MedImmune
A member of the AstraZeneca Group

Practical application of analytical tools for characterization of an impurity-related particle formation mechanism

Jared S. Bee, Ph.D.

Protein aggregation measurement in biotherapeutics

Maryland Center for Excellence in Regulatory Science and Innovation (MCERSI) and the Bio- & Nano-Technology Center of the University of Maryland.

05th December, 2016

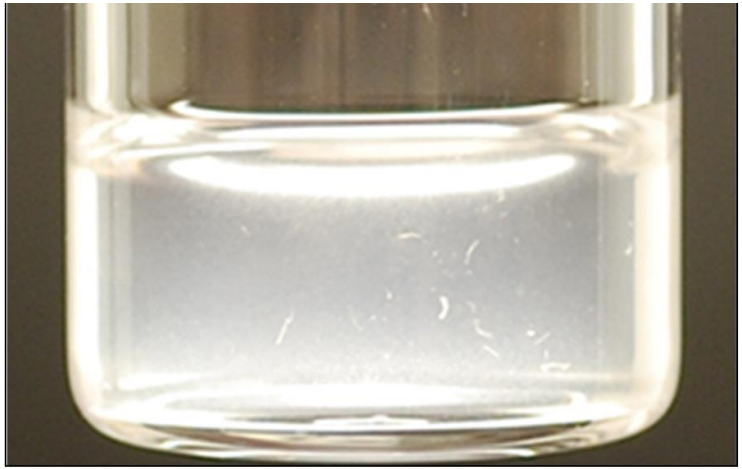
Manufacturing of monoclonal antibodies (mAbs)

Cell culture

Purification
Formulation
Fill-Finish

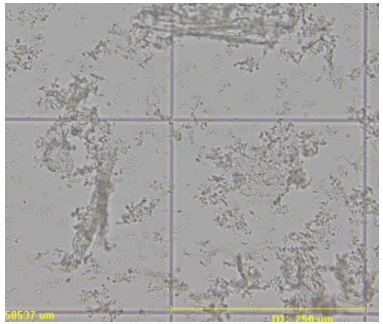
Drug Product

Unusually high levels of particles were observed for mAb-1

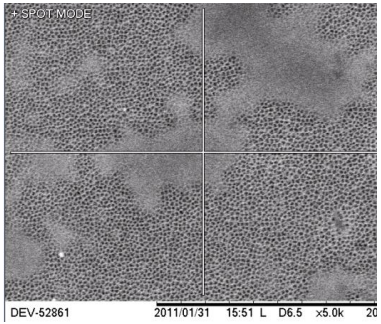


Particles formed over a period of weeks at 40°C and months at 2-8°C

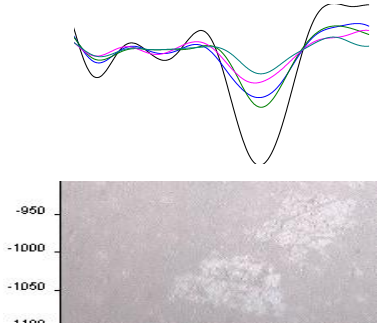
The particles contained mAb-1 and its fragments



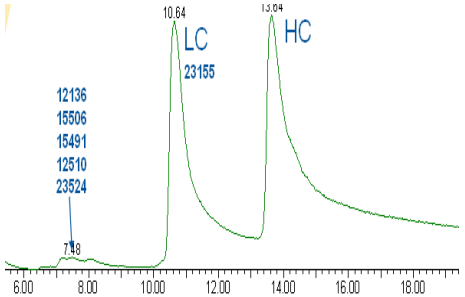
Light microscopy



SEM-EDX



FTIR microscopy



Mass spectrometry of pelleted particles

- Particles have a proteinaceous appearance
- No heavy elements were detected in the particles
- FTIR showed bands typical of near-native or native mAbs (β-sheet, turns)
- Particles contained mAb heavy chain (HC), light chain (LC) and fragments
- Elemental impurities not detected by ICP-MS
- No bioburden was detected

