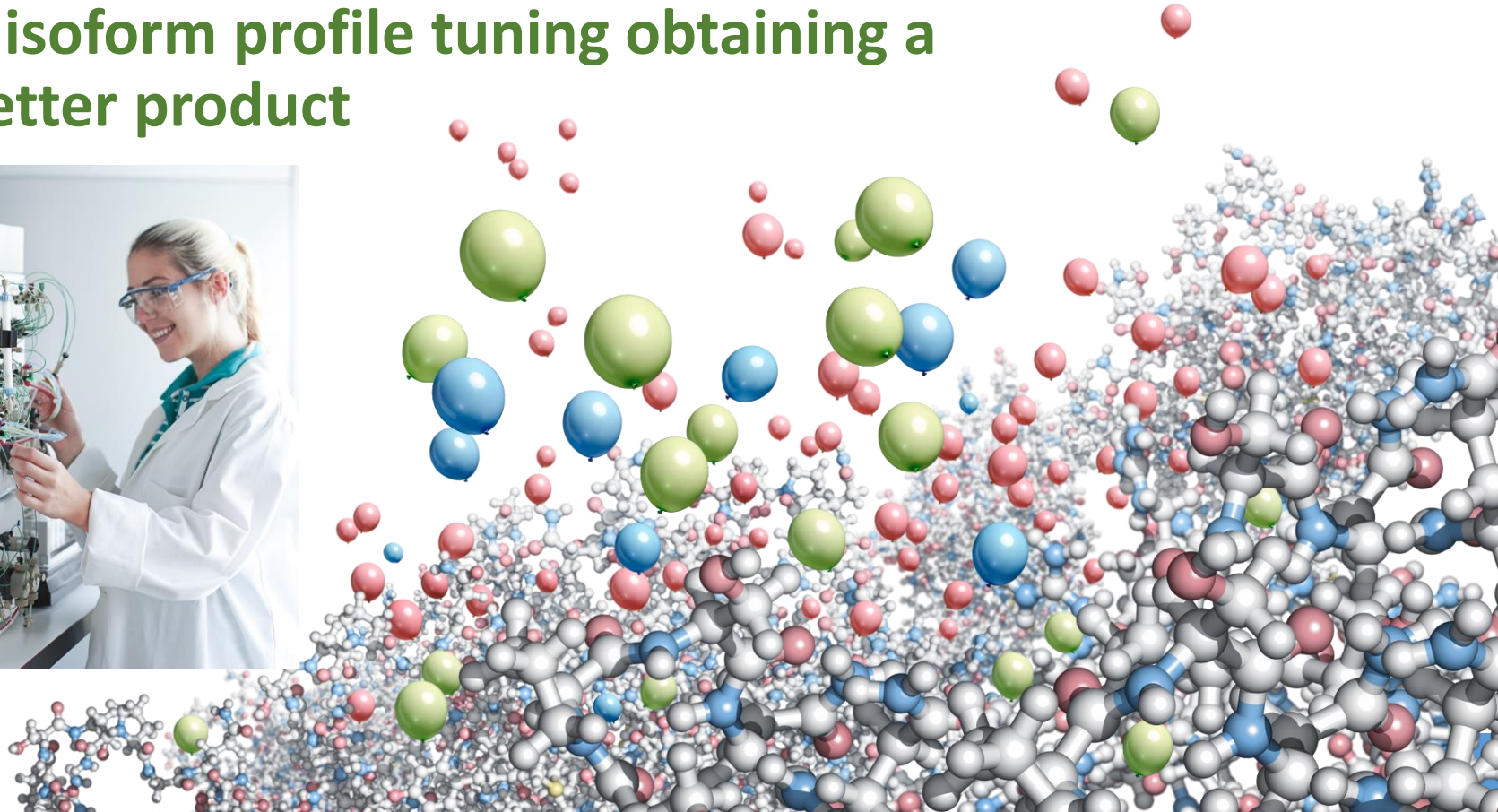
ChromaCon®
A new dimension in purification

