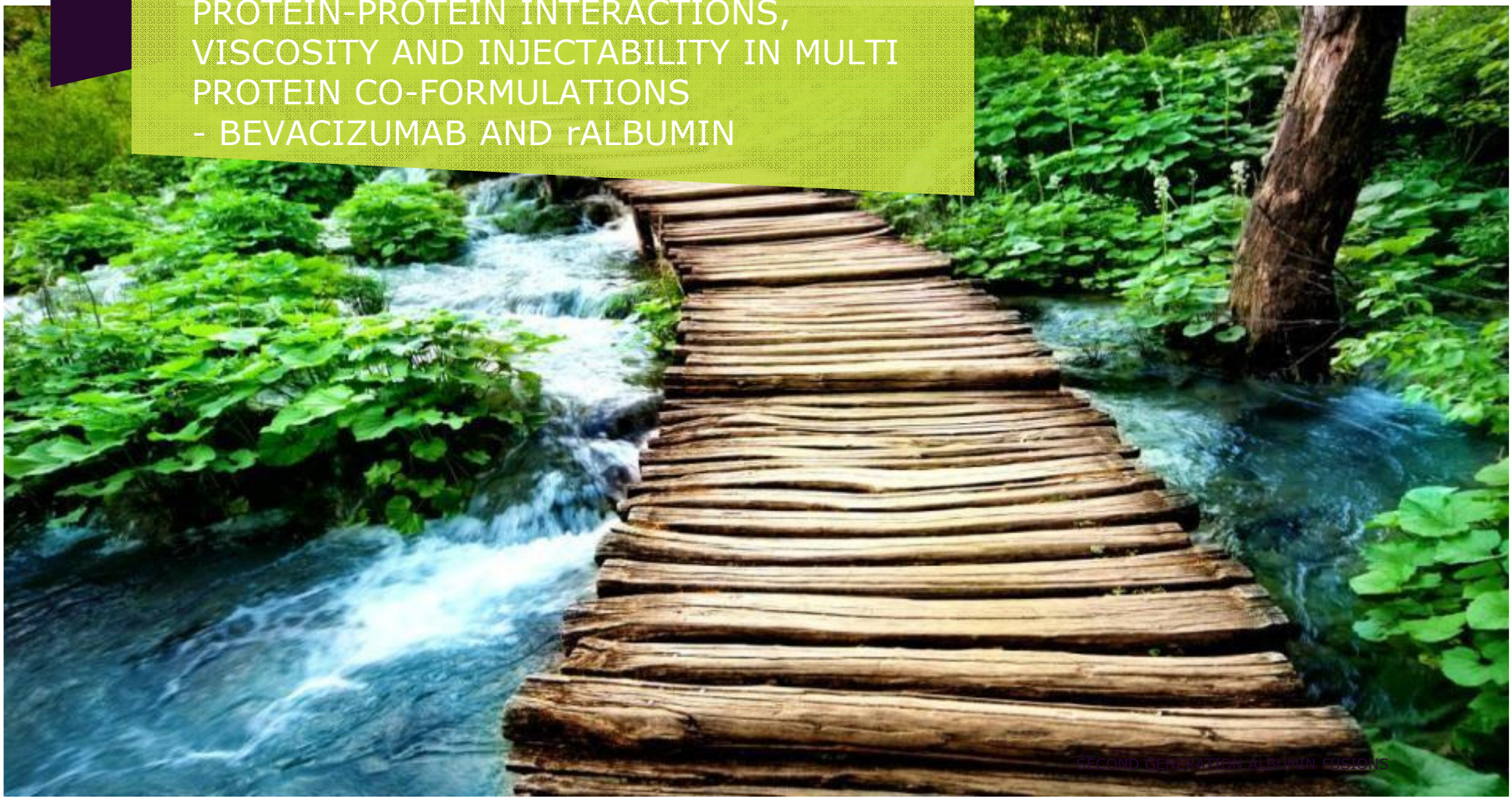
PROTEIN-PROTEIN INTERACTIONS, VISCOSITY AND INJECTABILITY IN MULTI PROTEIN CO-FORMULATIONS - BEVACIZUMAB AND rALBUMIN



Jens T. Bukrinsky, PhD
Senior formulation Scientist
At Novozymes Biopharma A/S

Cambridge October 2013

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AGENDA

Case study – on aggregation prevention

- Analytical challenges and preliminary data
- Mechanism
  - SAXS
  - DLS
  - AF4
  - Viscosity

HUMAN SERUM ALBUMIN - PROPERTIES

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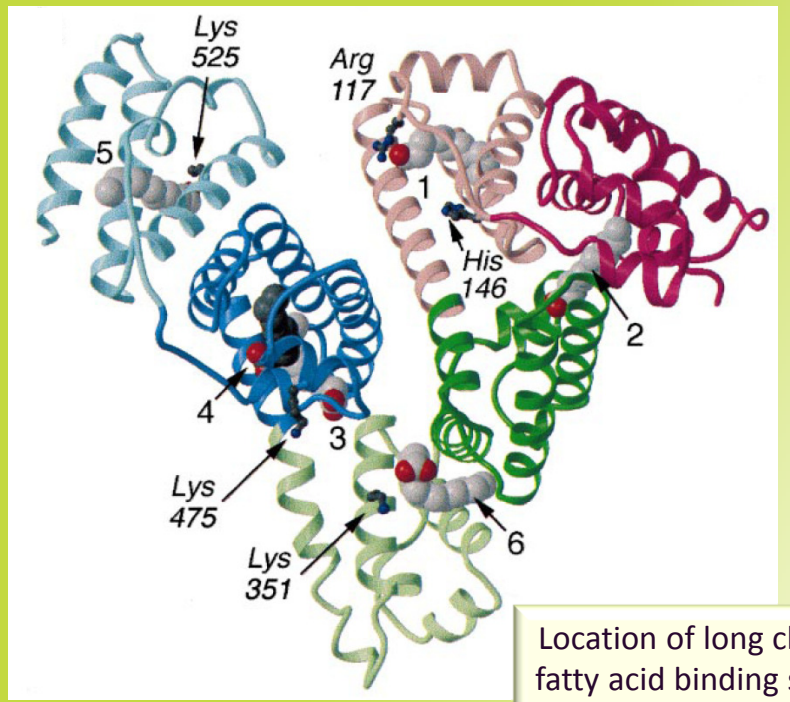
- Structure
  - 585 amino acids, Single chain
  - Three domains
  - 17 disulfide bridges
  - **Cys34 is unpaired**
  - One tryptophan
  - 66472 g/mol
  - **Hydrophobic patches/cavities**
- IpH = 5.9
- **Soluble up to >400 g/L**
- Approximately 50 g/L in blood
- High physical stability
- Long plasma ½ life
  - 19-20 days
- Inert
  - Safe



Location of long chain fatty acid binding sites

Curry *et al.* (1999)
BBA **1441**, 131-140

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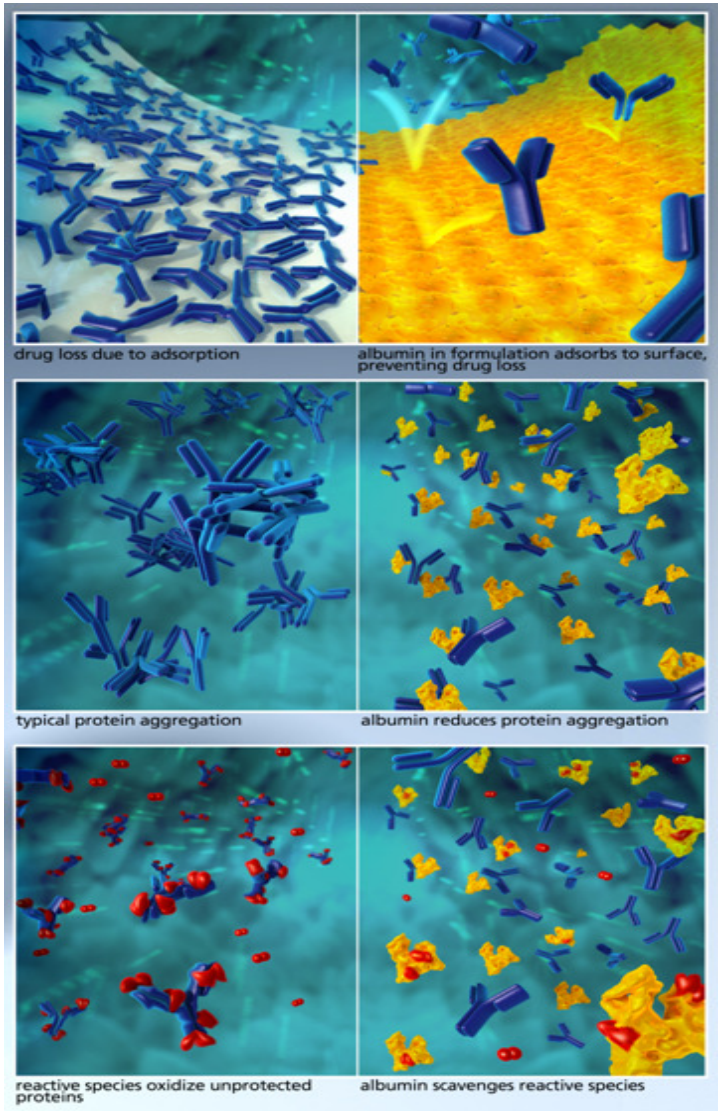
How does rAlbumin stabilize protein formulations