Stability and purity issues were observed for early mAb-1 lots

Lot	Process/ Scale	HCP Level (ng/mg)	Rate at 40°C (% per month)		Visual Appearance at 40°C	Visual Appearance at 5°C
			HPSEC Purity Loss	RP-HPLC Fragmentation		
A	1a/35L	428	6.6	4.3	Failed at 3 weeks	Failed at 6 months
B	1a/100L	120	1.9	2.9	Failed at 12 weeks	Failed at 6 months
C	1a/20L	263	3.5	3.7	Failed at 12 weeks	Failed at 3 months
D	1a/36L	157	1.6	2.6	Failed at 4 weeks	Failed at 2 months

Stability and purity issues observed for early mAb-1 lots

Lot	Process/ Scale	HCP Level (ng/mg)	Rate at 40°C (% per month)		Visual Appearance at 40°C	Visual Appearance at 5°C
			HPSEC Purity Loss	RP-HPLC Fragmentation		
A	1a/35L	428	6.6	4.3	Failed at 3 weeks	Failed at 6 months
B	1a/100L	120	1.9	2.9	Failed at 12 weeks	Failed at 6 months
C	1a/20L	263	3.5	3.7	Failed at 12 weeks	Failed at 3 months
D	1a/36L	157	1.6	2.6	Failed at 4 weeks	Failed at 2 months

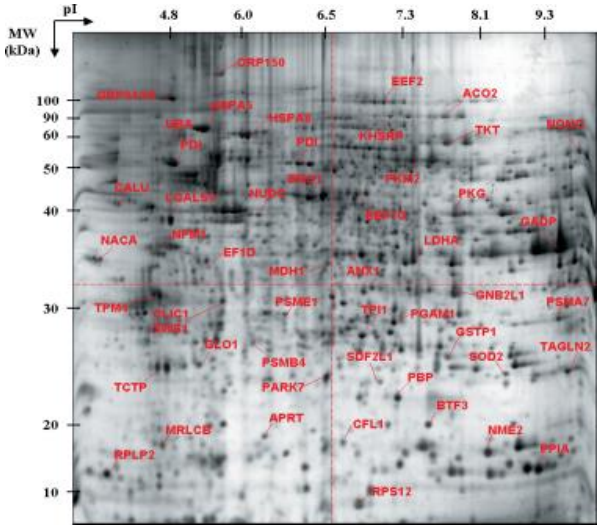
High HCP levels

High and variable fragmentation rates at 40°C

Formation of delayed-onset particles

Could high HCP levels be linked to particle formation and fragmentation?

A residual host cell protease?



2D Gel of CHO HCPs, Hayduk et al., 2004

- Proteome analysis has identified > 6000 chinese hamster ovary (CHO) HCPs (Baycin-Hizal et al., 2012)
- Some HCPs can bind to the mAb making them harder to remove during the purification process (Valente et al. 2015)

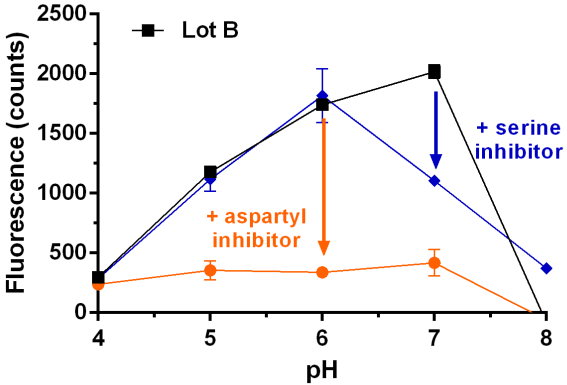
Baycin-Hizal, D. et al. Proteomic analysis of chinese hamster ovary cells. *Journal of Proteome Research* **2012**, *11*, 5265-5276.

Hayduk, E. J.; Choe, L. H.; Lee, K. H. A two-dimensional electrophoresis map of Chinese hamster ovary cell proteins based on fluorescence staining. *Electrophoresis***2004**, *25*, 2545-2556.