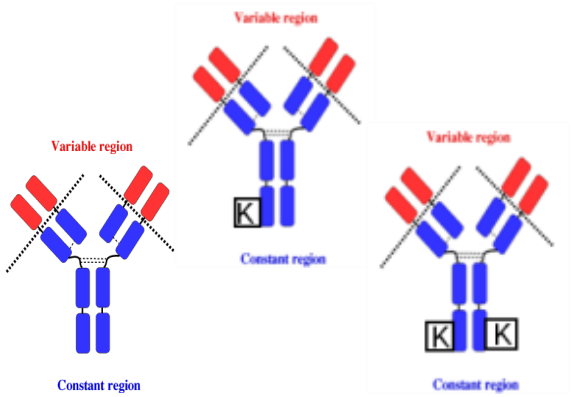
Contichrom® Twin-column FPLC Chromatography

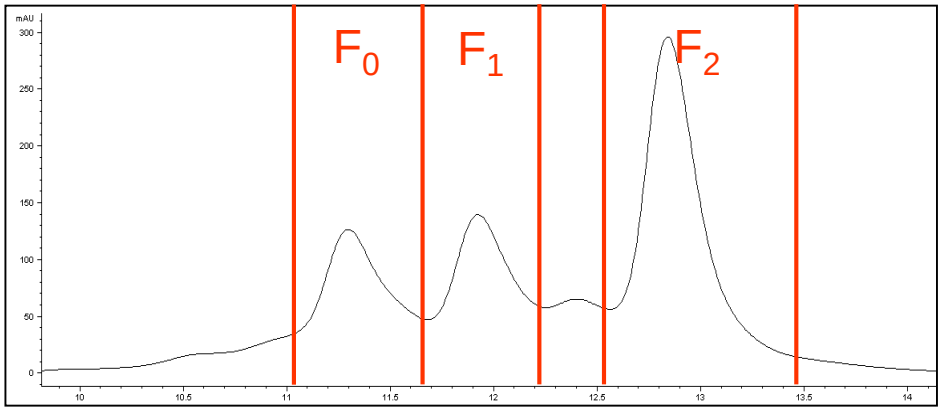
mAb isoform profile tuning obtaining a biobetter product



Case Study 1: MAb Lysine Isoform Separation

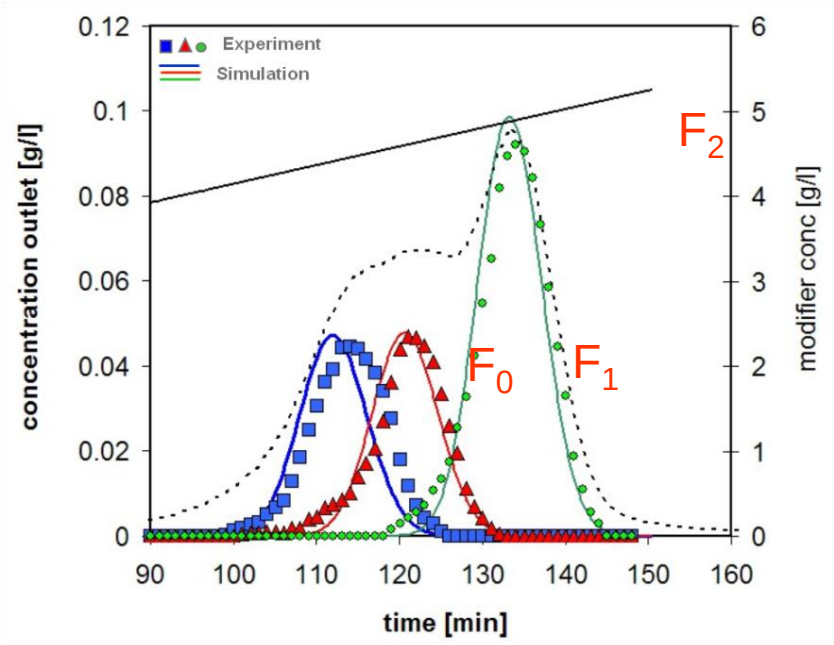
- Collaboration with Novartis
- Separation challenge:
  - Separate mAb isoform with 1 terminal lysine group (F1) from mAb variants with 0 and 2 terminal lysine group, i.e. (F0) and (F2)
- Analytical chromatogram:





Case Study 1: MAb Lysine Isoform Separation

- Preparative batch chromatogram (Cation Exchange chromatography):