HSA in blood	rAlb in formulation
Multiple hydrophobic binding sites	Coat hydrophobic surfaces in primary packaging materials
Increases the colloidal stability of blood	Prevent self-association of protein drugs
Natural antioxidant in blood	Prevent oxidation of protein drugs




ANALYSIS OF AGGREGATES
- A CHALLENGE FOR COMPLEX FORMULATIONS
- PRELIMINARY DATA
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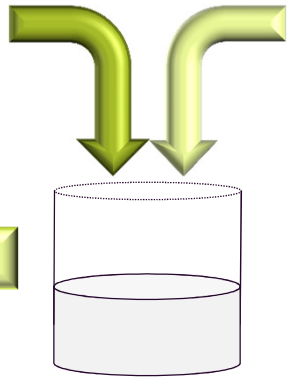
Prevention of protein aggregation in high concentration mAb formulations

Method set-up

Avastin (Bevacizumab)

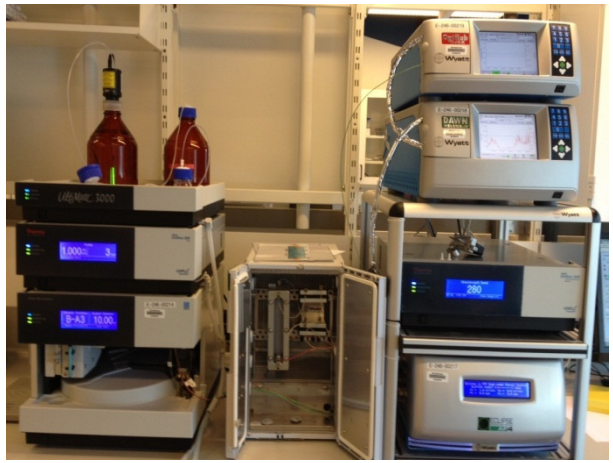
Recombumin Alpha (rAlb) or buffer



Formulation

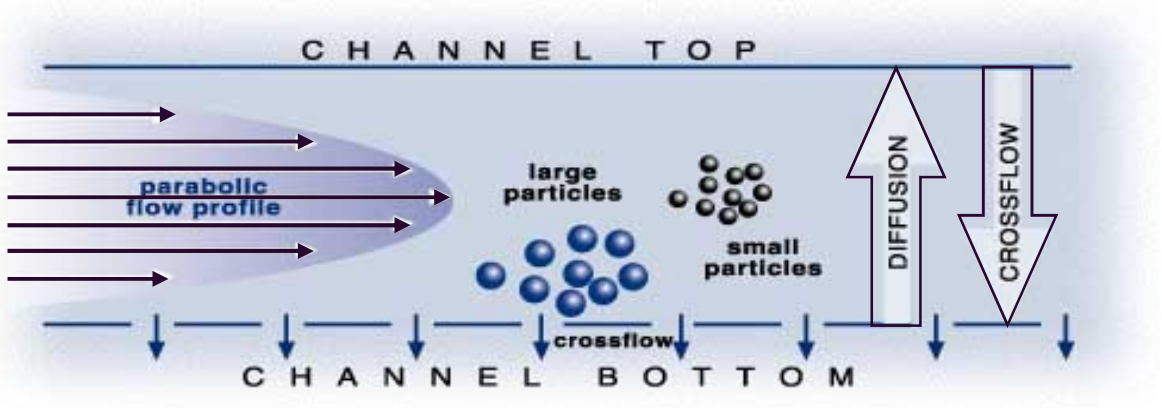
Transfer to Glass vial

29 days 40°C

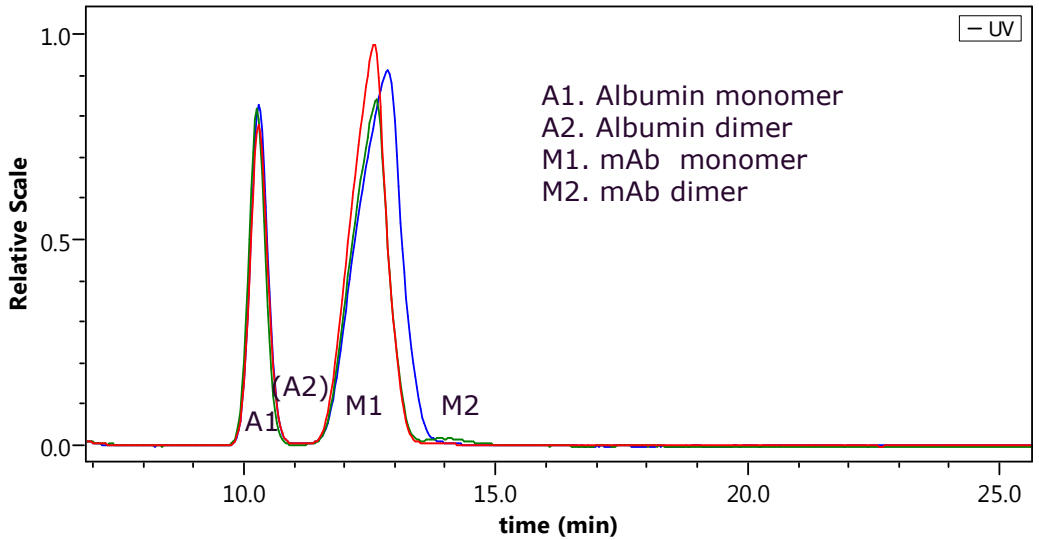


AF4-UV-MALS-RI analysis

AF4-UV-MALS-RI ANALYSIS



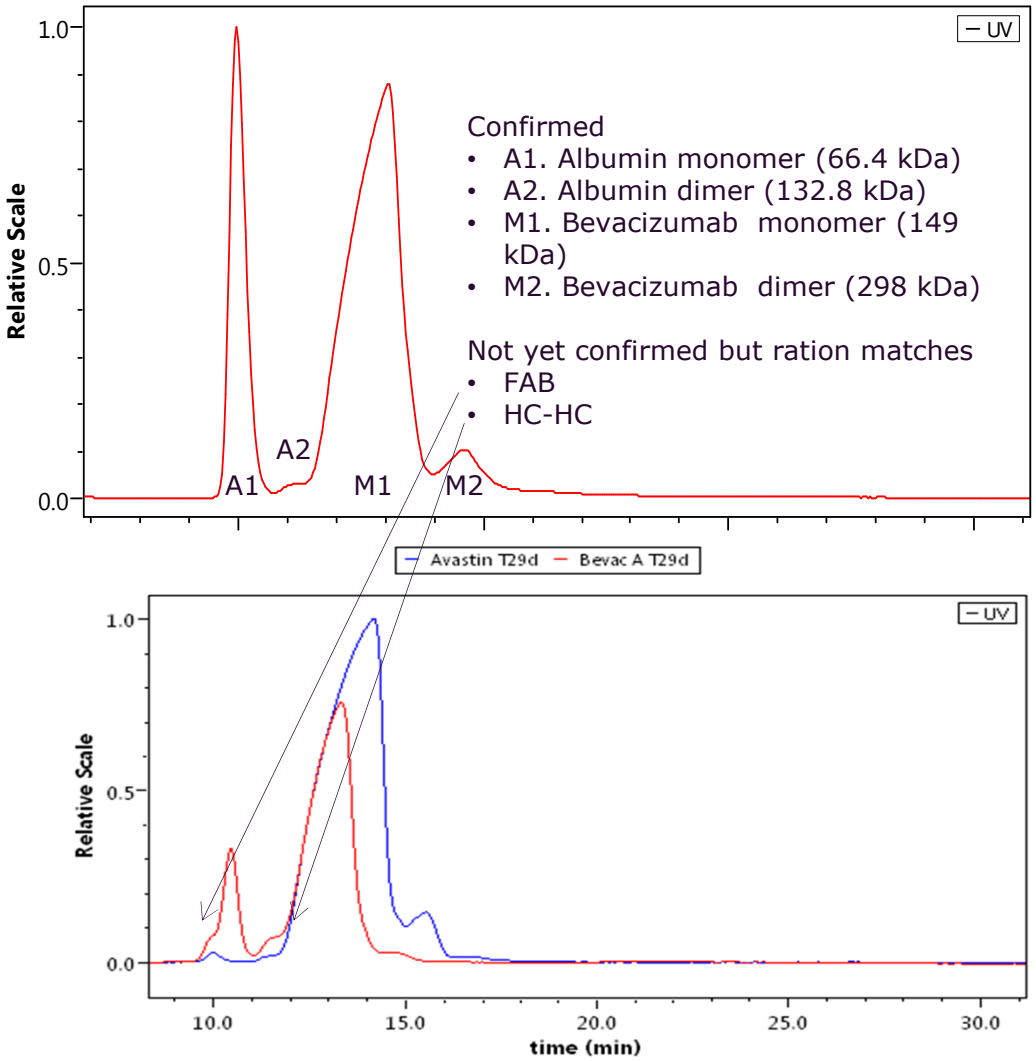
- **AF4: Asymmetric Flow Field Flow Fractionation**
- **Separation takes place in the channel based on differences in diffusion**



