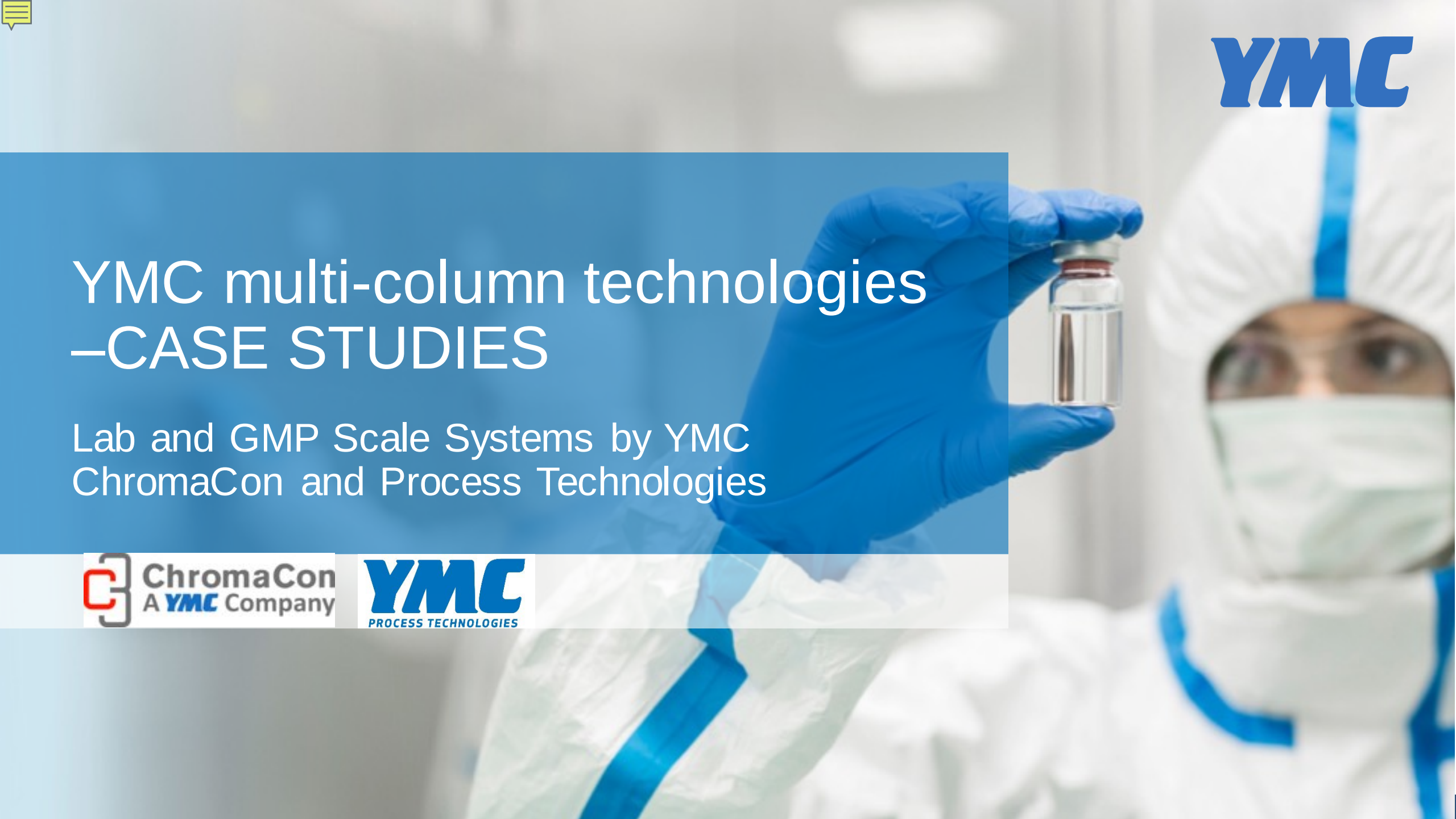
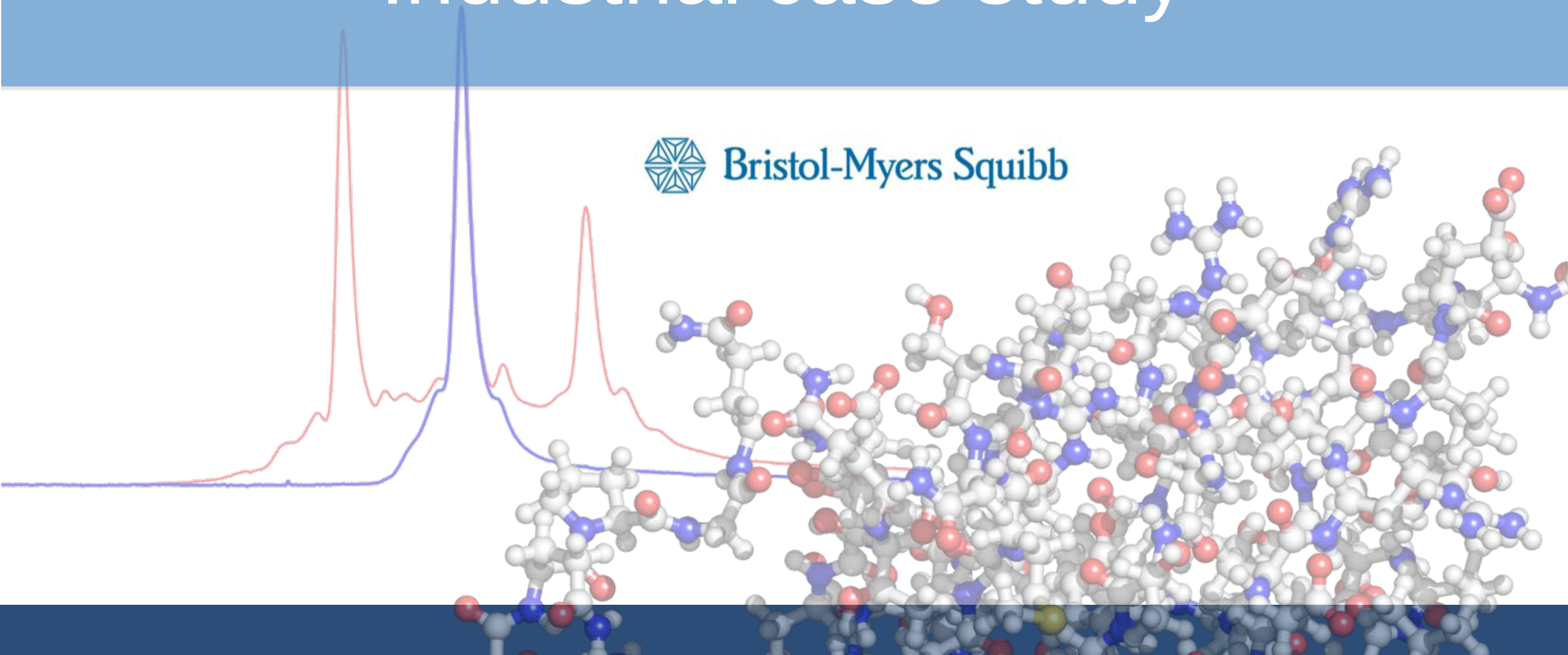
YMC multi-column technologies –CASE STUDIES

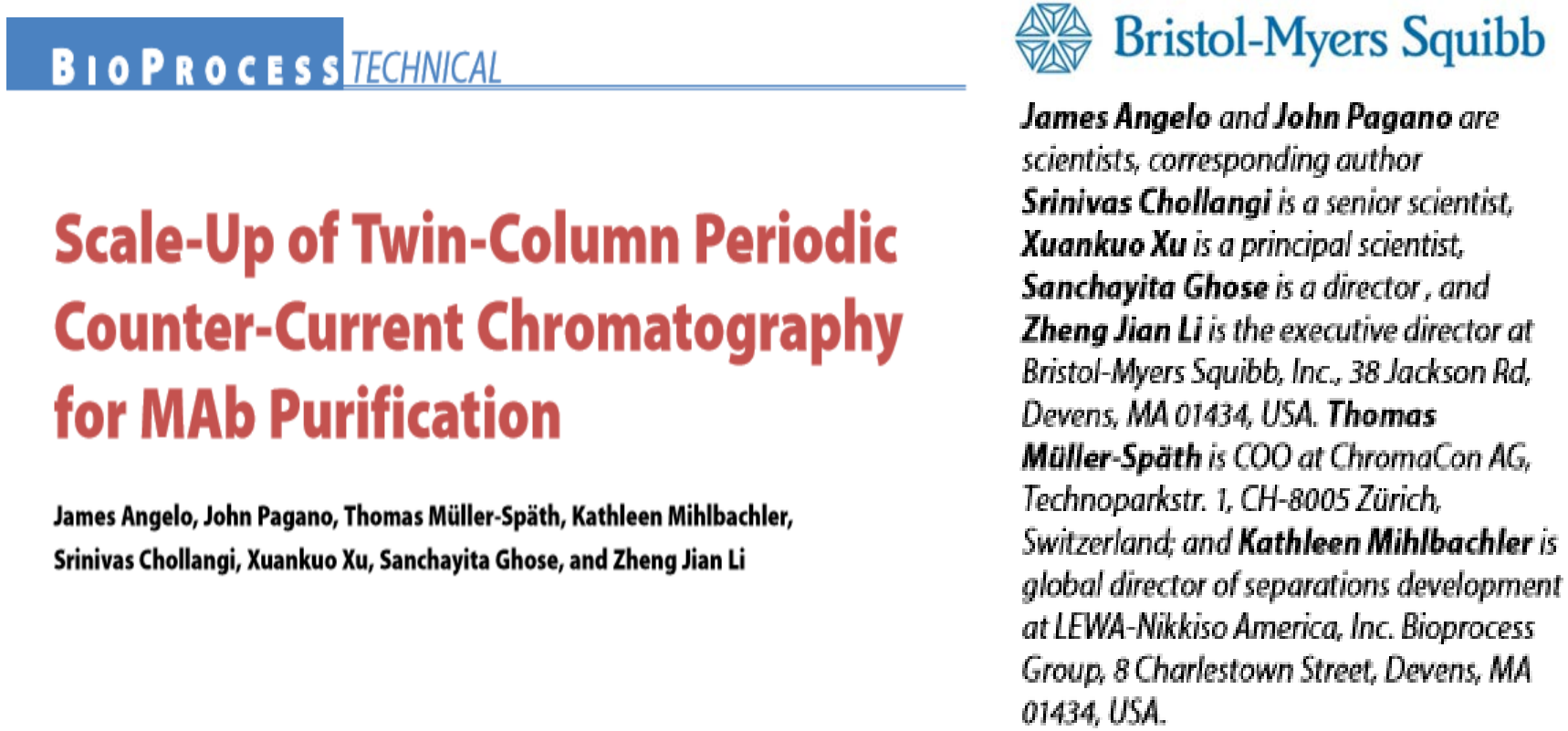
Lab and GMP Scale Systems by YMC ChromaCon and Process Technologies