Fractogel EMD COO 100x4.6 mm, d_p = 30µm

NOVARTIS

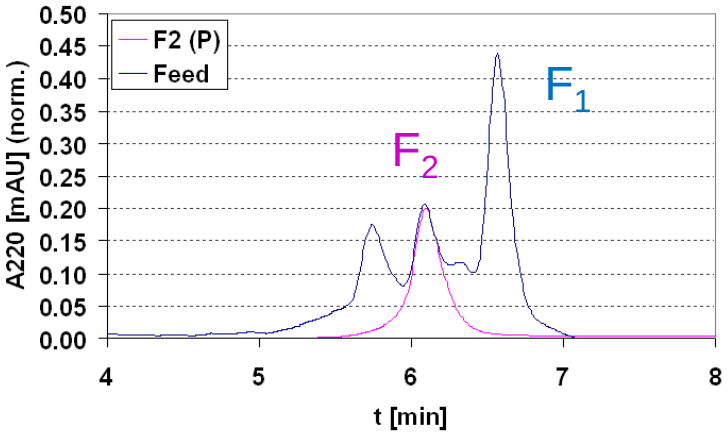
- Result: at 80% purity of F1 (main product) : only ⍰5% yield

Case Study 1: MAb Lysine Isoform Separation

- Apply MCSGP process instead of batch:
  - Fast MCSGP process conversion from batch using ChromIQ Software with Contichrom® equipment

- Experimental results:

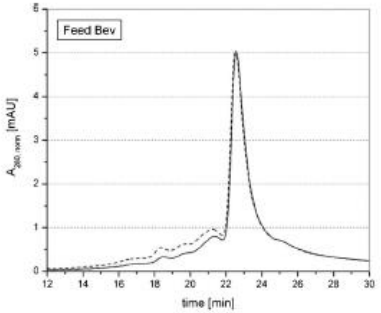
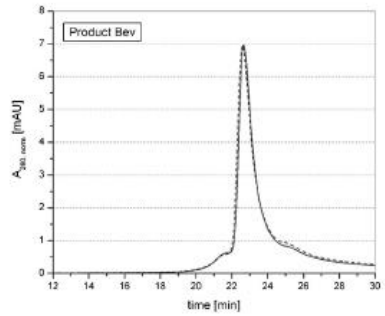
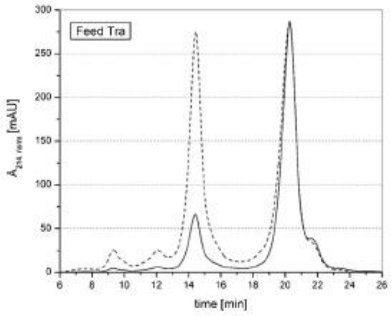
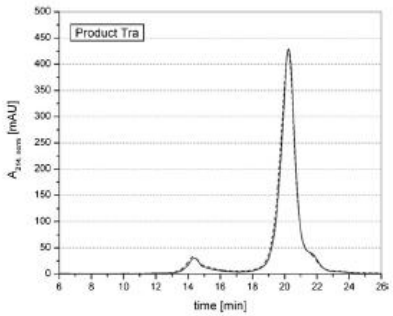
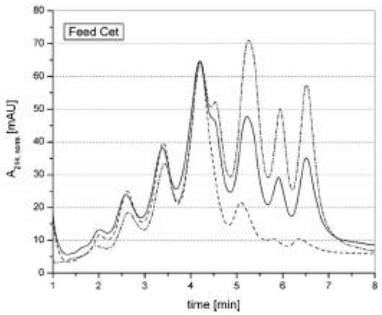
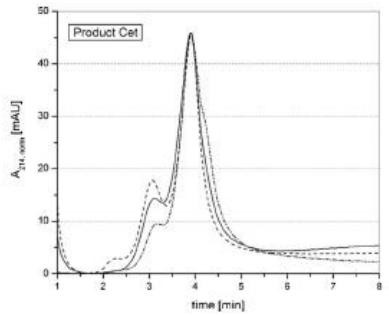
	MCSGP	Batch
Yield F_1	93%	5%
Purity F_1	93%	80%



- Analytical chromatogram:
  - Comparison of feed and MCSGP-purified product F2

Ref: Muller-Spath T, Aumann L, Melter L, Strohlein G, Morbidelli M. 2008. Chromatographic Separation of three Monoclonal Antibody variants using Multicolumn Countercurrent Solvent Gradient Purification (MCSGP), Biotechnology and Bioengineering 100 (6): 1166-1177.