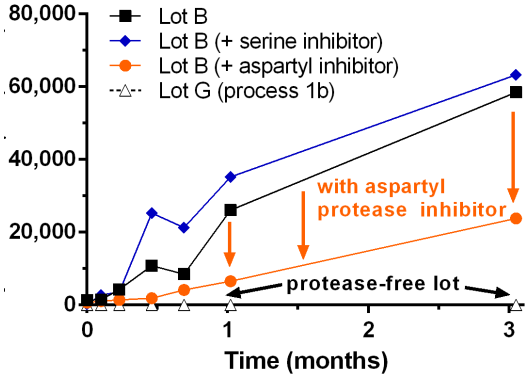
Valente KN, Lenhoff AM, Lee KH. Expression of difficult-to-remove host cell protein impurities during extended Chinese hamster ovary cell culture and their impact on continuous bioprocessing. *Biotechnol Bioeng*. 2015;112(6):1232-1242.3

Trace residual levels of an aspartyl protease was the cause of particle formation

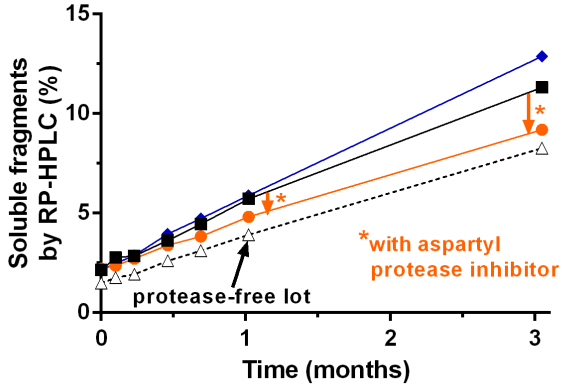
Protease activity assay



Sub-visible particle formation



***Soluble* fragmentation**



- Aspartyl protease inhibitor reduced protease activity, particle formation, and fragmentation rate (other inhibitors did not have same effect)
- Inhibitor only slightly decreased *soluble* fragment levels
- Mass spec detected multiple c-terminal heavy chain fragments in insoluble particles (these same fragments were not detected in soluble form by RP-HPLC)

With affinity enrichment, the aspartyl protease was identified as cathepsin D

mAb-1
Drug Substance

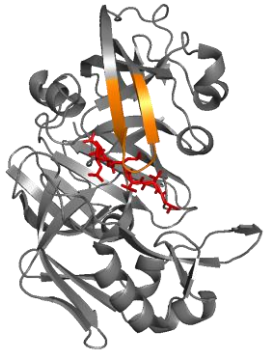
Affinity capture and enrichment
using immobilized pepstatin A resin

Enriched aspartyl
protease

MS

Mass spectrometry
Western blot

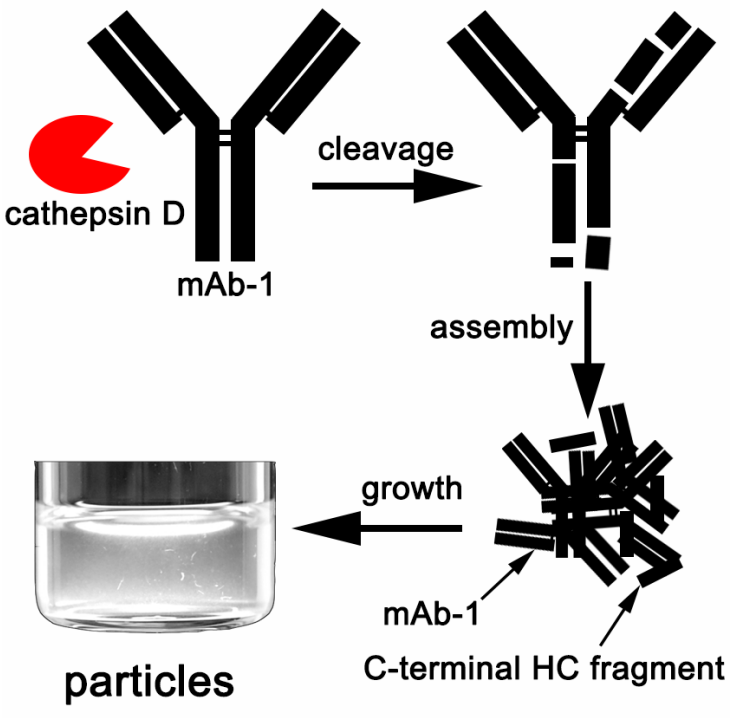
Positive identification
of cathepsin D



- Cathepsin D is a 48 kDa glycosylated aspartyl protease active at < pH 6
- Active site located in hydrophobic cleft; preferentially cleaves between two hydrophobic amino acid residues under slightly acidic or acidic conditions (Sun at al. 2013)
- Spiking this purified cathepsin D into mAb-1 caused particle formation