Industrial case study



Milestone 2018: Industrial GMP Scale-up of Continuous Downstream Capture Step



BIOPROCESS TECHNICAL

Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification

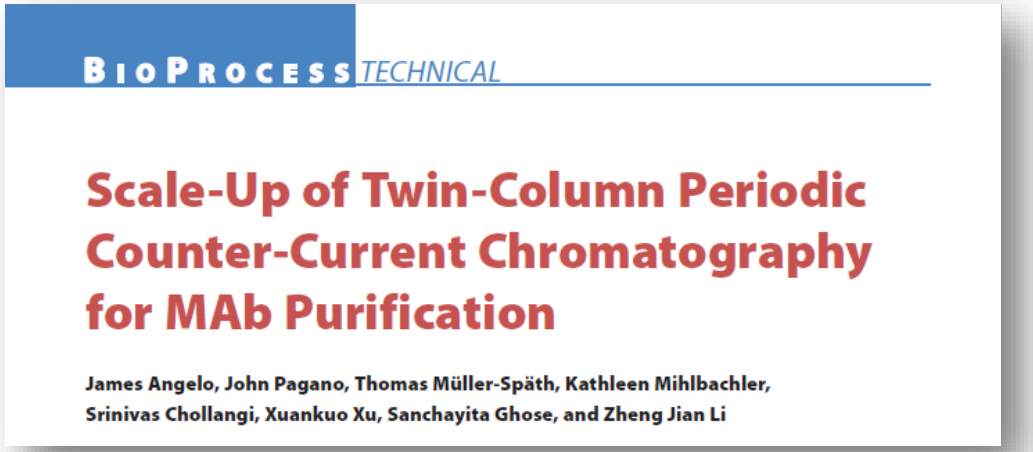
James Angelo, John Pagano, Thomas Müller-Späth, Kathleen Mihlbachler, Srinivas Chollangi, Xuankuo Xu, Sanchayita Ghose, and Zheng Jian Li

Bristol-Myers Squibb

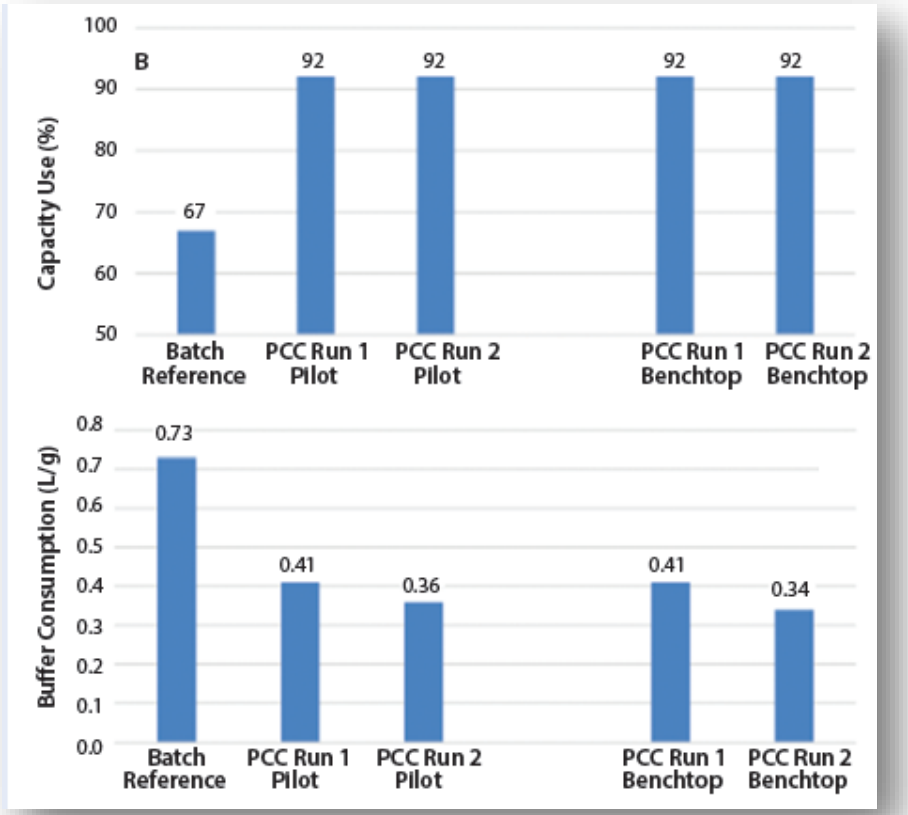
James Angelo *and* ***John Pagano*** *are scientists, corresponding author* ***Srinivas Chollangi*** *is a senior scientist,* ***Xuankuo Xu*** *is a principal scientist,* ***Sanchayita Ghose*** *is a director , and* ***Zheng Jian Li*** *is the executive director at Bristol-Myers Squibb, Inc., 38 Jackson Rd, Devens, MA 01434, USA.* ***Thomas Müller-Späth*** *is COO at ChromaCon AG, Technoparkstr. 1, CH-8005 Zürich, Switzerland; and* ***Kathleen Mihlbachler*** *is global director of separations development at LEWA-Nikkiso America, Inc. Bioprocess Group, 8 Charlestown Street, Devens, MA 01434, USA.*

1 Scale-Up of Twin-Column Periodic Counter- Current Chromatography for MAb Purification, J. Angelo et al, BioProcess International, Vol. 16(4) April 2018

User data shows greater than 2X productivity and ~50% buffer savings



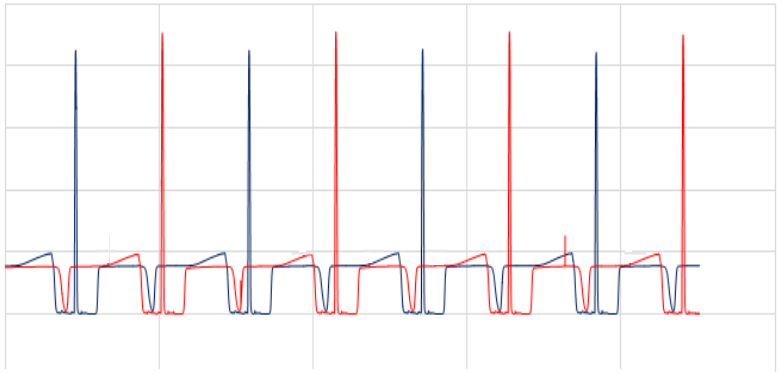
Successful 100x scale up



Paper published in February 2018: http://www.bioprocessintl.com/

Comparison of bench scale to GMP pilot scale performance

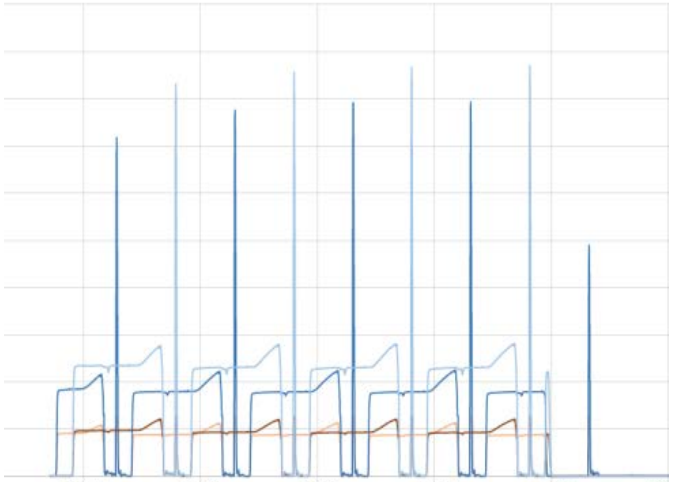
~30 mL/min



UV signals of four cycles (column 1 and column 2)

ChromaCon
Contichrom
CUBE Combined

~600 mL/min



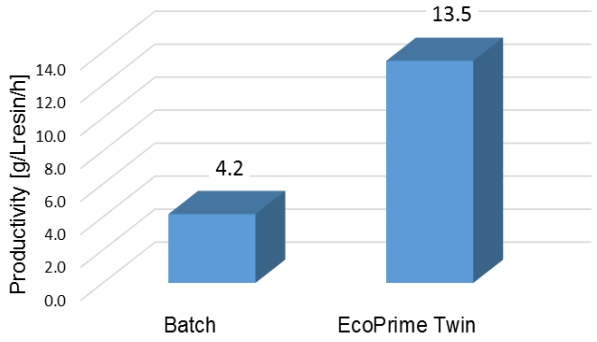
YMC Process
Technologies'
EcoPrime
Twin LPLC

Seamless scale-up of a continuous capture process, CaptureSMB verified

Continuous capture process at GMP scale

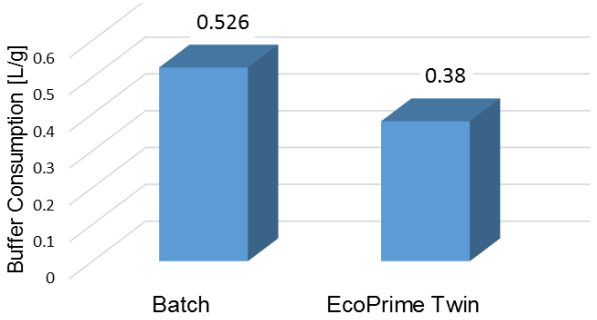
Customer 1

Productivity - Customer 1



1g/L titer mAb

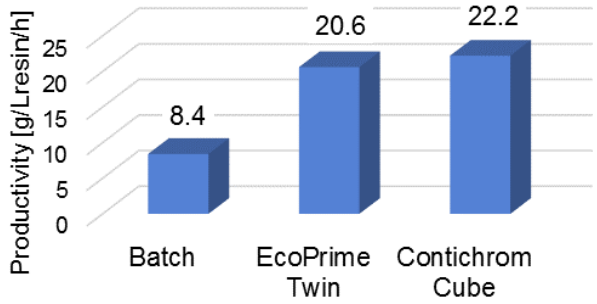
Buffer Consumption - Customer 1



Triple productivity or 67% less resin and ~ 30% buffer reduction

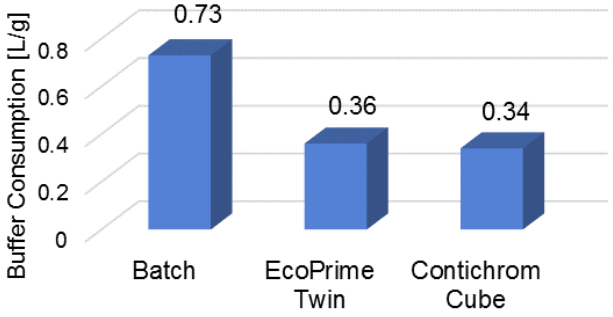
Bristol-Myers Squibb

Productivity - BMS



5g/L titer mAb

Buffer Consumption - BMS



> 2X productivity or 50% less resin and ~ 50% buffer reduction

mAb Product Quality Results: CaptureSMB vs. Batch

Table Impurity results for batch and pilot scale CaptureSMB

Sample Name	Concentration (g/L)	HCP (ppm)	DNA (ppb)	rProA (ppm)	HMW %	CE-SDS Purity %
Batch Run (product pool)	31.3	447	56	16	3.0	99.8
Run 1 (product pool)	30.2	464	42	18	2.9	99.8
Run 2 (product pool)	28.0	500	55	7	2.9	99.8

Single column batch reference

CaptureSMB

Comparable product quality with CaptureSMB and Protein A single-column, batch chromatography