AF4 separation works well with 4 out of 4 tested mAbs:

Omalizumab (**blue**)
Rituximab (**green**)
Tocilizumab (**red**)
Bevacizumab (**next slide**)

Aggregation prevention of ≥100 mg/mL Bevacizumab by 50 mg/mL rAlbumin



- AF4 separation:
  - Short channel
  - Spacer 350S
  - 10 kDa RC membrane
  - 50 mM $NaNO_3$, pH 7.2
  - Channel flow 1 ml/min
  - Cross flow gradient 3-1 mL/min (15 min)
- Bevacizumab:rAlb mixture ≥100:50 mg/mL (in red)
- Avastin 25 mg/mL(in blue)

	Monomer	Dimer
Avastin	93,0	6,4
Bevacizumab+albumin	92,3	2,2

Prevention of freeze-thaw induced formation of sub-visible particles in proteins/peptides?

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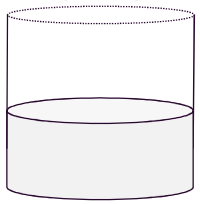
Method set-up

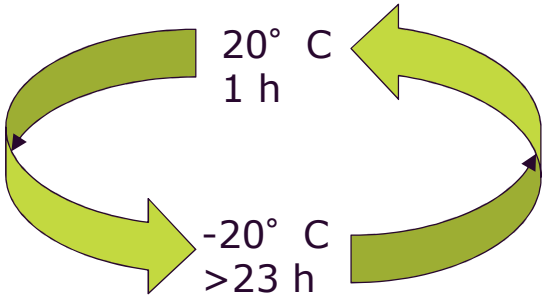
RoActemra (Tocilizumab)

Albucult (rAlb) or buffer

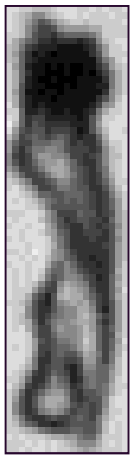


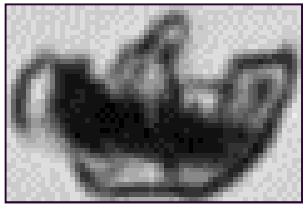
Transfer to Eppendorff tube

X 3



Analysis by Micro-flow Imaging (MFI)
Sub-visible particles (1-100 µm)





Albucult® prevents the formation of sub-visible particles in Tocilizumab (a monoclonal antibody)

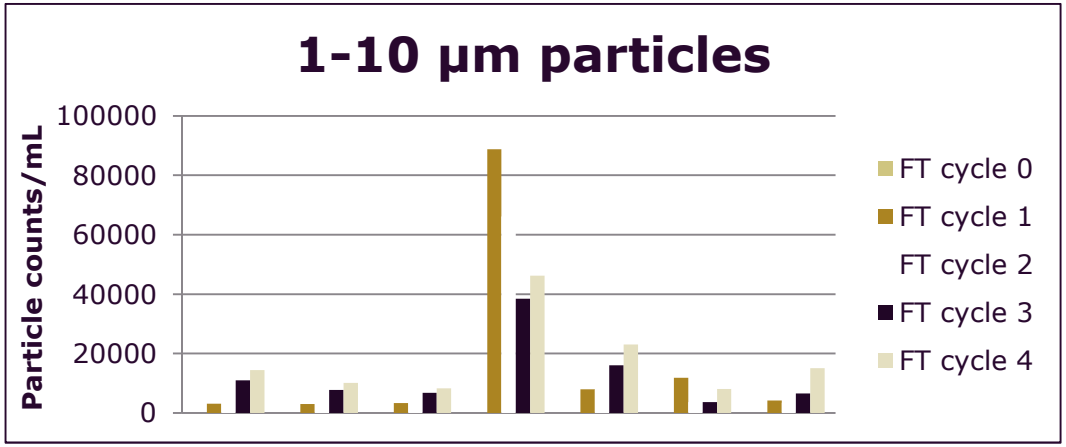
Reference formulations

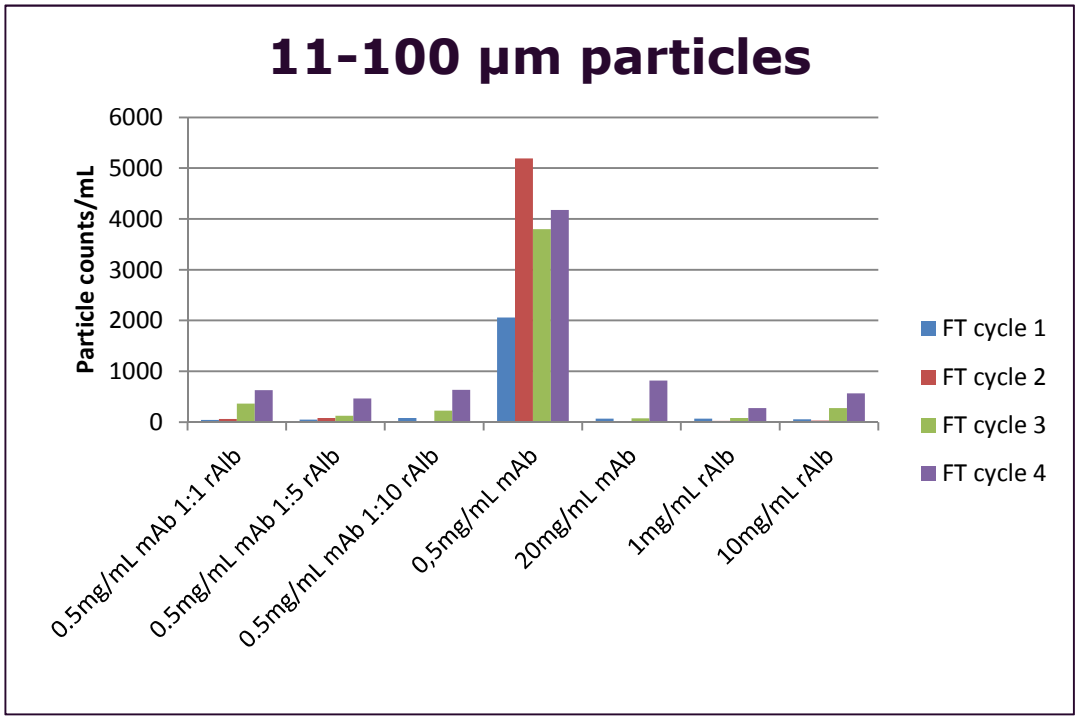
- 3.4 µm (0.5 mg/mL) Tocilizumab (diluted w milliQ)
- 137 µm (20 mg/mL) Tocilizumab (RoActemra®, Roche)
- 15 µm (1.0 mg/mL)Albucult (dil. w milliQ)
- 150 µm (10 mg/mL) Albucult (dil. w milliQ)

Test samples

- 0.5 mg/mL Tocilizumab formulated with Albucult in mAb:rAlb molar ratios
  - 1:1
  - 1:5
  - 1:10

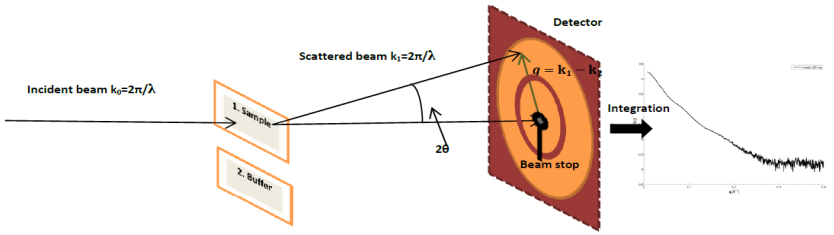
ALBUCULT® PREVENTS FORMATION OF FREEZE THAW INDUCED SUB-VISIBLE PARTICLES IN MAB FORMULATIONS