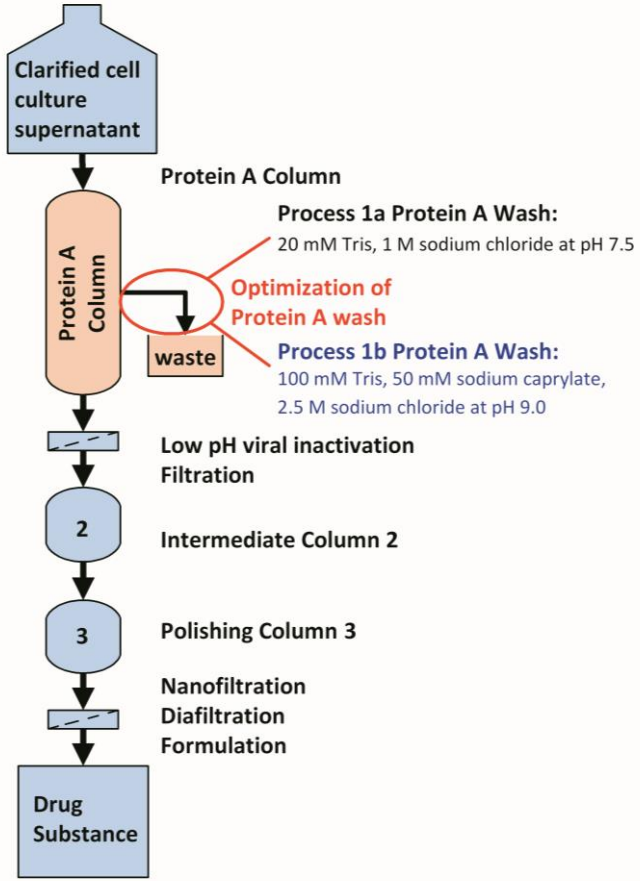
Sun H, Lou X, Shan Q, et al. Proteolytic Characteristics of Cathepsin D Related to the Recognition and Cleavage of Its Target Proteins. *PLoS ONE*. (6):e65733.

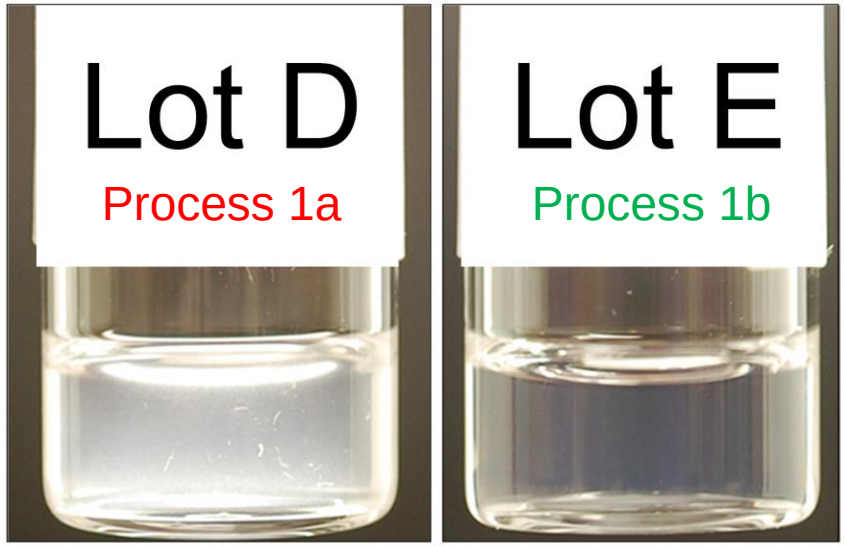
Trace residual levels of CHO cathepsin D caused particle formation in the final mAb-1 product



Bee JS, Tie L, Johnson D, Dimitrova MN, Jusino KC, Afdahl CD. Trace levels of the CHO host cell protease cathepsin D caused particle formation in a monoclonal antibody product. *Biotechnol Prog*. 2015;31(5):1360-1369.