Seamless transfer from batch to continuous capture

Customer B – pilot plant process comparison; 5 g/L titer mAb

BioProcess Technical
Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification

Batch capture
Load 50 g/L resin
Total CV 17.3 L

	CV	linear [cm/h]
Pre-Sanitization	3	300
Equilibration	3	300
Load	10	150
Wash 1	2	300
Wash 2	5	300
Wash 3	3	300
Elution	5	300
CIP	3	300
Neutralization 1	3	300
Sanitization	3	300
Neutralization 2	3	300
Storage	3	300

Continuous capture
Load 80 g/L resin
Total CV 1.6 L

	CV	linear [cm/h]
Pre-Sanitization	3	400
Equilibration	3	400
Load	4.6	100
Load interconnected	11.4	150
Wash 1	2	400
Wash 2	5	400
Wash 3	3	400
Elution	3.5	400
CIP	3	400
Re-equilibration	3	400
Sanitization	3	400
Neutralization 2	3	400
Storage	3	400

The same process steps enables simple process transfer

Shorter process time; more productive

Customer B – pilot plant process comparison; 5 g/L titer mAb

BioProcess Technical
Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification

Batch capture
Load 50 g/L resin
Total CV 17.3 L

	CV	linear [cm/h]
Pre-Sanitization	3	300
Equilibration	3	300
Load	10	150
Wash 1	2	300
Wash 2	5	300
Wash 3	3	300
Elution	5	300
CIP	3	300
Neutralization 1	3	300
Sanitization	3	300
Neutralization 2	3	300
Storage	3	300

Process time: 4.5 h

Continuous capture
Load 80 g/L resin
Total CV 1.6 L

	CV	linear [cm/h]
Pre-Sanitization	3	400
Equilibration	3	400
Load	4.6	100
Load interconnected	11.4	150
Wash 1	2	400
Wash 2	5	400
Wash 3	3	400
Elution	3.5	400
CIP	3	400
Re-equilibration	3	400
Sanitization	3	400
Neutralization 2	3	400
Storage	3	400

Cycle time: 2.5 h

Faster linear velocity results in shorter processing time

Higher load 10 vs 16 CVs results in higher resin utilization & reduce buffer consumption

Reproducibility and scalability

Customer B – 5g/L titer mAb: 80 g/L load

Similar chromatograms when scaling up and down

Peak #	Column 1 Peak area	Column 2 Peak area
1	0.239	0.276
2	0.255	0.283
3	0.259	0.285
4	0.260	0.286
Average	0.258	0.285
Error %	1.1	0.7

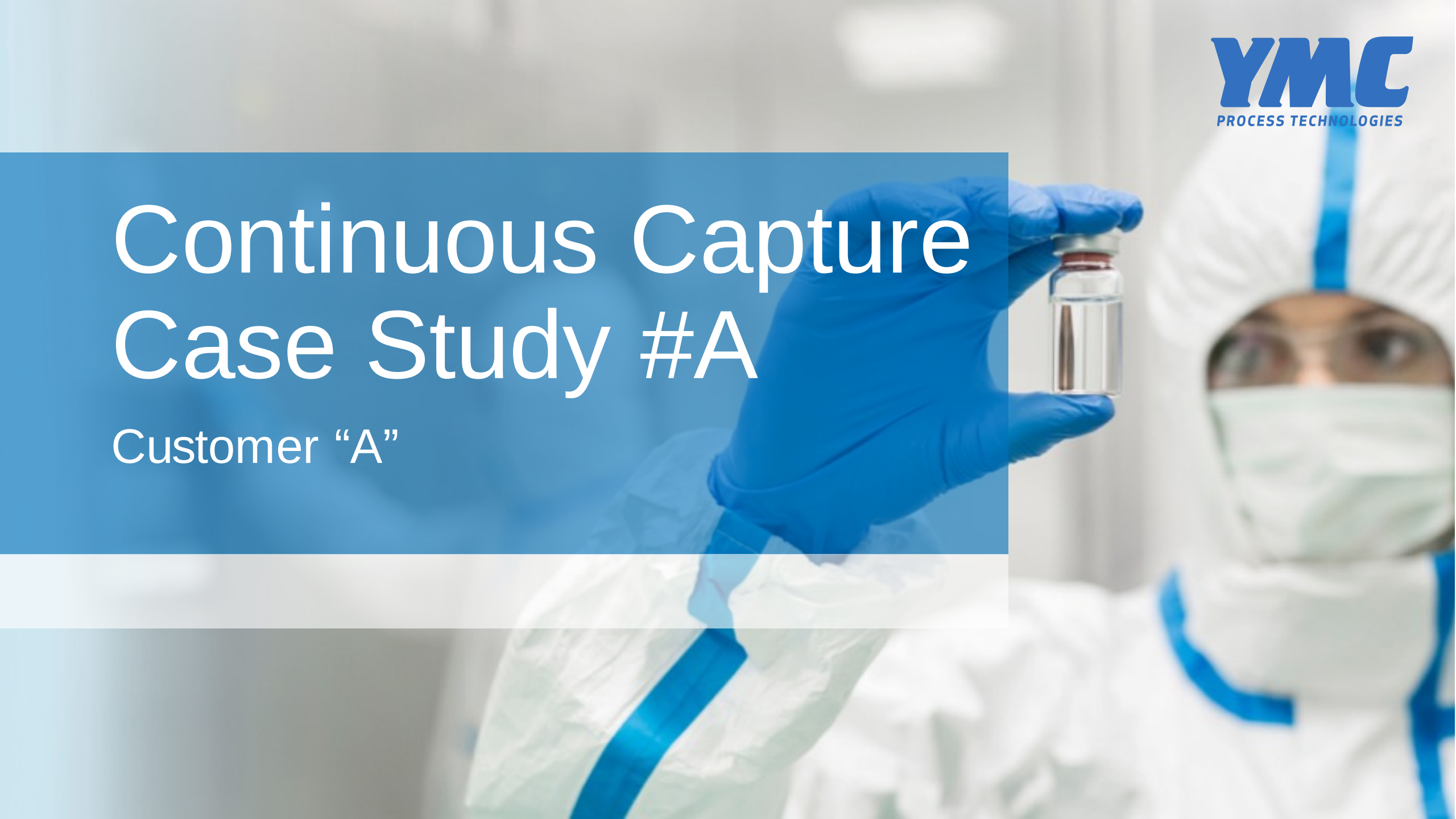
Steady state can be reached with first elution.

Variability of peak area is ~1 % at steady state

BioProcess Technical
Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification

Continuous Capture Case Study #A

Customer “A”



Simple transfer from batch to continuous capture

Customer A - process development run comparison with 1g/L titer mAb

Batch Capture
Total CV 7.85 L

	CV	Linear [cm/h]
Column Equilibration	3	250
Load	40	150
Wash 1	2	150
Wash 2	2	250
Wash 3	3	250
Elution	3	250
post wash 1	3	250
post wash 2*	2	250
Regen*	3	250

Continuous Capture
Total CV 1.6 L

	CV
Load Start-Up	15
Load Connected	27
Load Parallel	22
Wash 1 connected	1
Wash 1 Parallel	1
Wash 2	2
Wash 3	3
Elution	3
Post-Wash1	2
Post Wash 2	1
Regeneration	3
Re-Equilibration	5

Essentially same process steps = simple process transfer

The continuous capture advantage

Customer A - process development run comparison with 1g/L titer mAb

Batch Capture
Total CV 7.85 L

	CV	Linear [cm/h]
Column Equilibration	3	250
Load	40	150
Wash 1	2	150
Wash 2	2	250
Wash 3	3	250
Elution	3	250
post wash 1	3	250
post wash 2*	2	250
Regen*	3	250

(CIP)

Process time: 9 h

Continuous Capture
Total CV 1.6 L

	CV
Load Start-Up	15
Load Connected	27
Load Parallel	22
Wash 1 connected	1
Wash 1 Parallel	1
Wash 2	2
Wash 3	3
Elution	3
Post-Wash1	2
Post Wash 2	1
Regeneration	3
Re-Equilibration	5

(CIP after every 4th cycle)

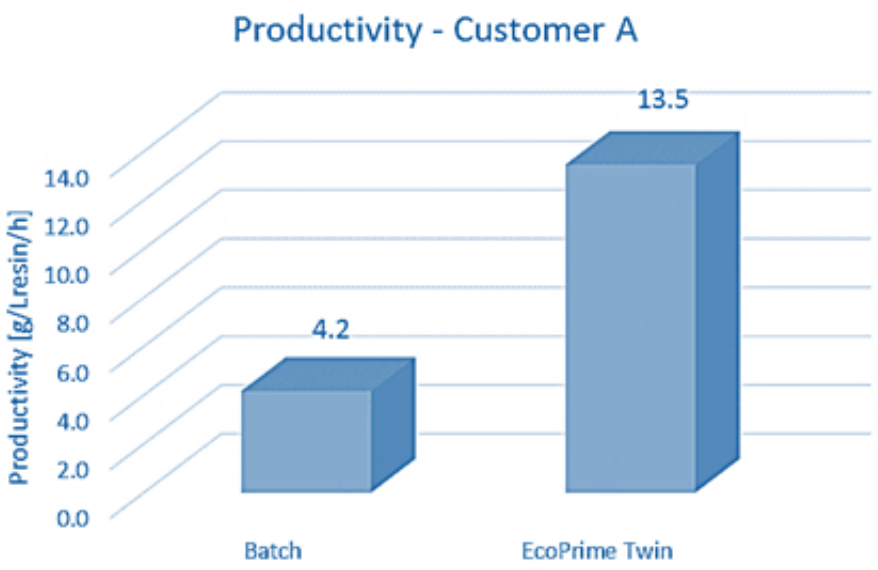
Cycle time: 4 h at linear velocity of 250 cm/h

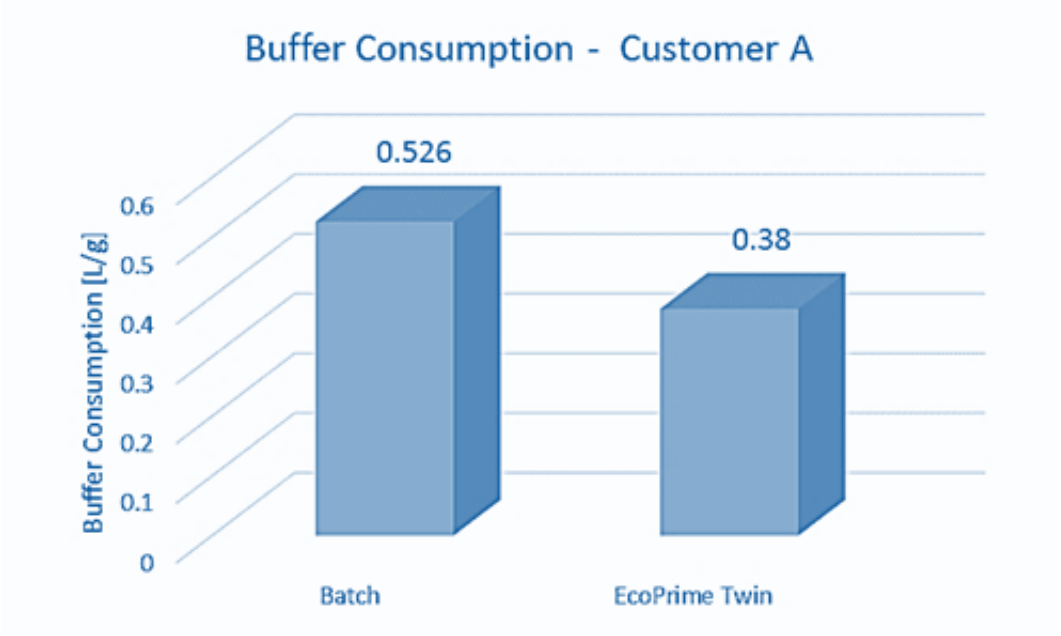
The same amount of material can be processed in 50% less time.