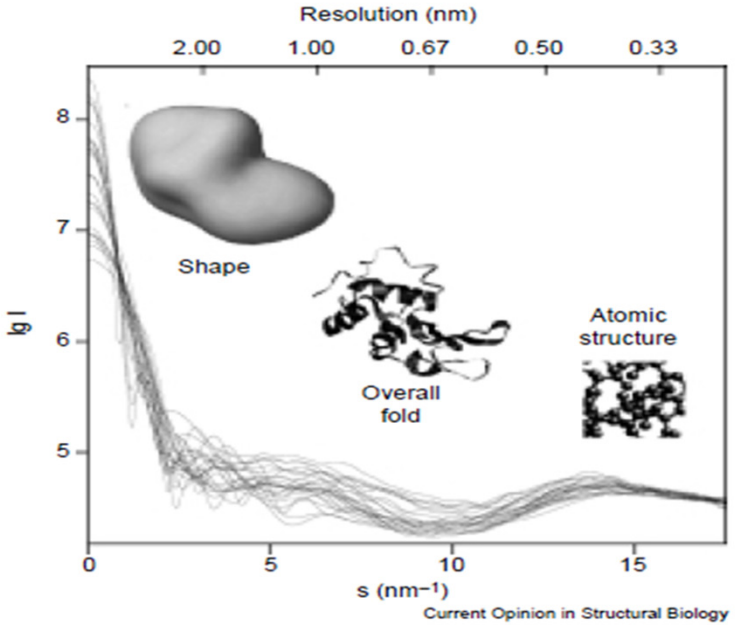




Mechanism behind aggregation prevention

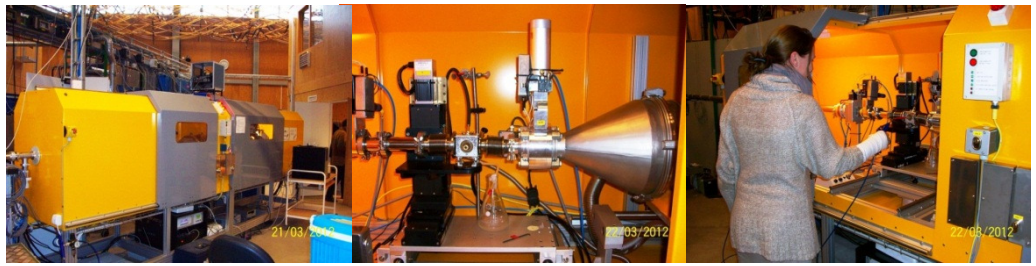
Small Angle X-ray Scattering Determination of solution structure



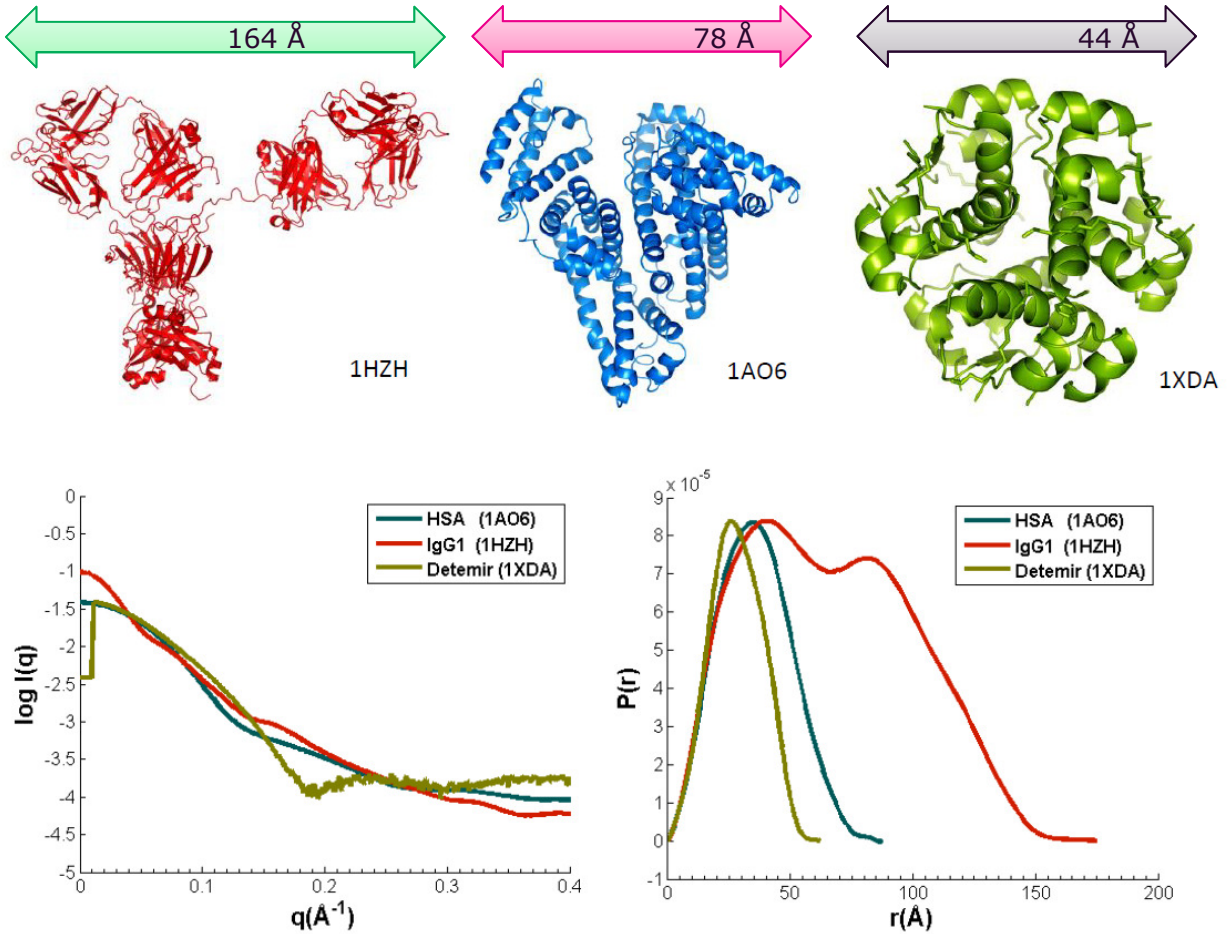


Svergun, D.I., Koch, M.H.J., 2002. Advances in structure analysis using small-angle scattering in solution. Current opinion in structural biology 12, 654–60.

MaxLab Beamline I911-SAXS

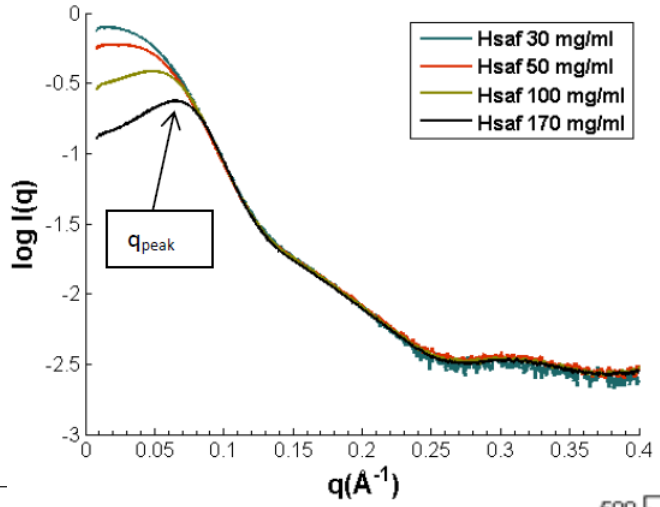


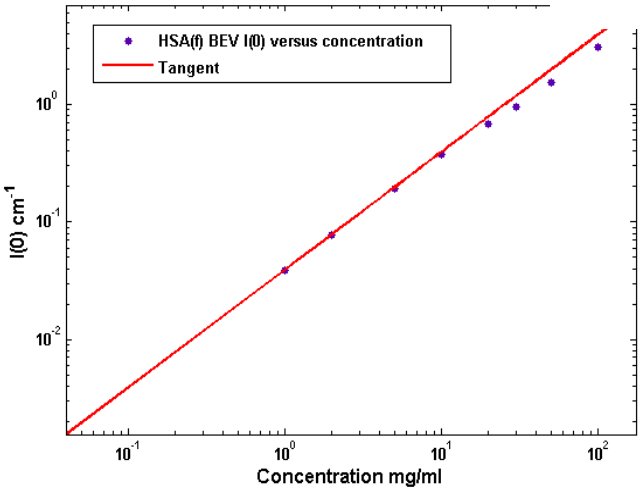
Pair distribution function

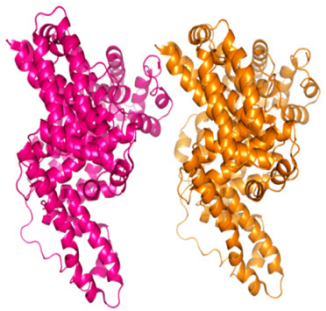


rAlb is a monomer with a concentration dependent repulsive behaviour

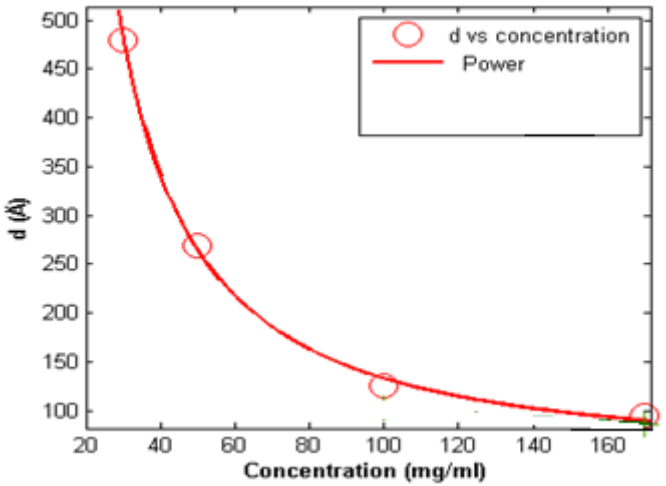
- Repulsion evaluated from I(0)