Case Study 2 : mAb isoform profile tuning

	Feed	Product
	(variable isoform content)	(Contichrom®-purified)
Avastin® (Bevacizumab)		
Herceptin® (Trastuzumab)		
Erbitux® (Cetuximab)		

- Specific
- more active isoforms can be enriched
- Consistent product quality : MCSGP product purity not significantly affected by change of feed isoform profile.*

*Muller-Spath T, Krattli M, Aumann L, Strohlein G, Morbidelli M. 2010. Biotechnology and Bioengineering 107(4):652–662

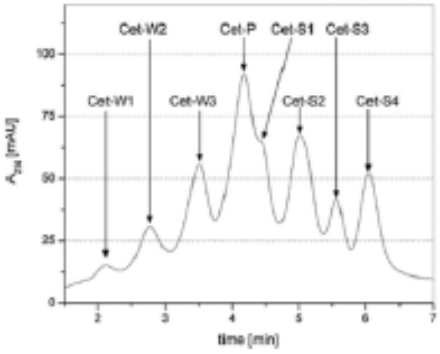
Case Study 2 : mAb isoform profile tuning

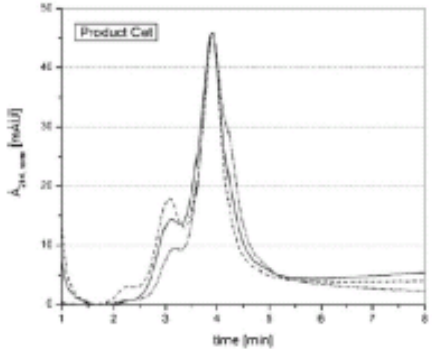
Challenge: low yields and purities due to very similar elution behavior of charge variants.

Performance results for monoclonal antibody charge variant separations (CIEX):

	MCSGP	*batch chromatography*
Yield	96%	41%
Purity	96.3%	95.0%

CIEX analytic of feed (left) and MCSGP purified product (right):





Case Study 3: Herceptin charge isoform separation

- Herceptin (Trastuzumab)
- IgG1, pI = 8.45
- Final product contains multiple isoforms

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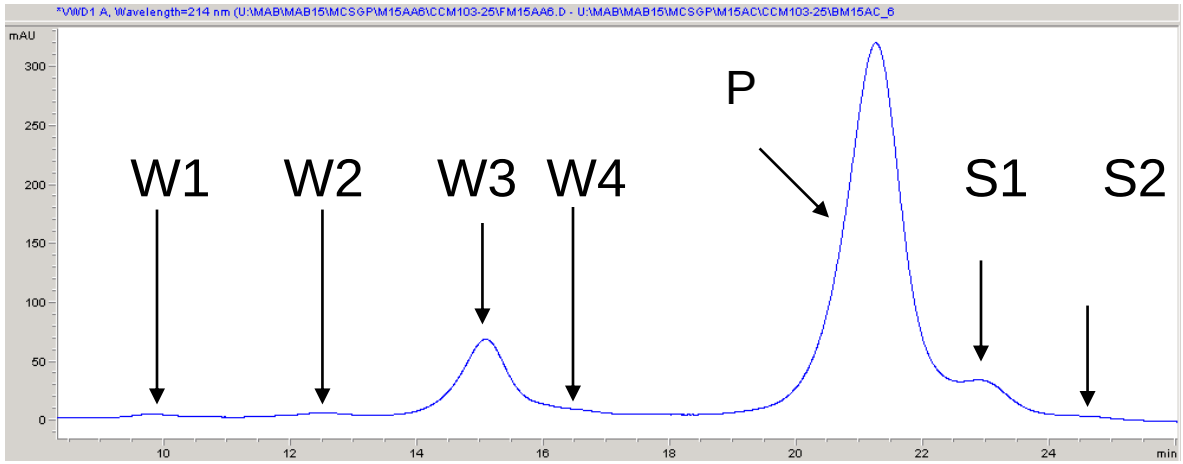
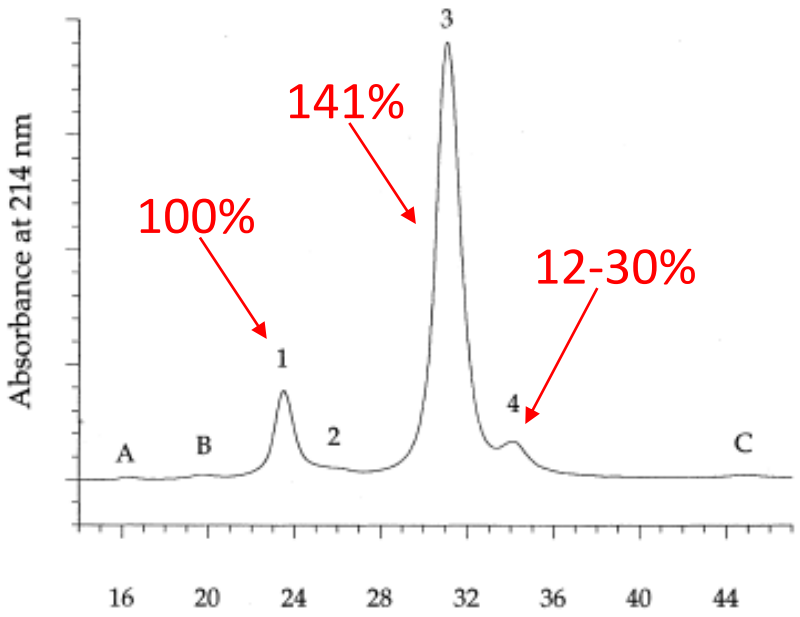