Higher load volume 40 vs 49 CVs → higher resin utilization & reduce buffer consumption
Shorter residence times → faster loading & shorter processing time

The continuous capture advantage

Customer A - process development run: 1g/L titer mAb





Triple productivity or 67% less resin and ~30% reduction in buffer consumption

Case Study #M

Customer “M”

Process Savings using Continuous Capture Chromatography

Customer produced this "poster" to report results to their management

YMC EcoPrime Twin 100: 100L of 3 g/L Product

	Batch	Twin Column
# of Columns	1	2
Column Diameter (cm)	20	10
Column Bed Height (cm)	20	10
Total Resin Volume (L_{resin})	6.3	1.6
Binding Capacity (g/L_{resin})	40	60

	Batch	Twin Column
Cycles	2	3
Process Time (hr)	6	9
Buffer Requirement (L)	300	150
Resin Cost ($16k/$L_{resin}$)	$100,800	$25,600
Productivity (g/ L_{resin} -hr)	10	20

YMC EcoPrime Twin 1000 : 2000L of 5 g/L Product

	Batch	Twin Column
# of Columns	1	2
Column Diameter (cm)	60	45
Column Bed Height (cm)	20	10
Total Resin Volume (L_{resin})	56	28
Binding Capacity (g/L_{resin})	35	65

	Batch	Twin Column
Cycles	6	5
Process Time (hr)	18	11
Buffer Requirement (L)	7100	3900
Resin Cost ($16k/$L_{resin}$)	$896,000	$448,000
Productivity (g/ L_{resin} -hr)	22	40

Process Savings using Continuous Capture Chromatography

Pilot data results – 3 g/L mAb product

YMC EcoPrime Twin 100: 100L of 3 g/L Product

	Batch	Twin Column
# of Columns	1	2
Column Diameter (cm)	20	10
Column Bed Height (cm)	20	10
Total Resin Volume (L_{resin})	6.3	1.6
Binding Capacity (g/L_{resin})	40	60

Twin pilot performance vs batch:

50% buffer reduction

75% less ProA

2X productivity

	Batch	Twin Column
Cycles	2	3
Process Time (hr)	6	9
Buffer Requirement (L)	300	150
Resin Cost ($16k/$L_{resin}$)	$100,800	$25,600
Productivity (g/L_{resin}-hr)	10	20

YMC EcoPrime Twin 1000 : 2000L of 5 g/L Product

	Batch	Twin Column
# of Columns	1	2
Column Diameter (cm)	60	45
Column Bed Height (cm)	20	10
Total Resin Volume (L_{resin})	56	28
Binding Capacity (g/L_{resin})	35	65

	Batch	Twin Column
Cycles	6	5
Process Time (hr)	18	11
Buffer Requirement (L)	7100	3900
Resin Cost ($16k/$L_{resin}$)	$896,000	$448,000
Productivity (g/L_{resin}-hr)	22	40

Process Savings using Continuous Capture Chromatography

Production scale model from pilot 2000L @ 5 g/L mAb product

YMC EcoPrime Twin 100: 100L of 3 g/L Product

	Batch	Twin Column
# of Columns	1	2
Column Diameter (cm)	20	10
Column Bed Height (cm)	20	10
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Total Resin Volume (L_{resin})	56	28
Binding Capacity (g/L_{resin})	35	65

	Batch	Twin Column
Cycles	6	5
Process Time (hr)	18	11
Buffer Requirement (L)	7100	3900
Resin Cost ($16k/$L_{resin}$)	$896,000	$448,000
Productivity (g/ L_{resin} -hr)	22	40

Scale up Twin vs batch:

40% time savings

~$600K / year buffer savings

>$400K less ProA

Process Savings Using Continuous Capture Chromatography

Customer produced this "poster" to report results to their management

EcoPrime Twin 100: 100L of 3 g/L Product

Column Design

	Batch	Twin Column
# of Columns	1	2
Column Diameter	20	10
Column Bed Height	20	10
Total Resin Volume (L_{resin})	6.3	1.6
Binding Capacity (g/L_{resin})	35	65

Process Requirements

	Batch	Twin Column
Cycles	2	3
Buffer Requirement (L)	300	150
Productivity (g/ L_{resin}-hr)	10	20

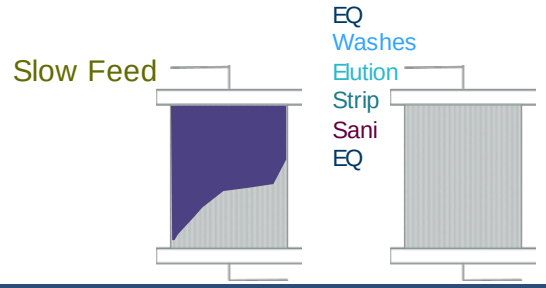
EcoPrime T

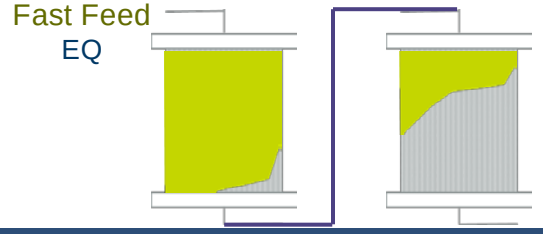
Column

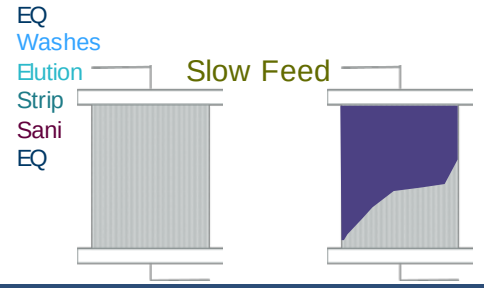
	Batch	Twin Column
# of Columns	1	2
Column Diameter	60	45
Column Bed Height	20	10
Total Resin Volume (L_{resin})	56	28
Binding Capacity (g/L_{resin})	35	65

Process Requirements

	Batch	Twin Column
Cycles	6	5
Buffer Requirement (L)	7100	3900
Productivity (g/ L_{resin}-hr)	22	40







Process Savings Using Continuous Capture Chromatography

Poster produced by Customer to report results to their management

YMC PROCESS TECHNOLOGIES

Lewa EcoPrime Twin 100: 100L of 3 g/L Product

Column Design

	Batch	Twin Column
# of Columns	1	2
Column Diameter	20	10
Column Bed Height	20	10
Total Resin Volume (L_{resin})	6.3	1.6
Binding Capacity (g/L_{resin})	35	65

Process Requirements

	Batch	Twin Column
Cycles	2	3
Buffer Requirement (L)	300	150
Productivity (g/ L_{resin} -hr)	10	20

Twin pilot performance vs batch:

2X productivity

50% buffer reduction

Lewa EcoPrime Twin 1000 : 2000L of 5 g/L Product

Column Design

	Batch	Twin Column
# of Columns	1	2
Column Diameter	60	45
Column Bed Height	20	10
Total Resin Volume (L_{resin})	56	28
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Process Requirements

	Batch	Twin Column
Cycles	6	5
Buffer Requirement (L)	7100	3900
Productivity (g/ L_{resin} -hr)	22	40

Process Savings Using Continuous Capture Chromatography

What this means in a full scale production environment….

YMC PROCESS TECHNOLOGIES

Lewa EcoPrime Twin 100: 100L of 3 g/L Product

Column Design

	Batch	Twin Column
# of Columns	1	2
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Total Resin Volume (L_{resin})	6.3	1.6
Binding Capacity (g/L_{resin})	35	65

Process Requirements

	Batch	Twin Column
Cycles	2	3
Process Wet Time (hr)	5	9
Buffer Requirement (L)	300	150
Productivity (g/ L_{resin} -hr)	10	20

Lewa EcoPrime Twin 1000 : 2000L of 5 g/L Product

Column Design

	Batch	Twin Column
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Process Requirements

	Batch	Twin Column
Cycles	6	5
Buffer Requirement (L)	7100	3900
Productivity (g/ L_{resin} -hr)	22	40

Twin economics vs batch:

$360,000 decrease per campaign in ProA resin

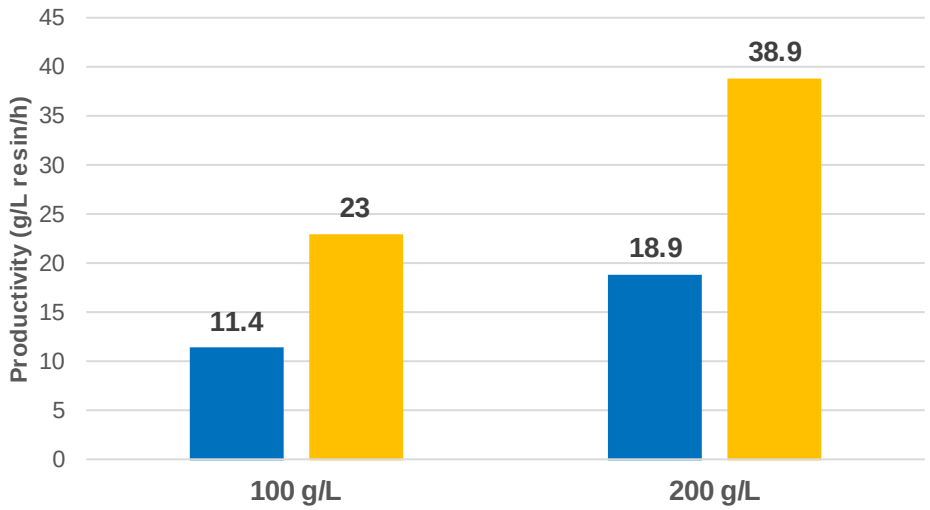
$640,000 yearly buffer savings (@$10/L x 20 (batches")