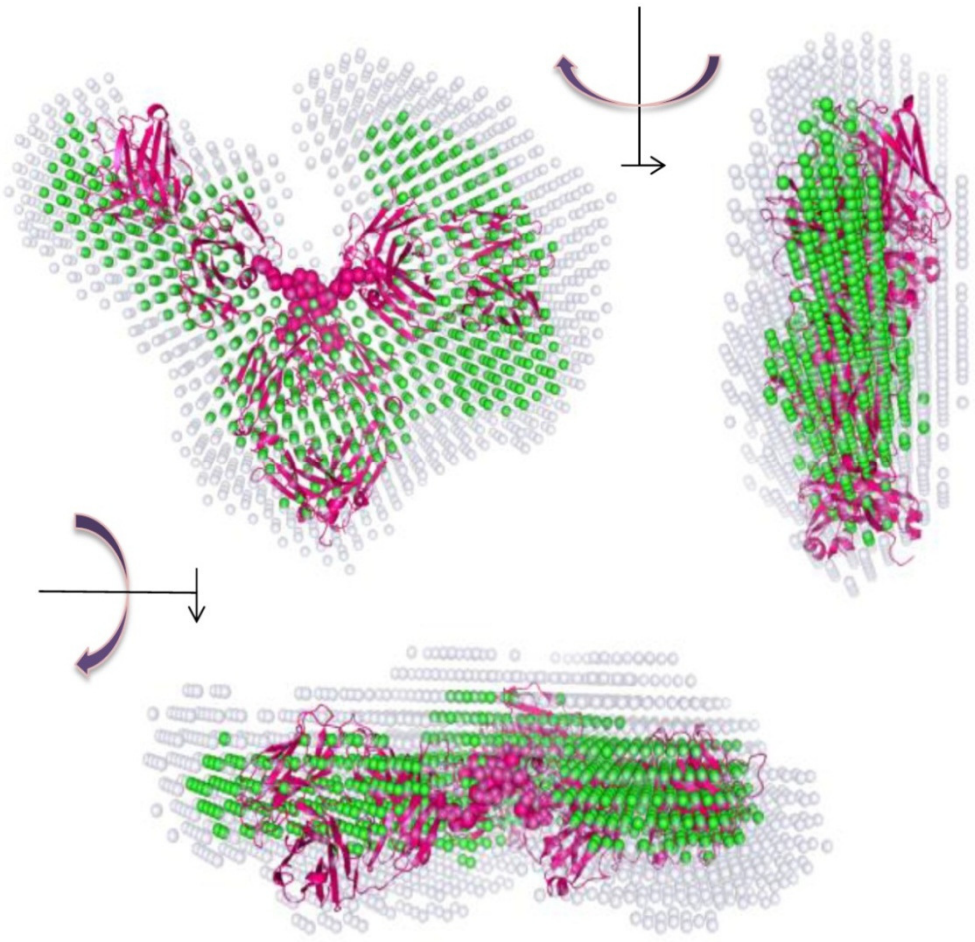


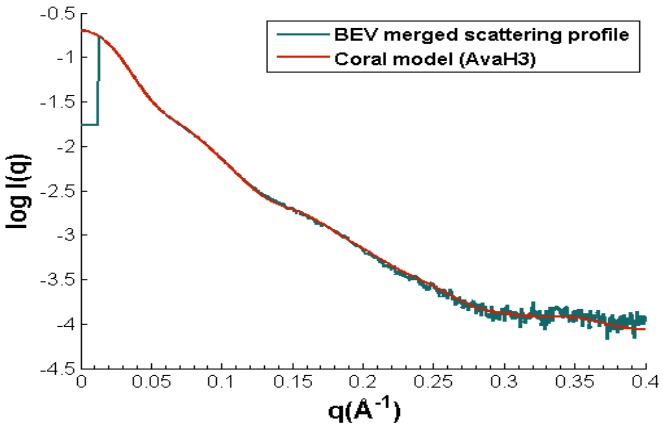


- Repulsion evaluated from peak



No concentration dependent self-association of Bevacizumab (up to 30 mg/mL)

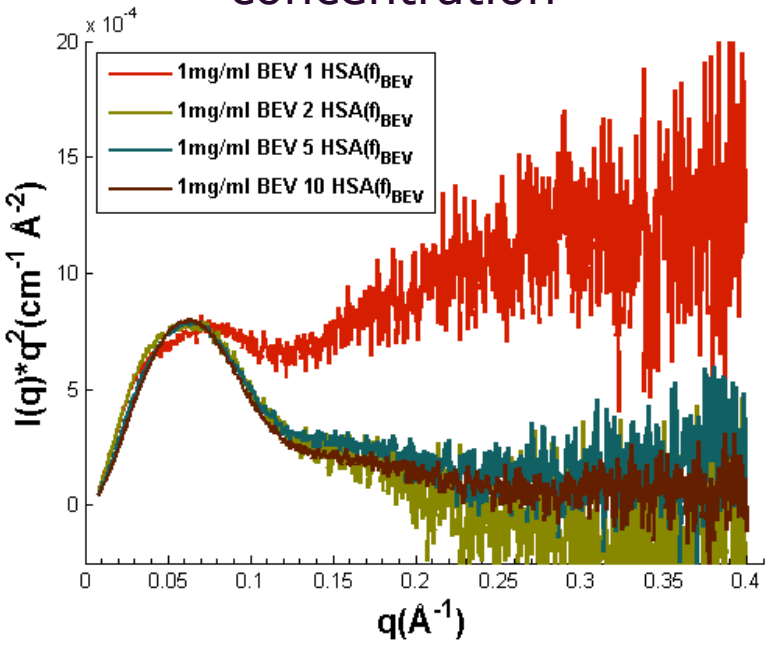




Co-formulation of Bevacizumab and rAlbumin

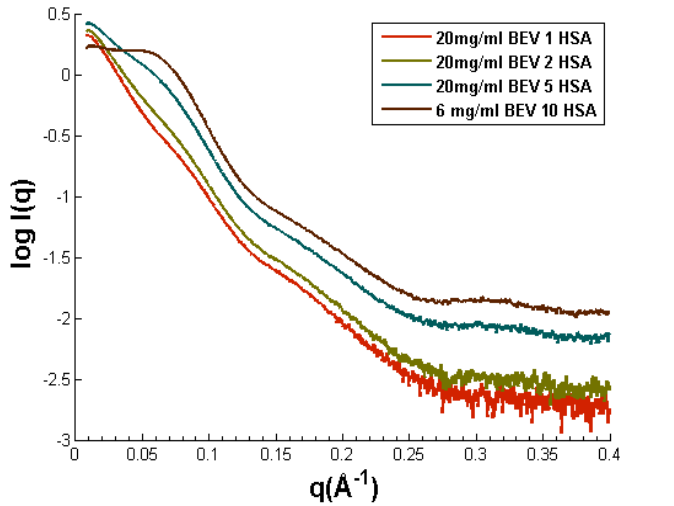
BEV mg/ml	HSA mg/ml	MM (kDa)
1	0.446	129.66
1	0.89	108.22
1	2.23	94.98
1	4.46	78.13
20	8.92	135.55
20	18	111.68
20	44.46	-
6	89.22	-

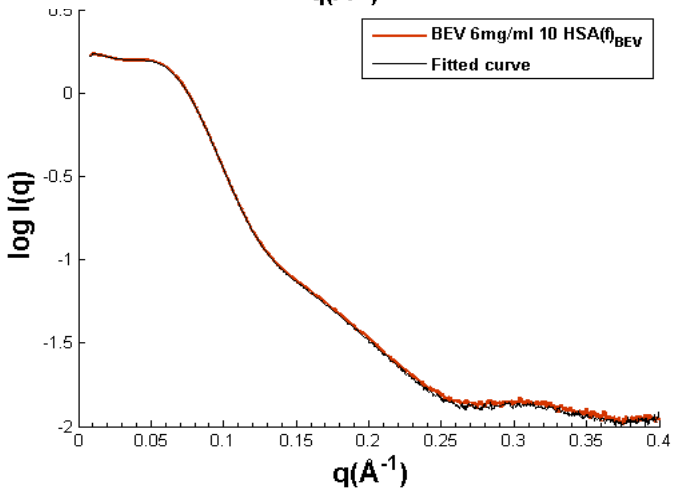
Bevacizumab flexibility decreases with increasing rAlbumin concentration



