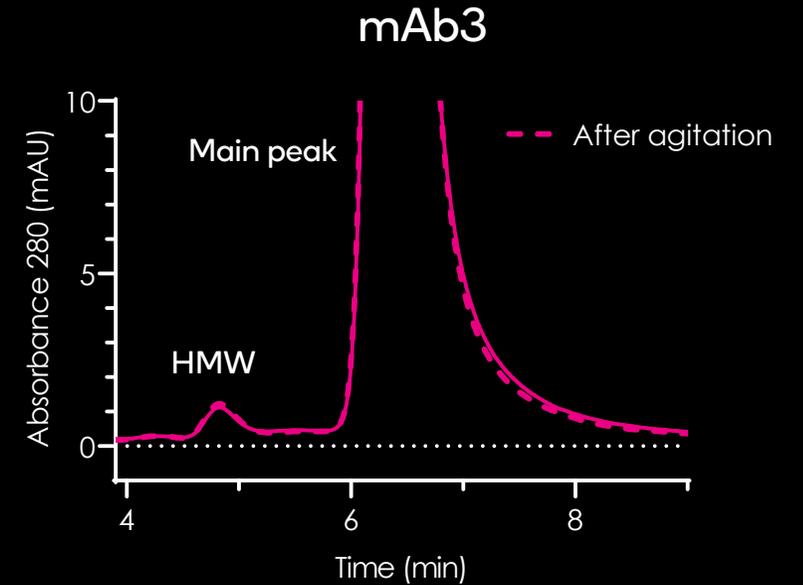
Peak Assignment	D0 @ 300 rpm	D2 @ 300 rpm
% HMW	1.0	1.1
% Main Peak	99.0	98.9

Forced Degradation Study: Agitation Stress
μCE-SDS

mAb1

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

mAb2

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

mAb3

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

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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

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