The fix: process was optimized to remove HCPs



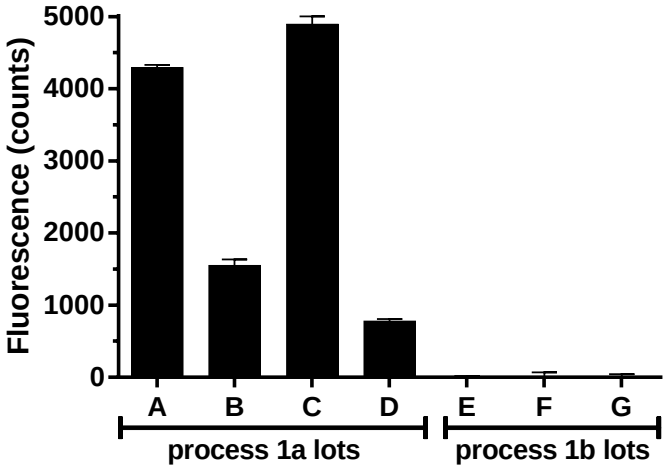


Process optimization focused on removal of HCPs was successful in eliminating particle formation in the final mAb-1 product.

'Caprylate wash' – developed by David Gruber and Richard Turner and applied to mAb-1 by Christopher Afdahl and Kristin Jusino

Gruber DE, Turner RE, Bee JS, Afdahl CD, Tie L, inventors. Purification of recombinantly produced polypeptides, United States Patent WO/2014/186350 (PCT/US2014/037821). 2014.

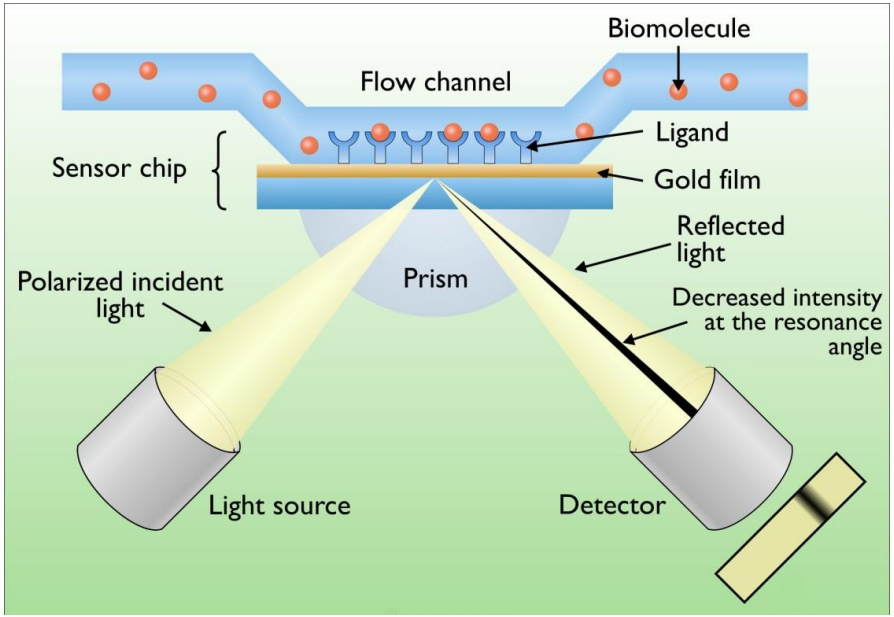
Process 1b lots were confirmed to be free of any detectable protease activity



In addition, the optimized process 1b lots did not form delayed-onset particles (12 weeks at 40°C and 12 months at 5°C)

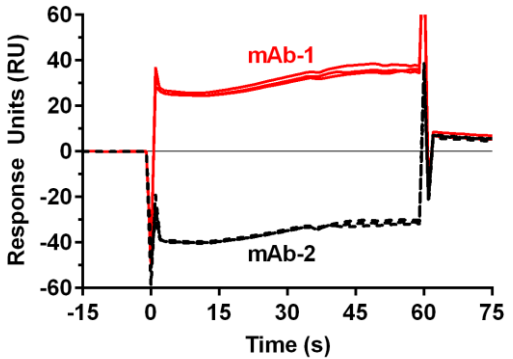
Why did mAb-1 have this problem?