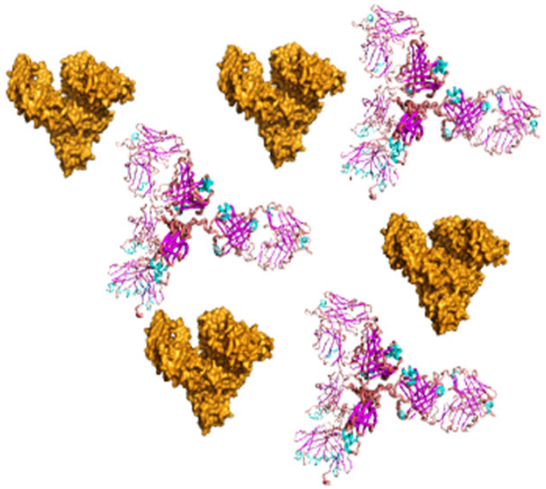
Kratky plot

rAlbumin stabilizes Bevacizumab through molecular crowding





- curve fitting by linear combination
- Repulsive behavior of rAlbumin not perturbed by Bevacizumab
- No interaction between rAlb and mAb



Hypothetical model

Evaluation of mAb-rAlbumin protein-protein interactions by DLS

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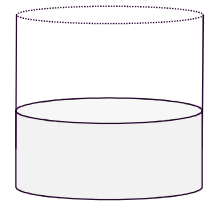
Method set-up

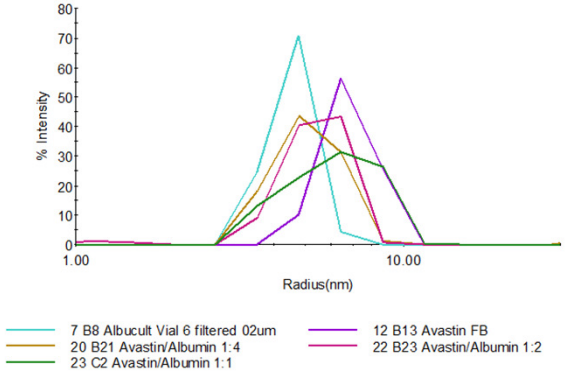
Avastin (Bevacizumab)

Recombumin Alpha (rAlb)

Formulation in different rAlb:mAb ratios



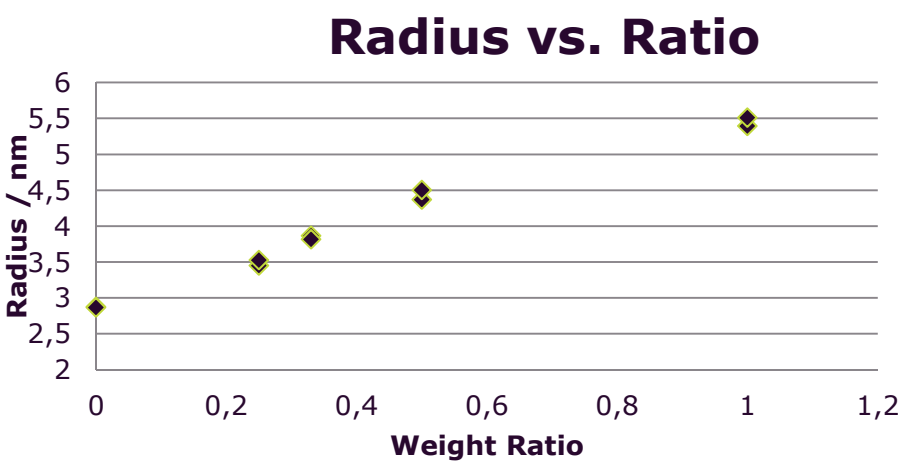
Mix



Wyatt DynaPro Plate Reader (DLS)

The protein-protein interaction is too weak to detect

Item	Mass Ratio	Radius (nm)	Expected Radius (nm)	Ratio
7 B8 Albucult	0	2.9		
8 B9 Albucult	0	2.9		
19 B20 Avastin/Albucult 1:4	0.25	3.5	3.5	1.02
20 B21 Avastin/Albucult 1:4	0.25	3.5	3.5	1.00
21 B22 Avastin/Albucult 1:2	0.33	3.9	3.8	0.97
22 B23 Avastin/Albucult 1:2	0.33	3.8	3.8	0.98
23 C2 Avastin/Albucult 1:1	0.50	4.4	4.2	0.96
24 C3 Avastin/Albucult 1:1	0.50	4.5	4.2	0.93
11 B12 Avastin FB	1	5.4		
12 B13 Avastin FB	1	5.5		



- Observed radius of mixture comparable to expected radius of two non-interacting species

VISCOSITY

- A CHALLENGE FOR PROTEINS IN HIGH CONCENTRATIONS

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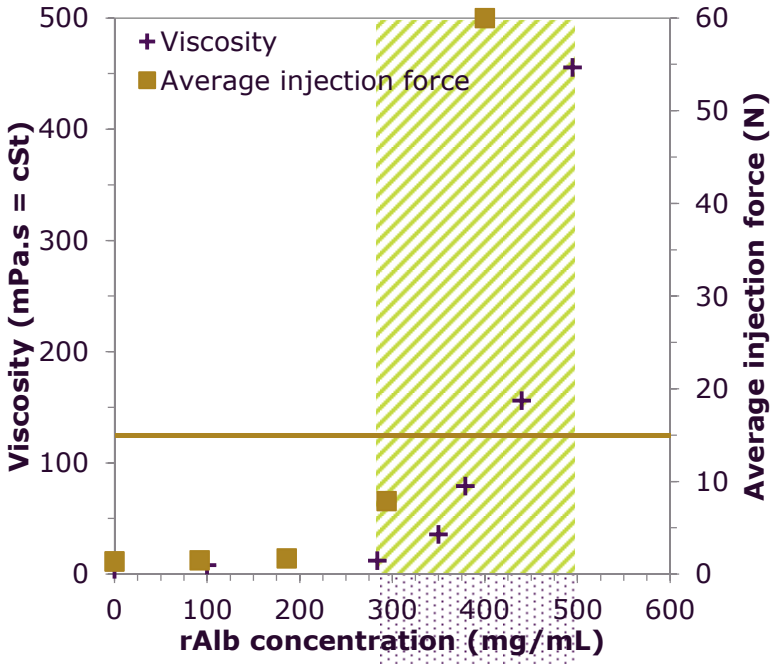