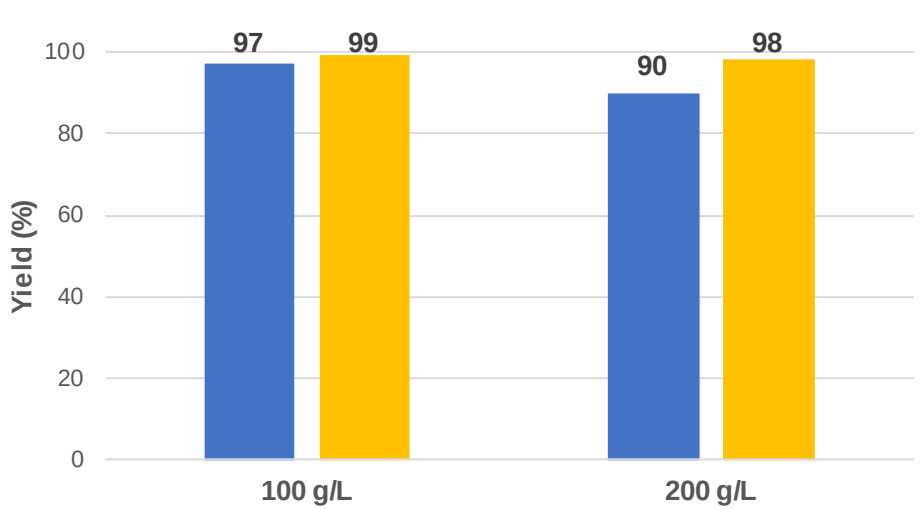
YMC
PROCESS TECHNOLOGIES

Sequential Polishing

Case Study

Double productivity with sequential processing





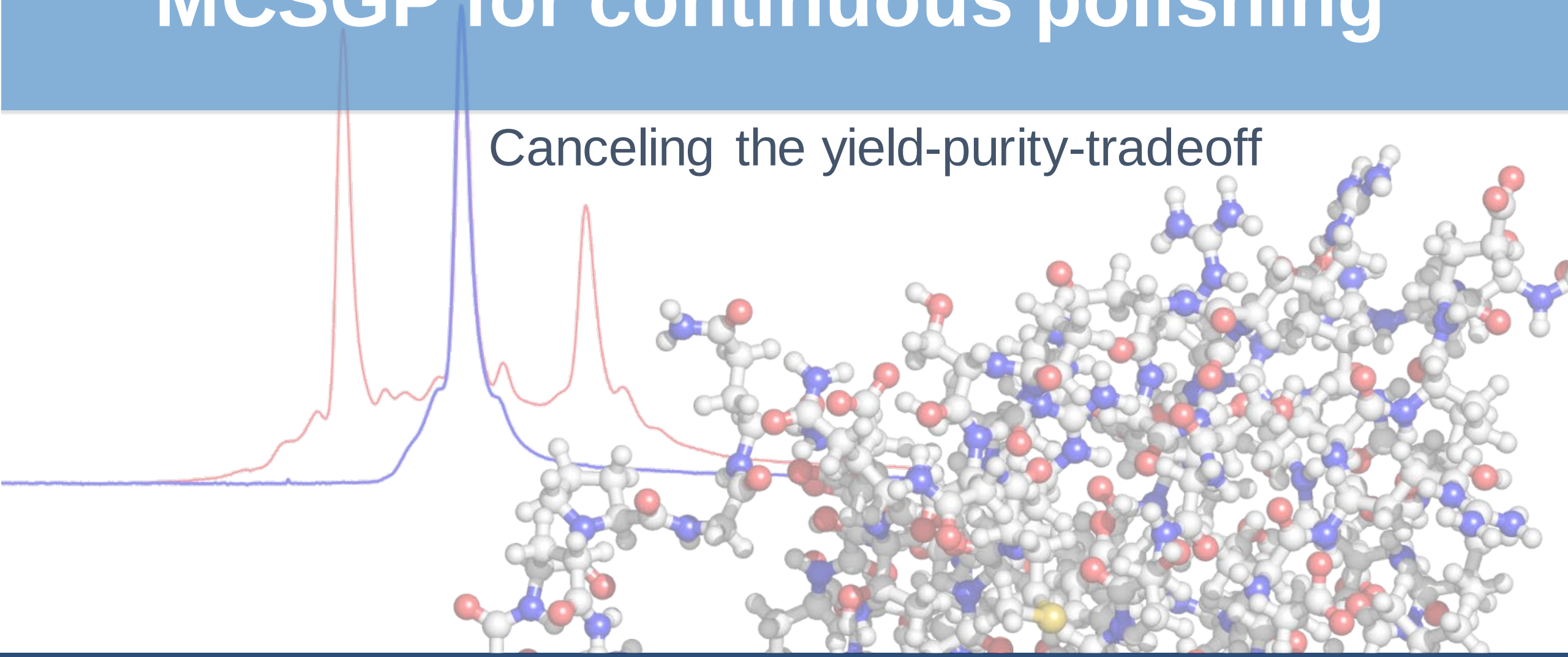
Batch Sequential

Up to 2-fold increase in productivity when using sequential over batch

- Increased further when accounting for changeover time between unit operations

MCSGP for continuous polishing

Canceling the yield-purity-tradeoff

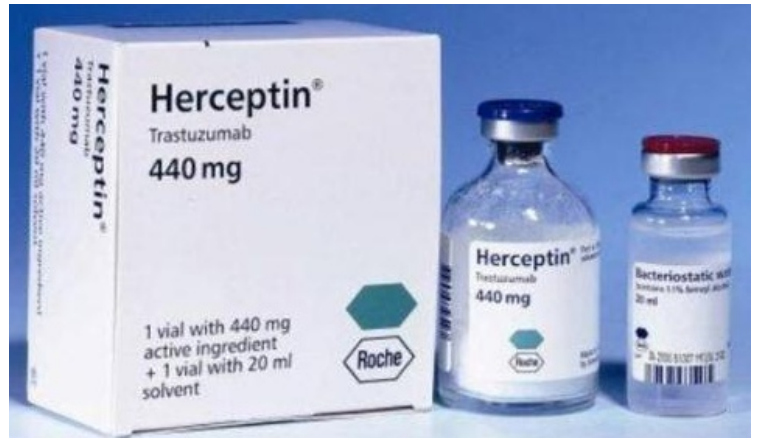


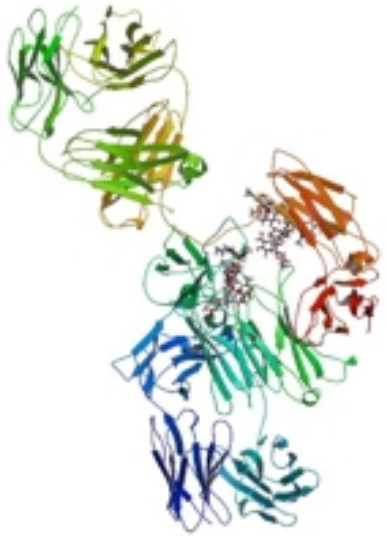
Case study: Herceptin charge isoform separation

Herceptin (Trastuzumab)

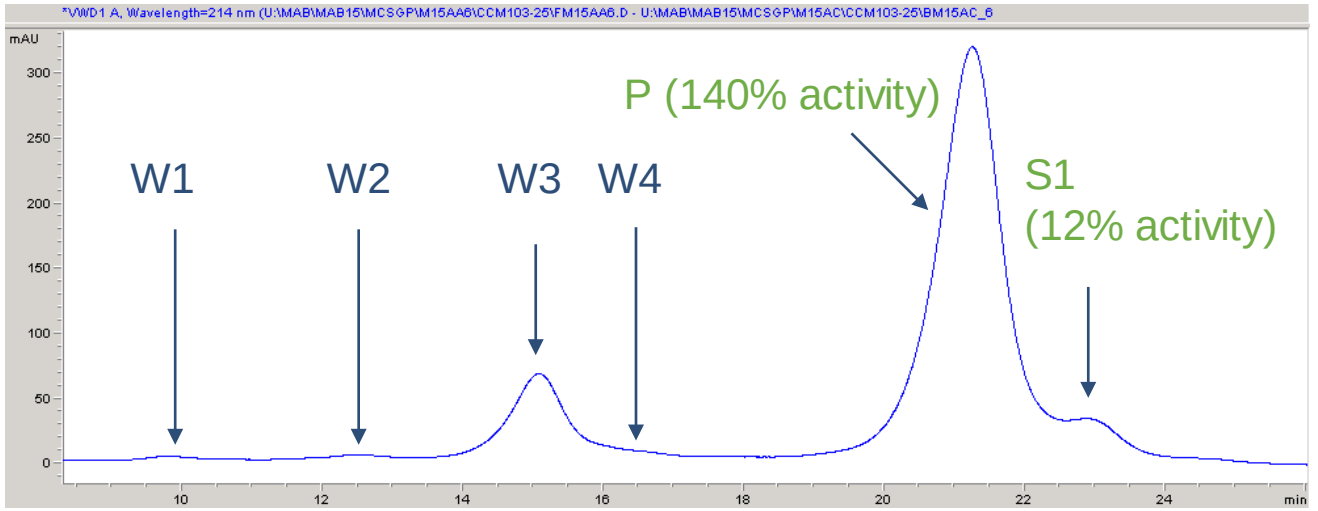
IgG1, pI = 8.45

Final product contains multiple isoforms with different activities.



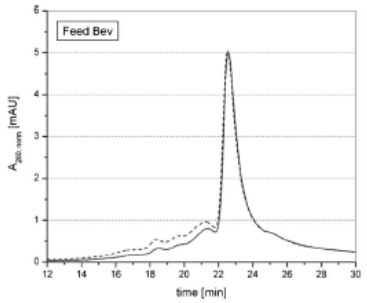
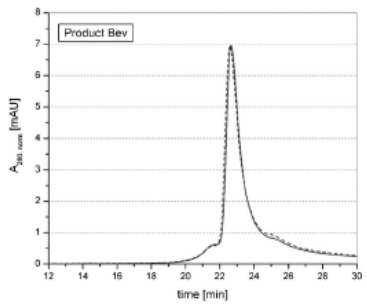
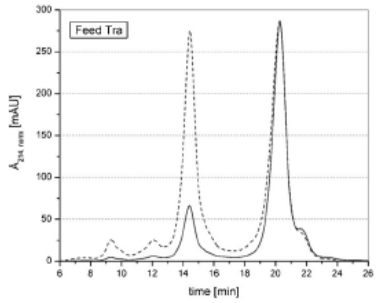
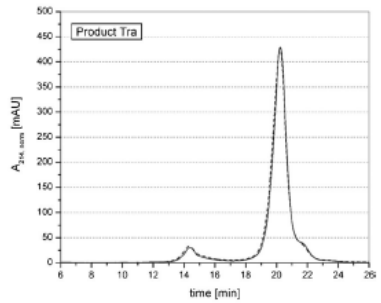
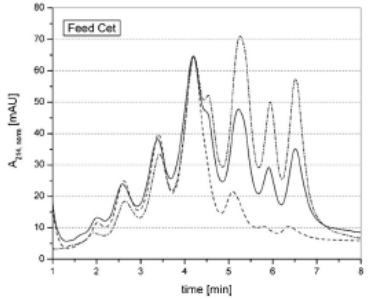
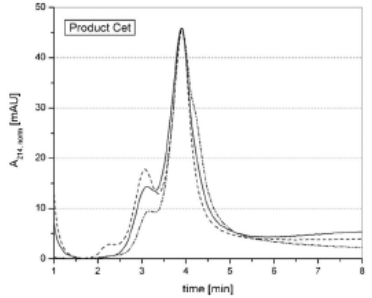


www.drugbank.ca



Analytical weak cation exchange chromatogram

Case study: mAb isoform profile tuning

	Feed	Product
Avastin® (Bevacizumab)		
Herceptin® (Trastuzumab)		
Erbitux® (Cetuximab)		