Does it bind cathepsin D?

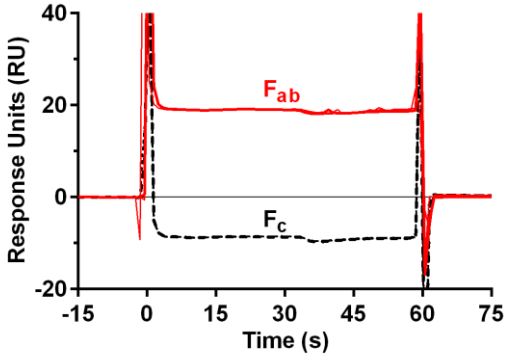


SPR of immobilized CHO cathepsin D was used to detect its binding to a panel of mAbs

mAb-1 bound to cathepsin D



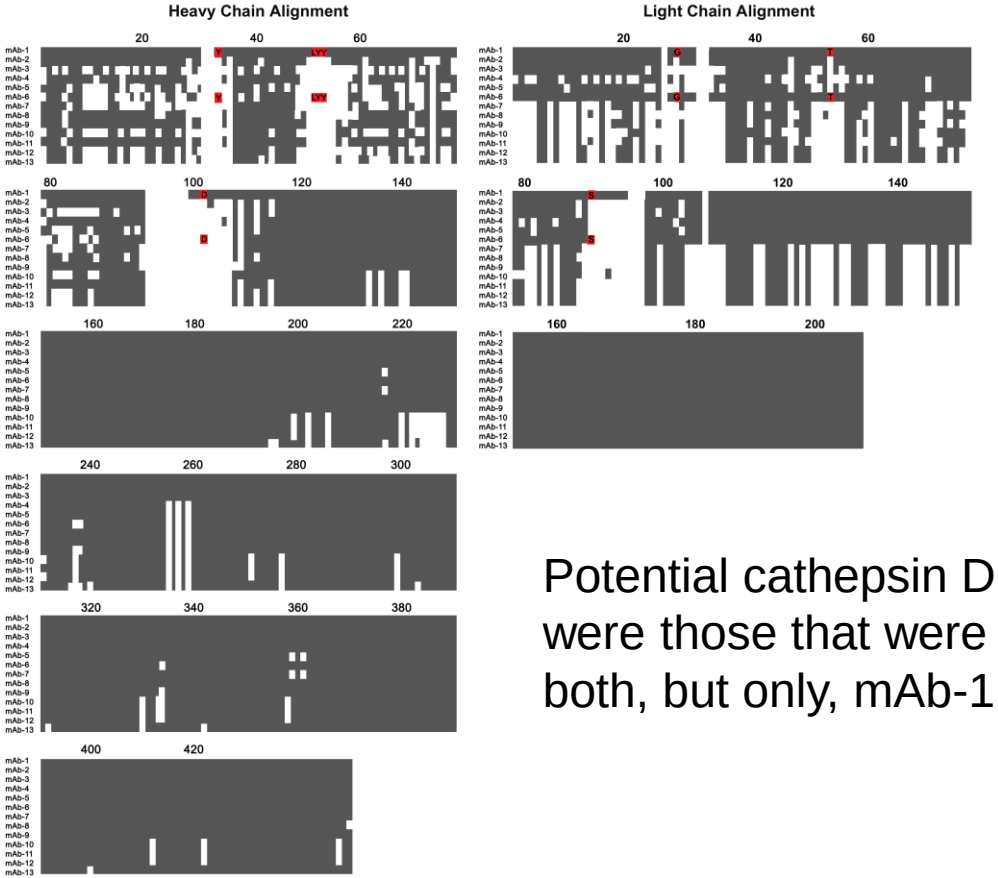
mAb-2 did not bind, even though it has 94% identity to mAb-1