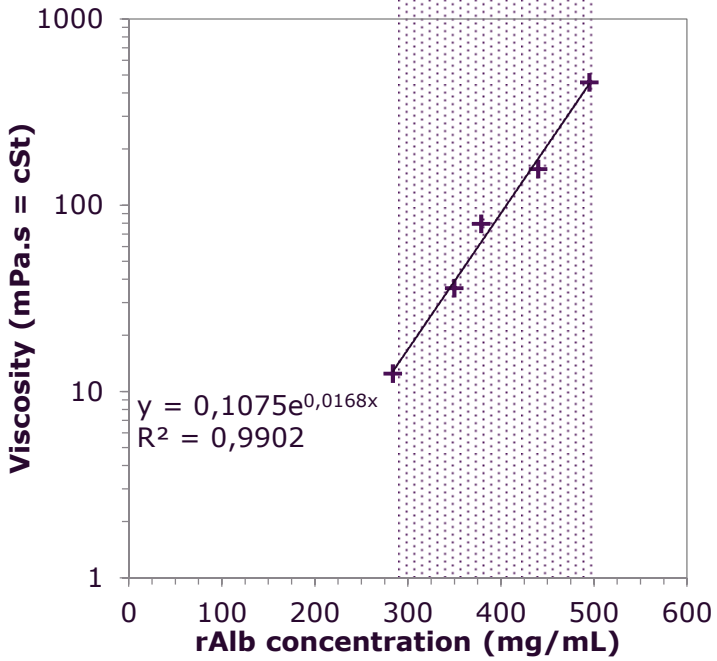

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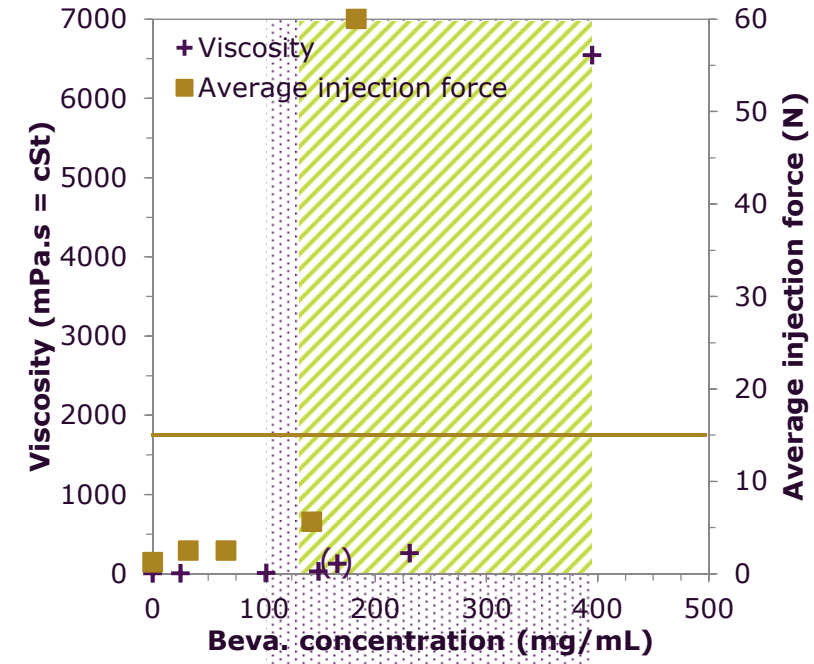
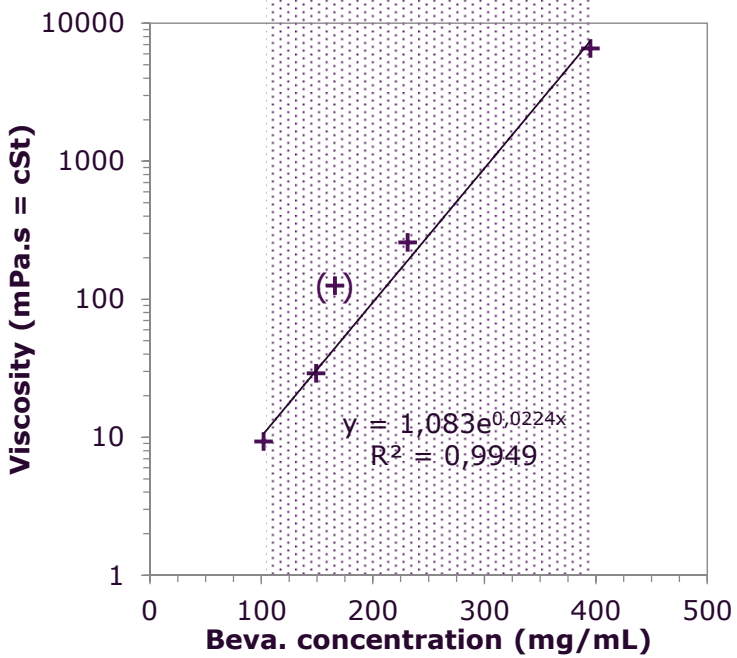
CONCENTRATED rALB FORMULATIONS PRESENT AN EXCELLENT INJECTABILITY* UP TO AT LEAST 300 MG/ML

- Viscosity and injectability are very well correlated properties for rAlb formulations
- The concentration of critical injectability is between 300 and 400 mg/mL where the viscosity markedly levels up
- Between 280 and 500 mg/mL, the viscosity of rAlb in solution increases exponentially with protein concentration

*30G ½'', 2 mL/min





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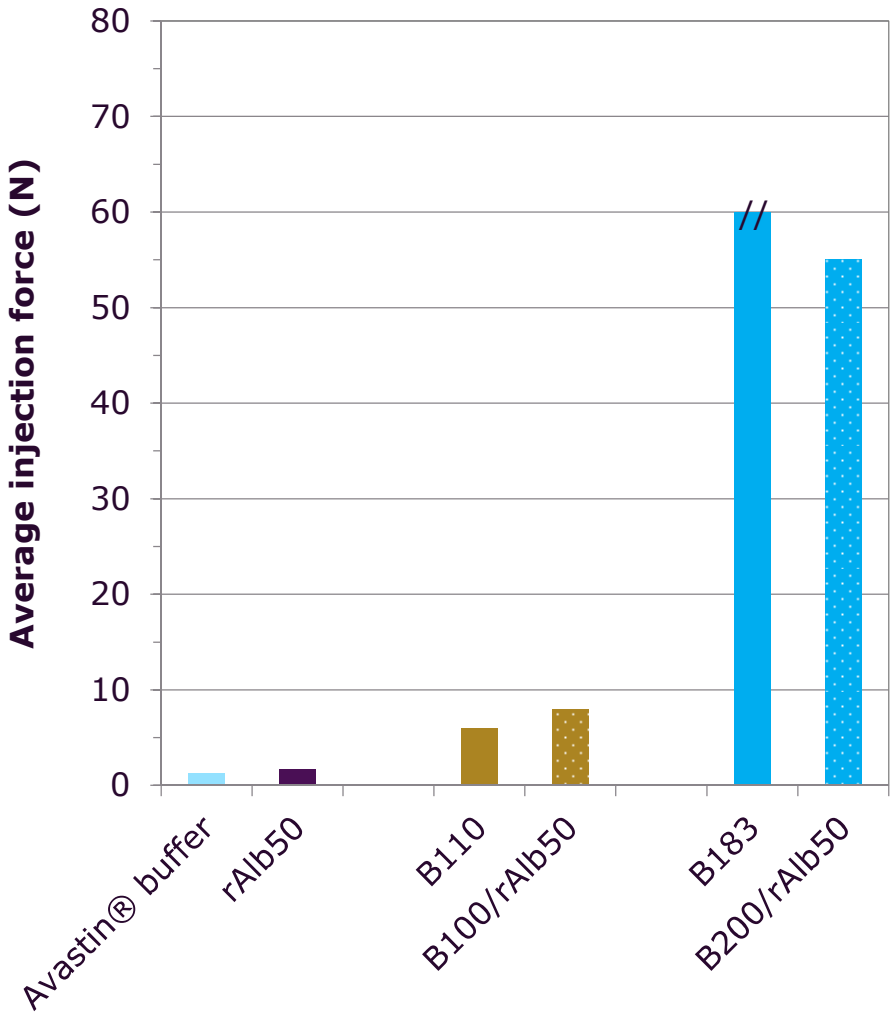
THE INJECTABILITY* OF BEVACIZUMAB BECOMES CRITICAL BETWEEN 140 AND 180 MG/ML

- Viscosity and injectability are well correlated properties for Bevacizumab formulations
- The concentration of critical injectability is between 140 and 180 mg/mL
- This is in agreement with the viscosity increase between 150 and 230 mg/mL
- Between 100 and 400 mg/mL the viscosity of Bevacizumab in solution increases exponentially with protein concentration

*30G ½'', 2 mL/min



THE ADDITION OF rALB TO BEVACIZUMAB DOES NOT LEAD TO A SIGNIFICANT INCREASE OF THE INJECTION FORCE*

- The injection force of rAlb (50 mg/mL) is comparable to that of the buffer
- At a total protein concentration of 150 mg/mL, the addition of rAlb leads to a minor increase of the injection force
- At a total protein concentration of 250 mg/mL, it seems that rAlb eases the injection of Bevacizumab to a measurable value

*30G ½'', 2 mL/min

MAJOR CONCLUSIONS

Rheology and viscosity

- The viscosity of rAlb formulations is comparable to buffer solutions up to more than 100 mg/mL
- An exponential increase in viscosity and injection force is observed for rAlb and two mAbs at high concentrations
- Adding rAlb to high concentration mAb formulations does not give an exponential increase in injection force

Analytics

- Af4 (FFF) is applicable to mixed formulations of mAbs and rAlb
- Combining IEX and SEC is a feasible method for analysis of 4 out of 5 mAbs separating mAbs and rAlb

Safety

- GMP manufacturing in yeast (animal free)
- Phase I clinical trial performed with no adverse events (50 /dose IV and 65 mg/dos IM)
- Used in marketed products

Aggregation prevention

- Recombumin significantly reduces heat and freeze thaw induced particle formation in formulations of mAbs

Mechanism behind aggregation prevention (one case study)

- Repulsive behavior and crowding effect as **determined** by SAXS **and confirmed** by DLS, AF4 and Rheological studies

Acknowledgements

SAXS analysis are performed in collaboration with **DTU Chemistry**, Technical University of Denmark

- Professor Pernille Harris
- Ph.D. stud Pernille Sønderby

Small Angle X-ray scattering (SAXS) data collection was performed at **MAX-IV** Beam-line I-911-4

- Mette-Marie List Jensen
- Corinne Eenschooten
- Mette Larsen
- Anne Marie Scharff-Poulsen
- Stina Engelhardt
- Mikael Bjerg Caspersen
- Paul Luigi Gargani Weisbjerg