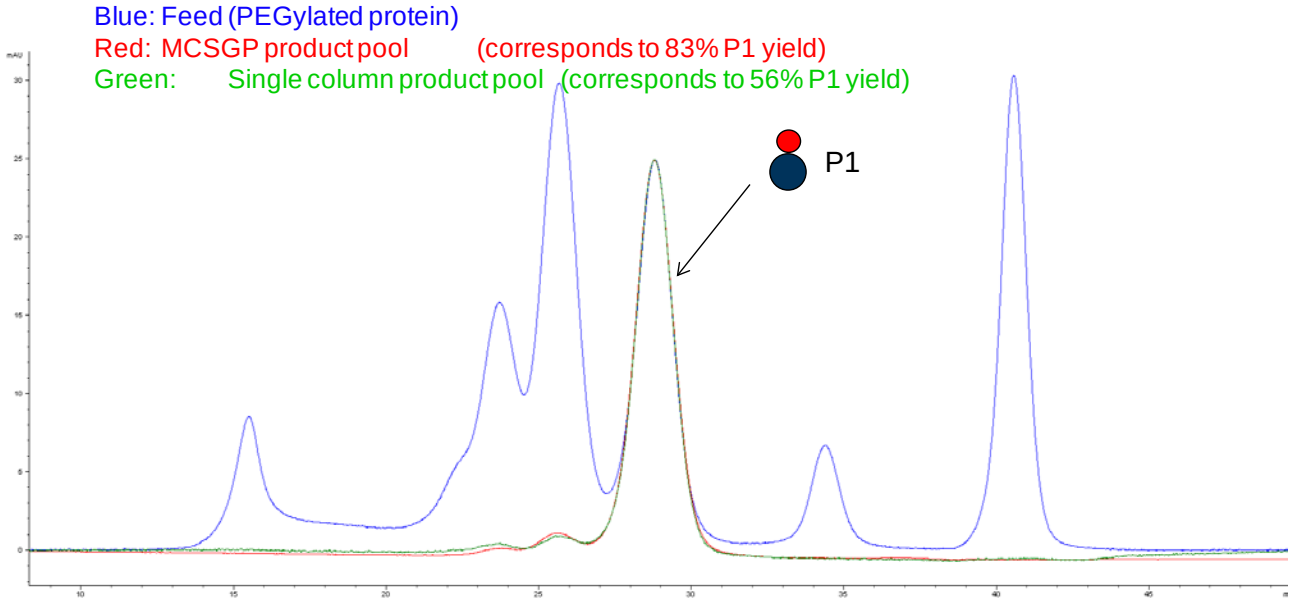
- Specific, more active isoforms are enriched
- Consistent product quality even with changing feed

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

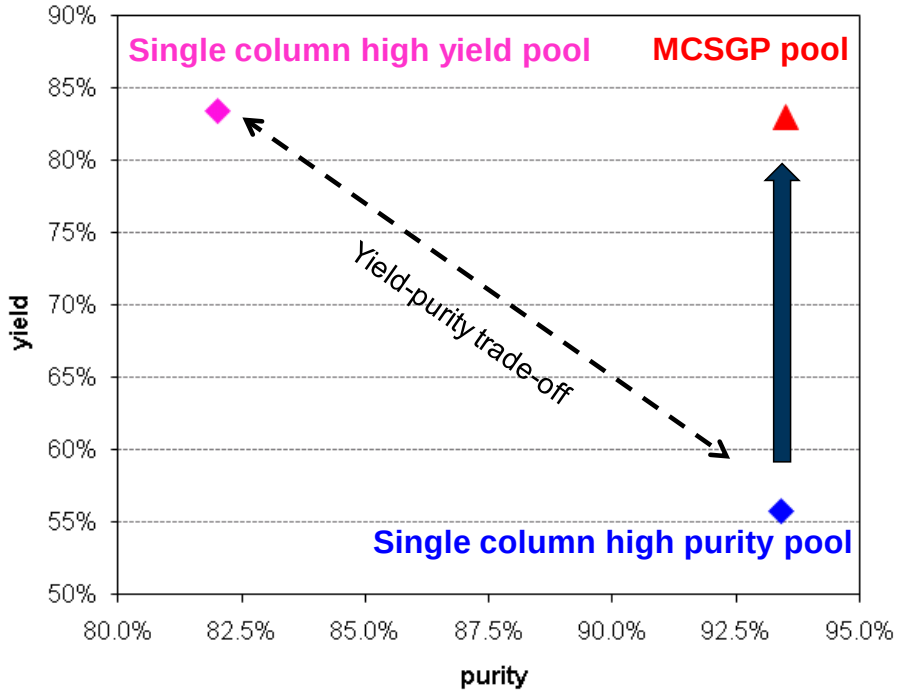
Case study: MCSGP purification of mono-PEGylated proteins

Yield	
Batch process	MCSGP process
56%	83%

Anion-exchange using POROS® HQ



SEC-analytics



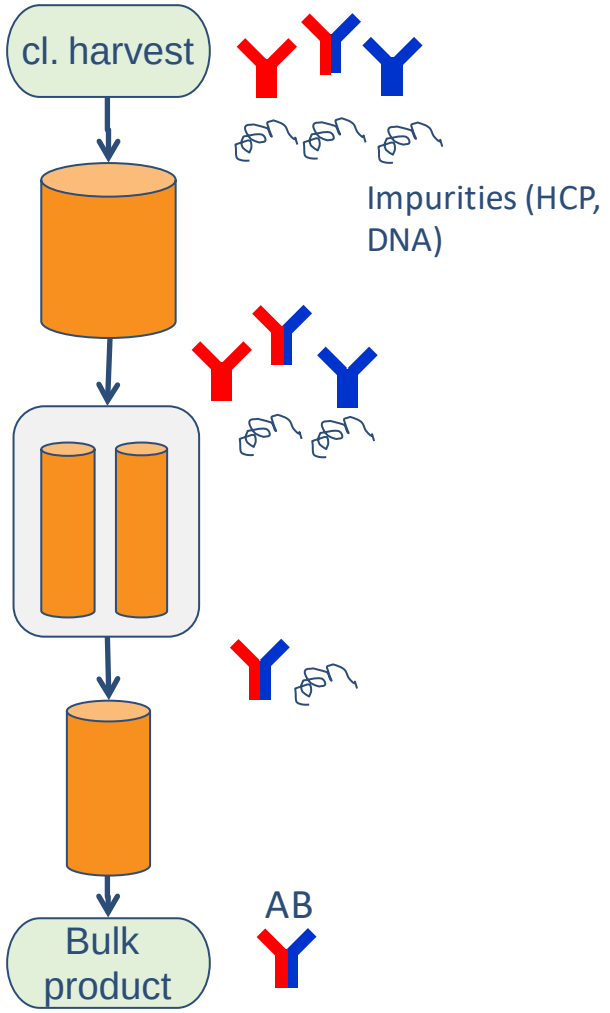
Economically attractive scenario can be established within a short development time.

Case study: bispecific antibody purification

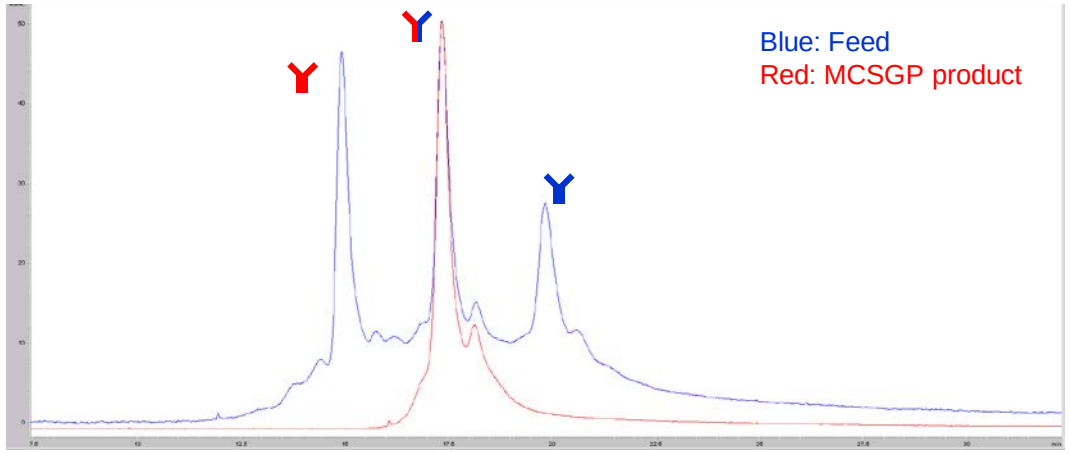
Protein A capture
(bind/elute mode)

CIEX-MCSGP
(bind/elute mode)

Multimodal / AIEX polish
(flowthrough mode)



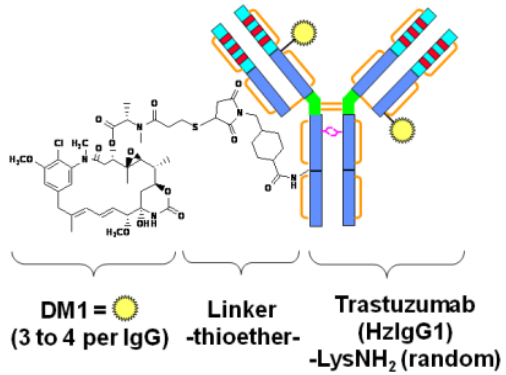
Purify bispecific antibody AB from PER.C6 harvest, remove aggregates, HCP, DNA, and parental antibodies AA and BB