The Fab region of mAb-1 was involved in binding

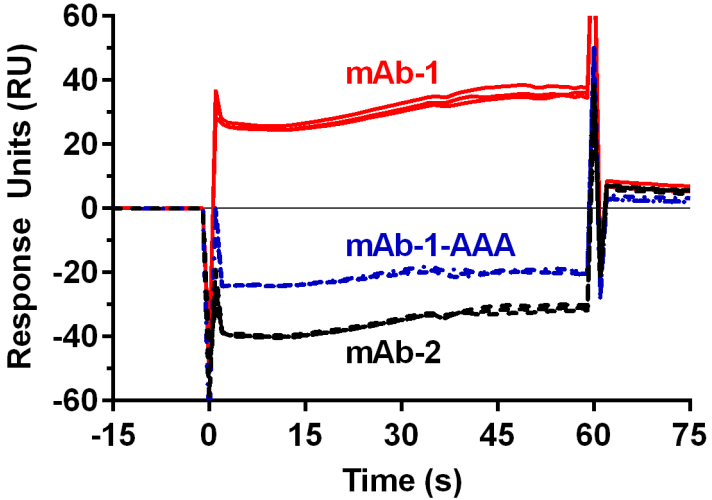
Bee at al. Identification of an IgG CDR sequence contributing to co-purification of the host cell protease cathepsin D. *Submitted, under review*

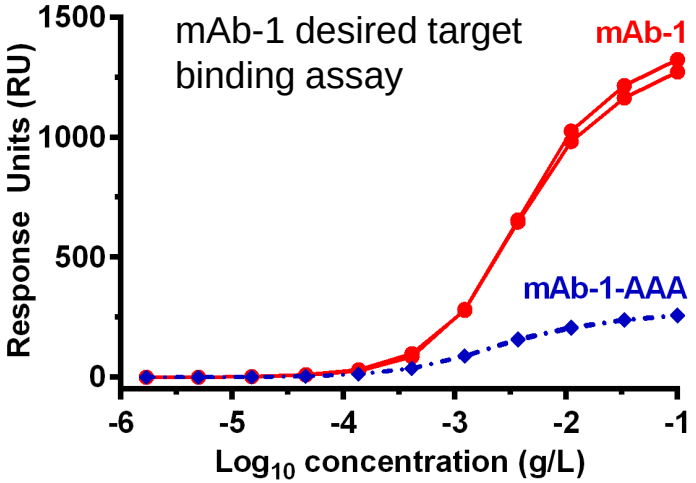
An ‘LYY’ motif was a unique match for the 2 mAbs (out of 13 tested) that bound cathepsin D



Potential cathepsin D binding sequences were those that were a unique match to both, but only, mAb-1 and mAb-6.

Mutation of ‘LYY’ to ‘AAA’ eliminated binding to cathepsin D, but unfortunately also eliminated target binding





Mutation confirmed that the LYY motif in the HC CDR2 was involved in weak binding to CHO cathepsin D.

Summary

- Particles formed in mAb-1 were found to contain mAb-1 and its HC fragments using microscopy, SEM-EDX, FTIR microscopy, and mass spectrometry
- The presence of trace amounts of an aspartyl protease, cathepsin D, was the cause of particle formation
- Optimization of the purification process was able to reduce the HCP levels resulting in a stable product
- Further studies identified an ‘LYY’ motif in mAb-1 that could bind to cathepsin D, resulting in its trace co-purification

Acknowledgments

Kristin C. Jusino/Chris Afdahl/Matthew Dickson – purification of cathepsin D
Yoen Joo Kim – FTIR microscopy and SEM-EDX
Shravan Gattu and Paul Santacroce – stability study support
Douglas Johnson – gel electrophoresis
Hung-Yu Lin/Jenny Heidbrink Thompson/Liu Tie – mass spectrometry
LeeAnn M. Machiesky and Ken Miller– SPR work
Jeffrey Gill – Fab and Fc generation
Li Peng – design and making of AAA mutant
Richard L. Remmele Jr. and Mariana Dimitrova – hypothesis generation and expt. design

Confidentiality Notice