The organizing committee

- For organizing such fine a event
- For giving me the opportunity to present

DLS analysis are performed in collaboration with **Wyatt Tecnology**

- Roger Scherrers

- For further information please contact

Jens Thostrup Bukrinsky
JTBU@novozymes.com

IEX-SEC METHOD FOR rALB:mAb MIXTURES - IN-LINE COLUMN CAPTURE OF rALBUMIN

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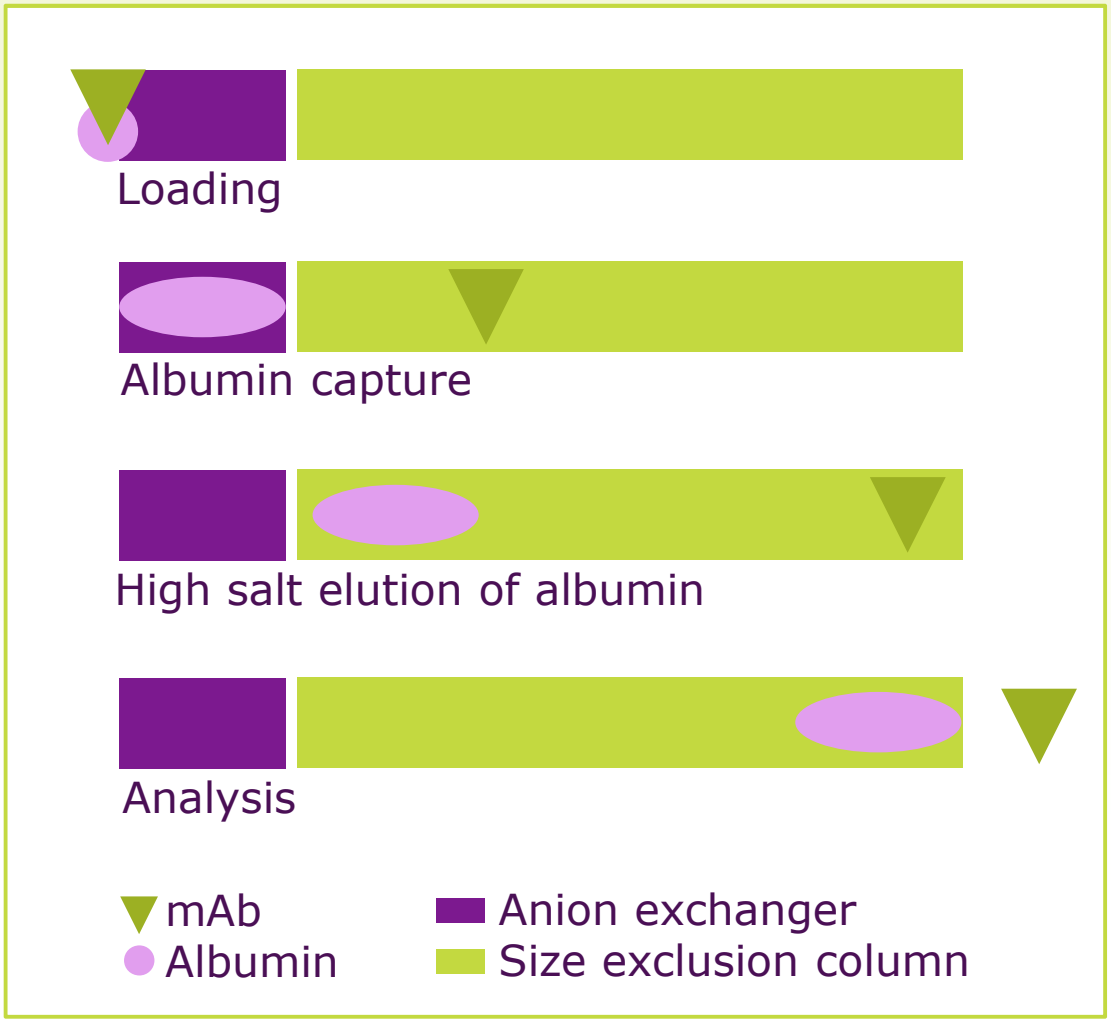
Strategy

- Separate albumin from mAb by in-line column capture of albumin under conditions where mAb elution is not affected
- When mAb has sufficiently eluted, a high salt (or pH shift) is applied to elute albumin from the capture column
- mAb aggregation can be analyzed, while albumin is either captured or undergoing elution

Column system

- One anion exchanger with minimal void volume (capture column)
- One size exclusion column



mAb AGGREGATION SUCCESSFULLY ASSESSED BY NEW IEX-SEC METHOD

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Materials

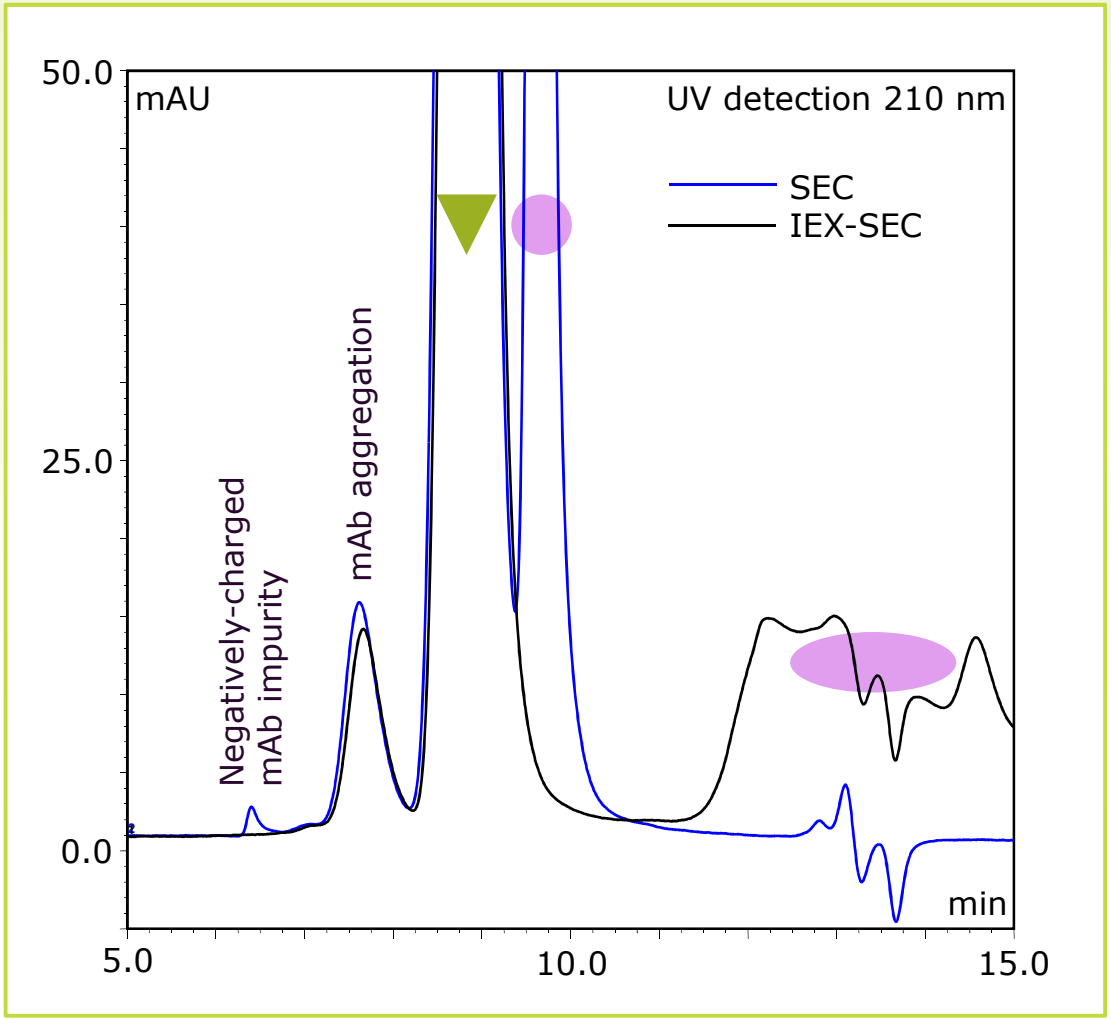
- mAbX/albumin mixture (1:1 w/w)

Method

- Mobile phase A (pH 7.5): Tris (25 mM), NaCl (0.2 M)
- Mobile phase B (pH 7.5): Tris (25 mM), NaCl (1.0 M)
- Anion exchanger: ProSwift SAX-1S 4.6 x 50 mm
- Size exclusion column: TSK G3000 SWXL 7.8 x 300 mm

Results

- Baseline separation between the mAb and albumin can only be achieved when using the new IEX-SEC method



THE NEW IEX-SEC METHOD CAN BE APPLIED TO A RANGE OF mAbs

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- The new IEX-SEC method was successfully applied to a range of mAbs and mAb/albumin weight ratios

mAb tested	IpH	Min. weight ratio for successful separation	Max. advised albumin weight ratio for formulation
mAb X	5.8	1:100	100 x
Omalizumab	8.3	1:30	30 x
Bevacizumab	10	1:5	5 x
Tocilizumab	>10	N/A*	N/A

The size exclusion column is not operable at high pH. When pH is lower than IpH, the positively-charged mAbs interact with the size exclusion column (cation exchanger) which hampers the analysis