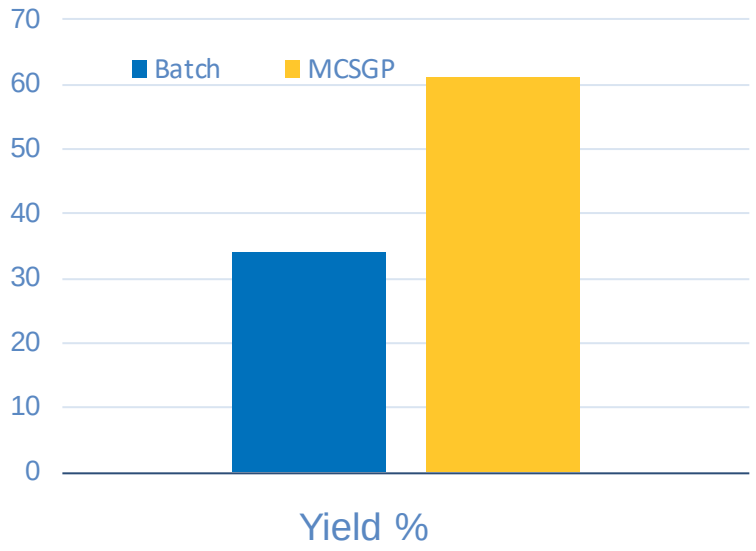
Analytical CIEX chromatogram

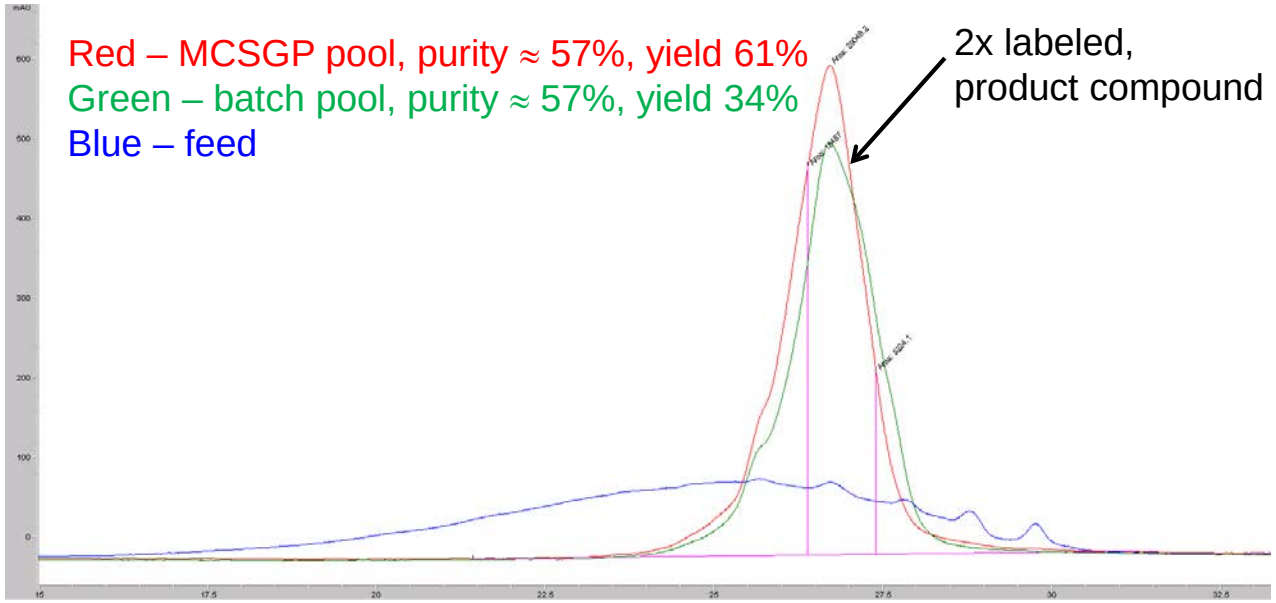
With MCSGP, the CIEX step yield was increased from 37% to 87%.

Case study: MCSGP for ADC (antibody-drug-conjugate) purification



Model system: Atto-488 instead of DM1



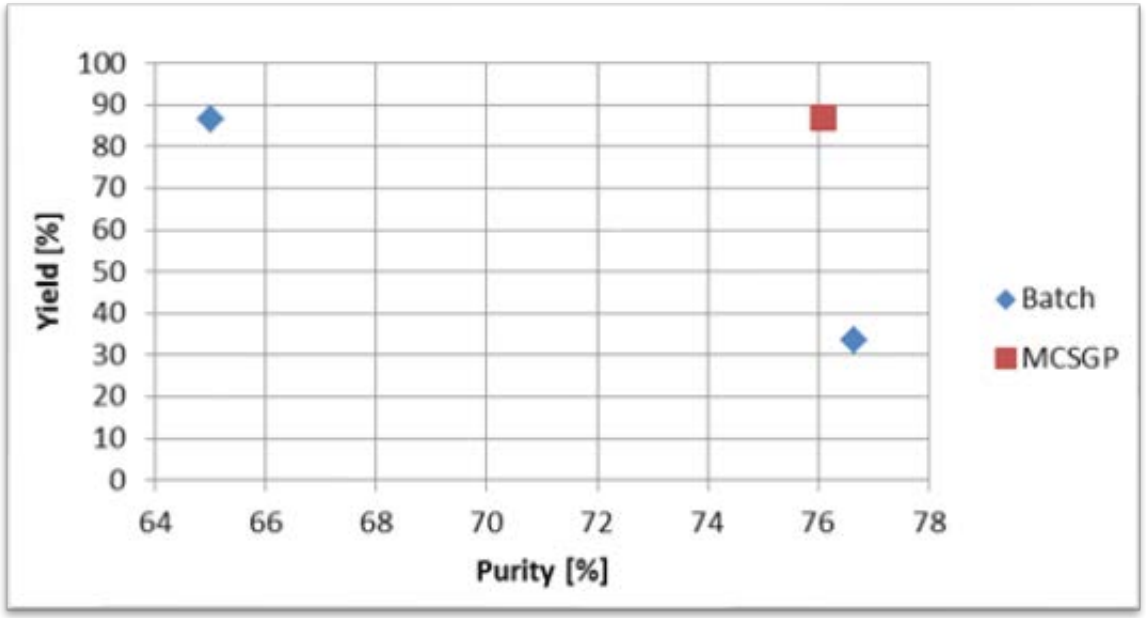


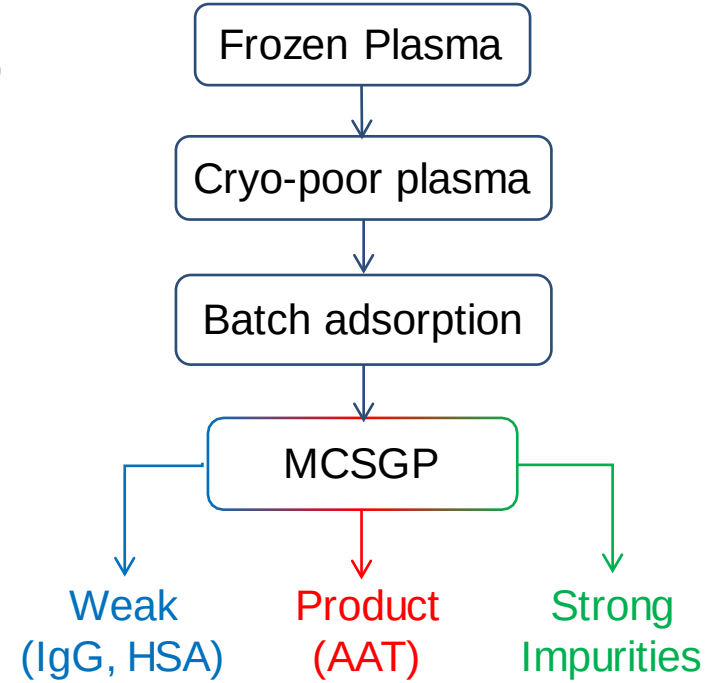
With MCSGP, yield increase from 34% to 61% with the same purity compared to traditional batch chromatography

- 80% productivity increase
- 55% reduction in buffer comsumption

Case study: α1-AT purification from human plasma

Replacement of a batch DEAE chromatography step by MCSGP chromatography (same resin)



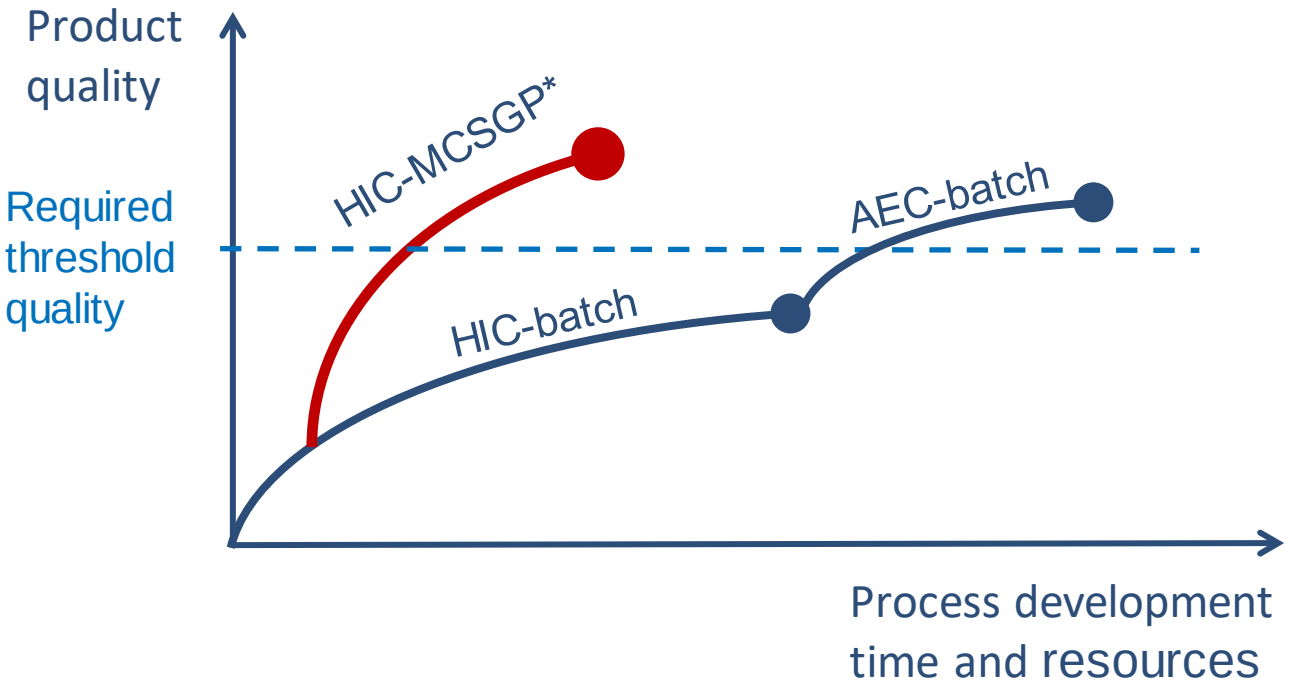


	Purity (%)	Yield (%)
Batch (max. P)	76.7	33.4
Batch (max. Y)	65.0	86.5
MCSGP	76.1	86.7

ETH Eidgenössische Technische Hochschule Zürich Swiss Federal Institute of Technology Zurich

D CHAB Department of Chemistry and Applied Biosciences

Case study: process development time savings with MCSGP



- To reach required quality with a batch process, extensive process development must be performed.
- Switching to MCSGP from a simple, non-optimized batch process results in the required product quality in a shorter time.