Analytical weak cation exchange chromatogram

Case Study : mAb isoform profile tuning

- Originator mAb product «Herceptin» contains 7 isoforms with different activities (10%-150%)
- Using Contichrom®, a homogeneous biobetter product has been isolated with high yield and purity, having 140% activity
- Potential for a Biobetter „Herceptin“ with lower dosing and better safety profile shown
- Isoform heterogeneity applies to all therapeutic mAbs,

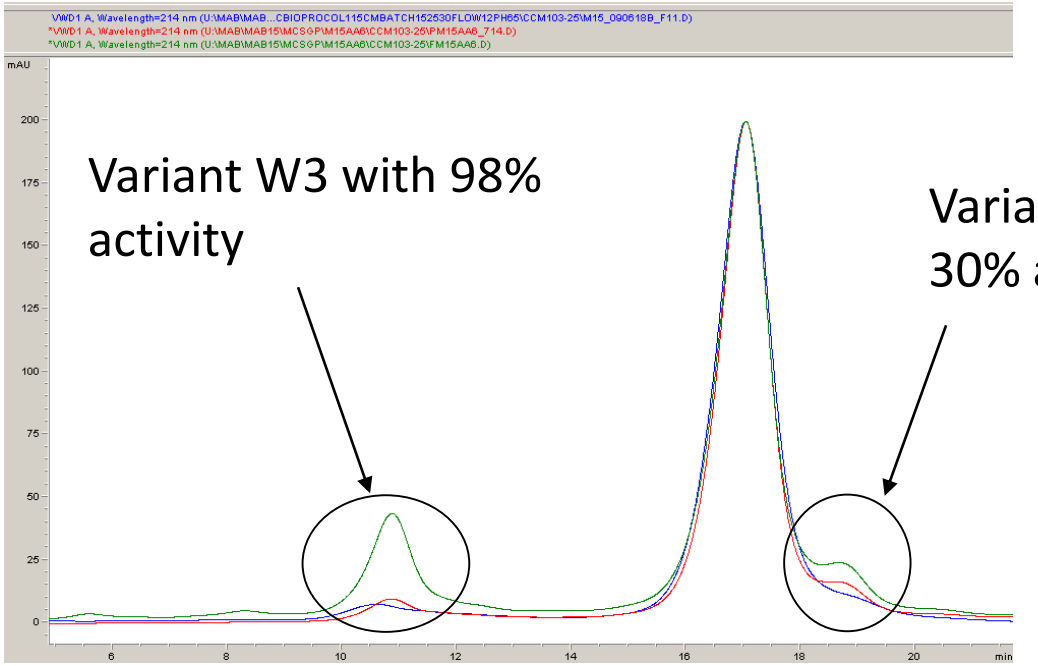


Herceptin isoforms

Case Study 3: Herceptin charge isoform separation

- Enrichment of most active mAb variant by MCSGP

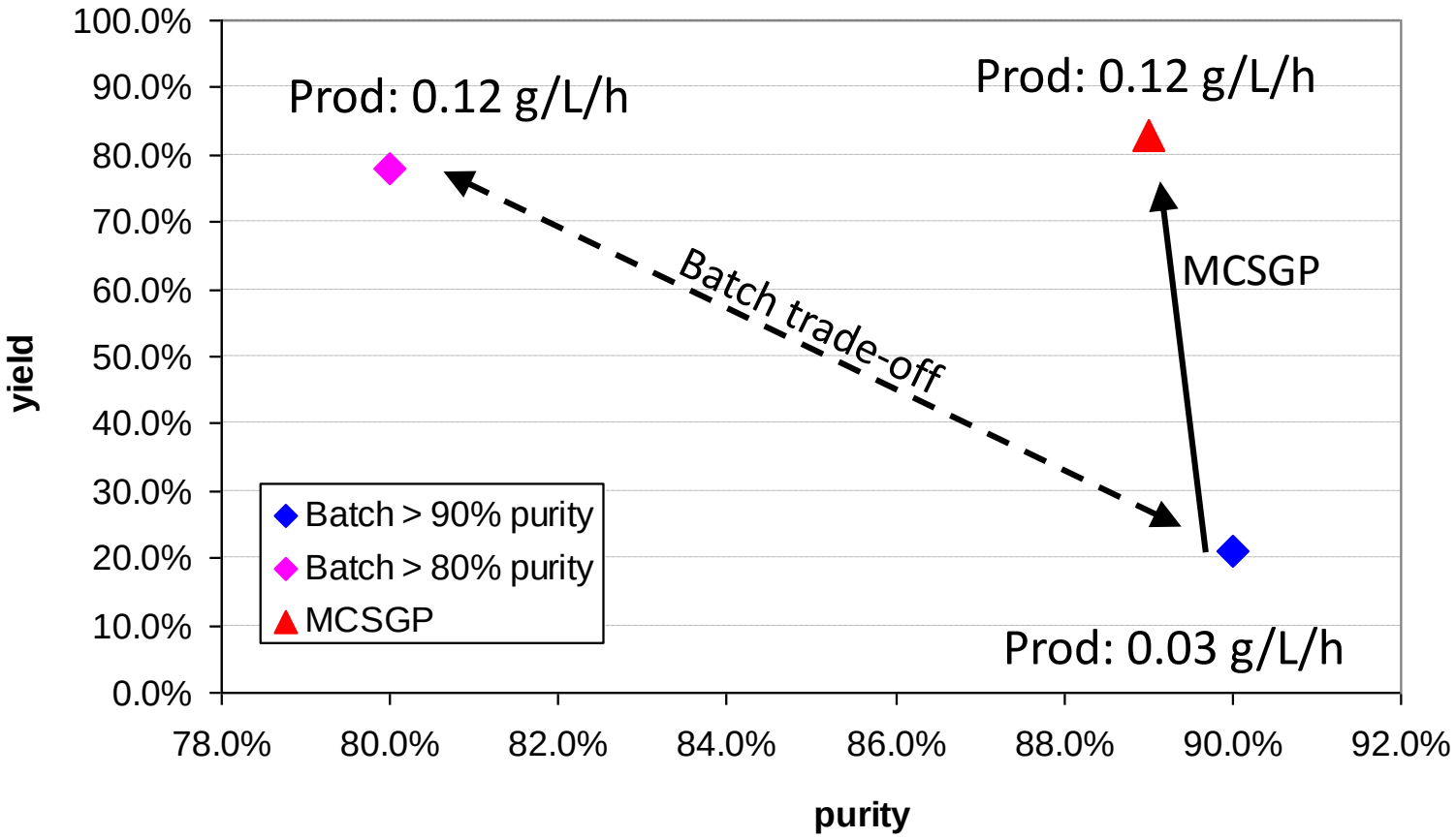
Green: Feed
Blue: Purest fraction batch proc.
Red: MCSGP product



	content of S1	at yield	activity [%]
Feed	100.0%	100%	100%
MCSGP CIEX	61.2%	82.8%	133%
Batch CIEX	24.7%	21.6%	136%

Case Study 3: Herceptin charge isoform separation

- Herceptin: Yield-Purity trade-off: Inherent to batch chromatography, not for MCSGP



Applications of high resolution MCSGP

- Matching a Biosimilar Product profile with an Originator Product: The profile and the activities can be adjusted using MCSGP both qualitatively and quantitatively

- Removing less active isoforms of the Originator Product to generate Biobetters

- Isolation and better characterization of isoforms and product-related impurities for de-risking regulatory CMC data

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