*could use a generic AEC step with zero development time

Accelerated process development time

Step	Activity	Batch Duration	MCSGP Duration
HIC	Solubility screen	2 weeks	2 weeks
HIC	Resin screen #1 (capacity and recovery as function of resin)	2	2
HIC	Resin screen #2 (resolution as function of resin)	2	1
HIC	Batch optimization (loading density, gradient shape, pooling criteria) MCSGP development and optimization	4	6
AEC	Resin screen (resolution as function of resin and pH)	2	
AEC	Optimization (loading density, gradient shape, pooling criteria)	6	
	Total	**17**	**11**

Case study: Comparison of process economics for Biologic Y

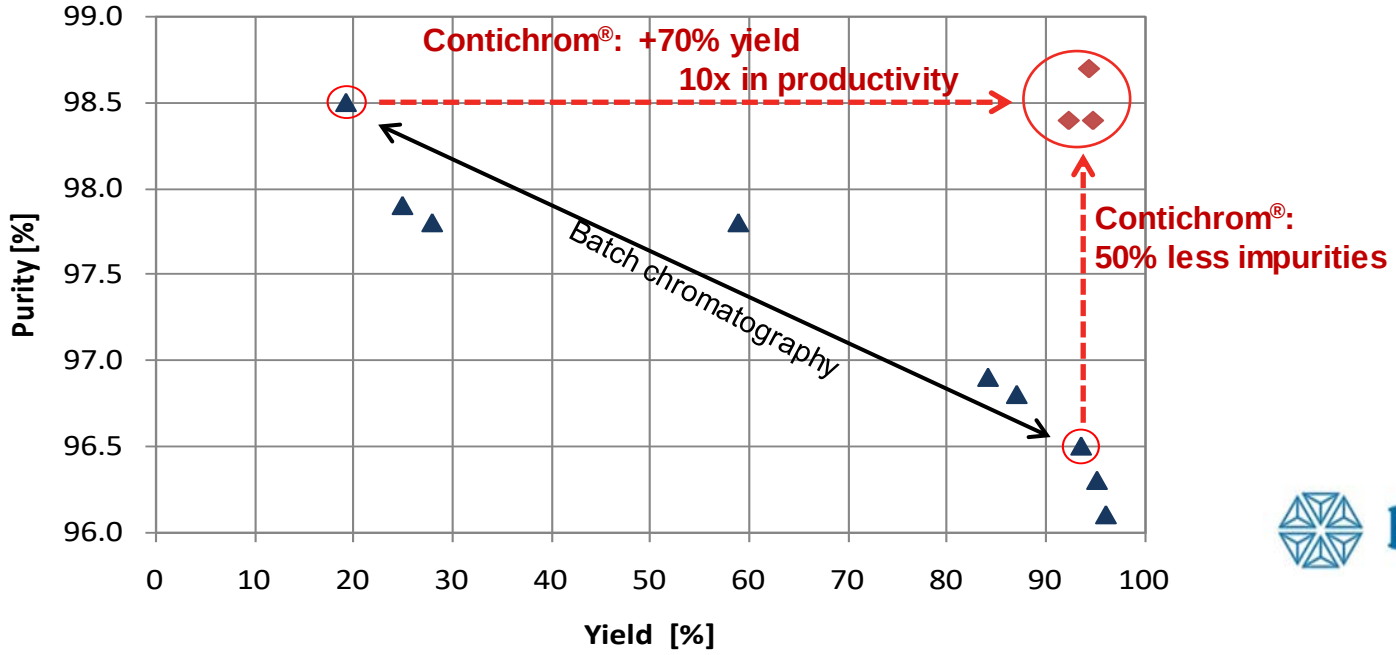
3-Step Chromatographic Purification Process	
Batch – Batch - Batch	Batch – MCSGP - Batch
Each step having 60% yield	Yields: 60% - 90% - 60%
Overall yield is 21.6%	Overall yield is 32.4%

Process economics

- COGs: Batch-Batch-Batch $20/g, Batch-MCSGP-Batch $14.7
- Fermenter 10,000L, 2 g/L titer
- Fix cost per year: $10M
- 40 batches per year
- Overall amount per year: 800 kg/year
- Savings per year with MCSGP is >$4M

Case: Therapeutic peptide purification by MCSGP

- Study showed substantial performance improvement through use of MCSGP resolving the yield/purity trade-off problem of batch chromatography



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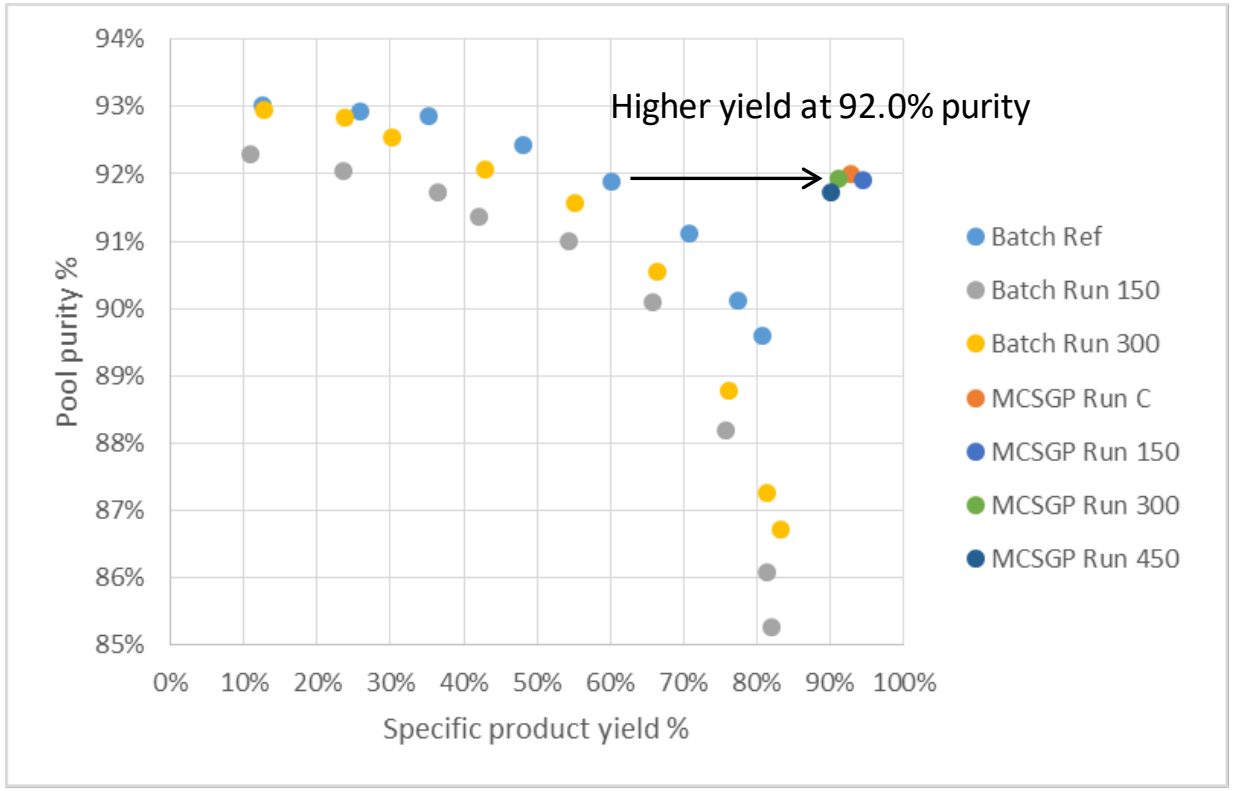
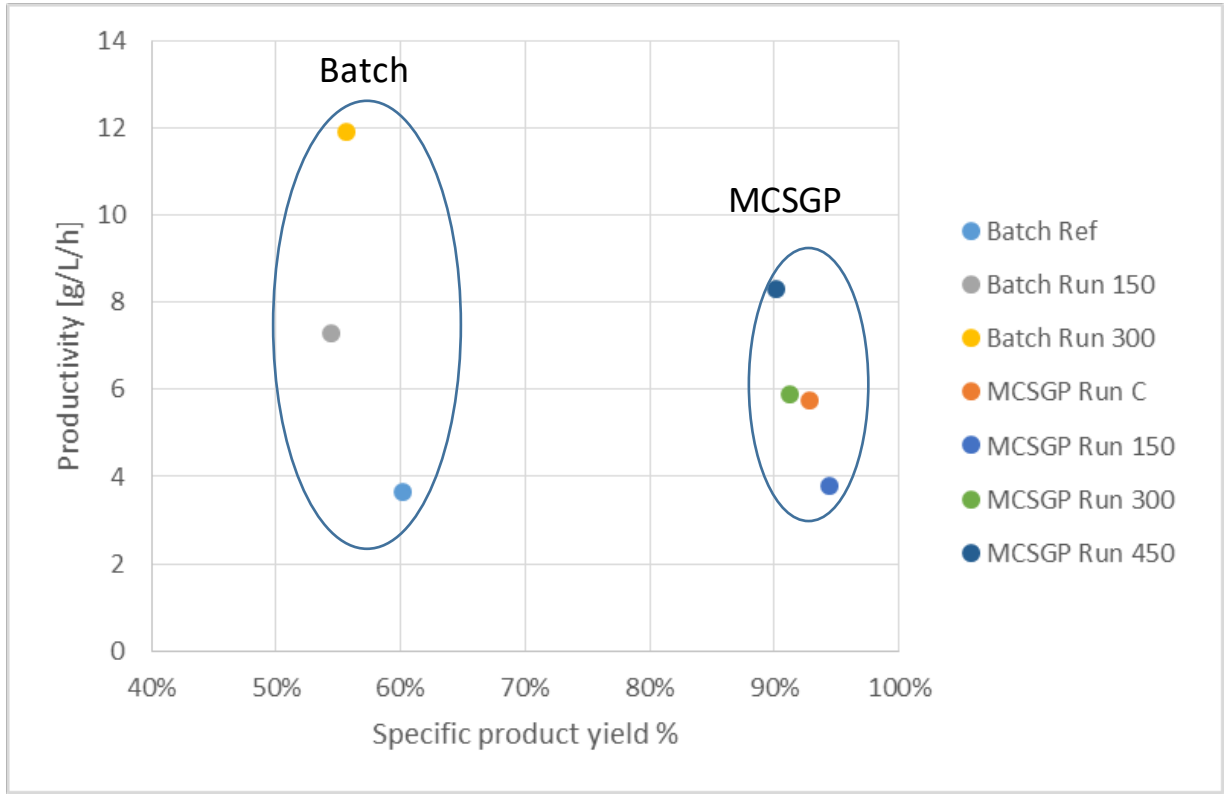
Bristol-Myers Squibb

Oligonucleotide purification: Process comparison - Batch vs. MCSGP

Pareto curve – Yield vs. Purity



Yield vs. productivity



Advantages of MCSGP:

- Yield improved from 60% to 90-95% at similar purity (92%) compared to batch chromatography
- Productivity improved from 3.7 to 8.3 g/L/h (see next slide)

Case study: Case study biologic Y

Scenario batch/batch/batch:

- 3-step chromatographic purification process with each batch having 60% yield ⇨ 21.6% overall yield

Scenario batch/MCSGP/batch:

- Sequence Batch-MCSGP-batch with yields 60% / 90% / 60% -> 32.4% overall yield (relative increase by 50%)

- COGS from downstream: batch $20/g, batch/MCSGP/batch: $14.7/g
- Fermenter 10‘000L, 2g/L titer
- Fix cost per year: $10M
- 40 batches per year
- Overall amount per year: 800kg/year

Savings per year with MCSGP: >$4M

Case study: purification of biologic Y

Study in collaboration with Sandoz, 2011

- Results reported in an MIT master thesis, http://dspace.mit.edu/handle/1721.1/66045

Goals of thesis

- Investigate financial impact of changing DSP steps for manufacturing of a complex biologic
- Evaluated processes: MCSGP, BioSC

Summary:

- COG could be decreased by 25% using MCSGP
- eNPV for use of MCSGP >$25M

Results of Monte-Carlo risk analysis:

- Only 5% chance of negative NPV for use of MCSGP

The future provides broader solutions for our customers

YMC Co., Ltd. assumed all rights and production for the EcoPrime suite of systems in late 2018 from LEWA-Nikkiso America, Inc. and the Contichrom portfolio from ChromaCon AG in 2019. These acquisitions bring a broad spectrum of chromatographic resins, and columns ideal for large and small molecule purification in a continuous process format.

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