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Purification of Peptides by Twin-column Countercurrent Chromatography

Thomas Müller-Späth[1,2]

[1]Institute for Chemical and Bioengineering, ETH Zurich, 8093 Zurich, Switzerland

[2]ChromaCon AG, 8005, Zurich, Switzerland

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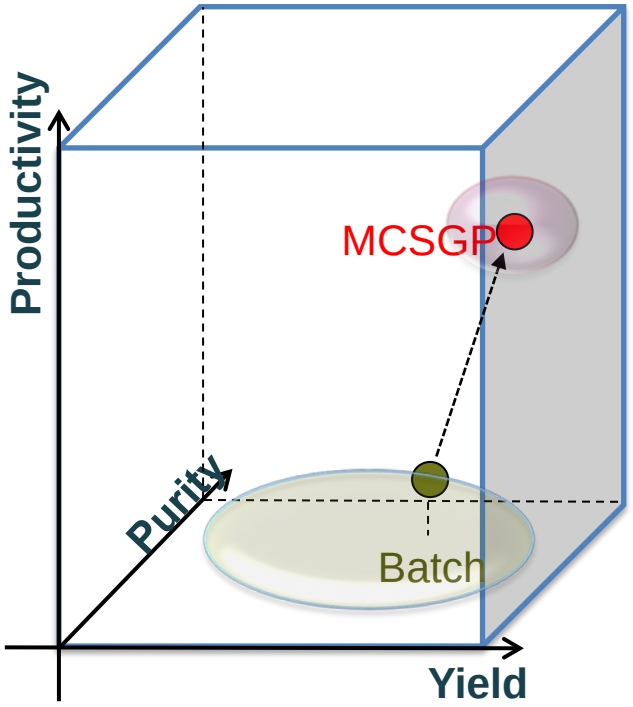
Presentation Highlights

MCSGP is a counter-current chromatography process that simultaneously achieves high yield and purity in difficult peptide purifications.

- The increased yield of MCSGP …
  - ✓ allows downscaling of the upstream chemical synthesis steps
  - ✓ improves productivity of the downstream process leading to smaller columns required
  - ✓ reduces solvent consumption
  - ✓ eliminates the need for re-chromatography
  - ✓ avoids generation of side-fractions to be stored and analyzed (reduction of analytical burden)

Presentation Outline

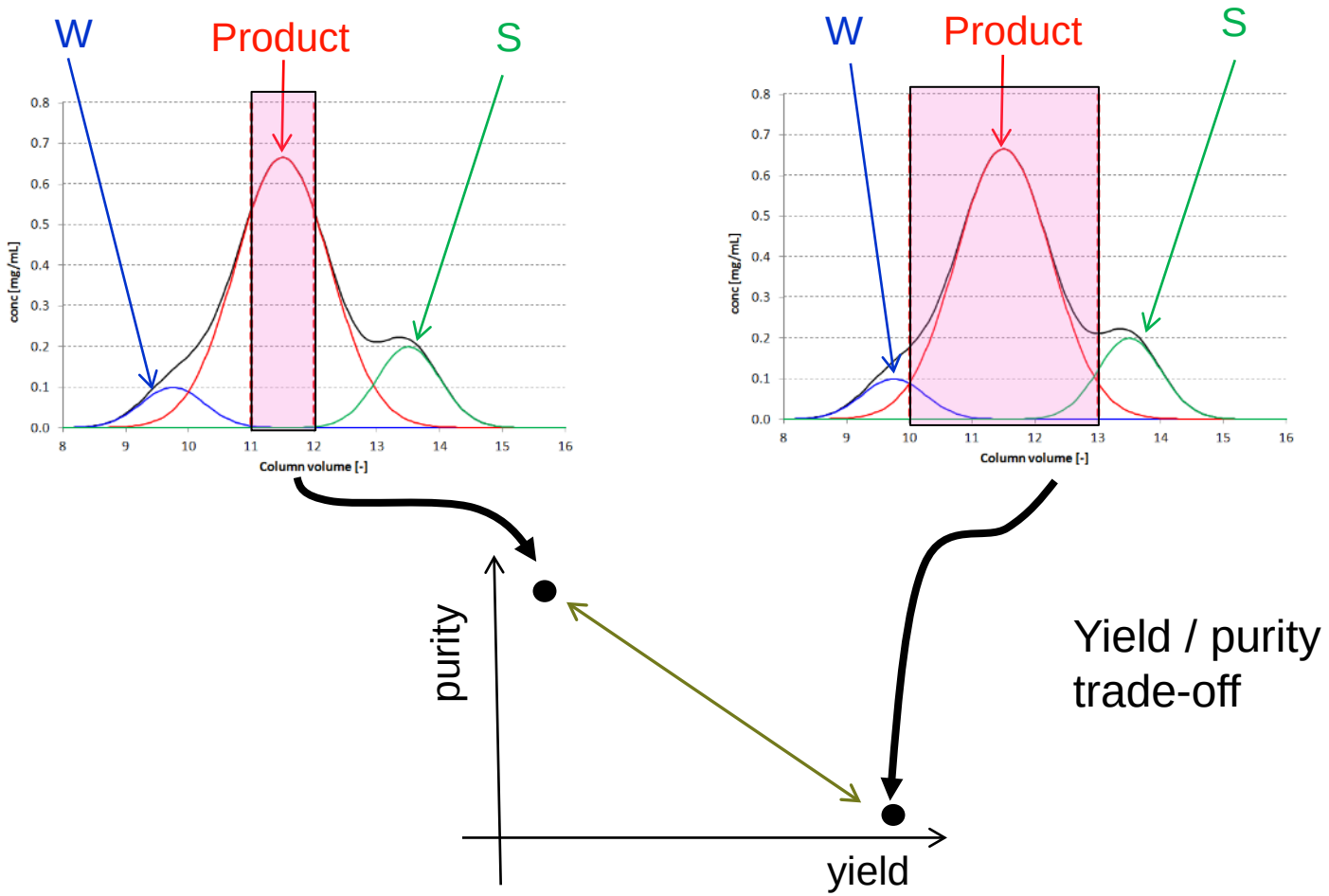
- Multicolumn chromatography (MCSGP) process introduction
- MControl - Dynamic process control for MCSGP
- MCSGP for peptide purification – case studies
- Economic evaluation of MCSGP



MCSGP - Multicolumn Countercurrent Solvent Gradient Chromatography

Purification Challenge

- Yield-purity trade-off for ternary separations

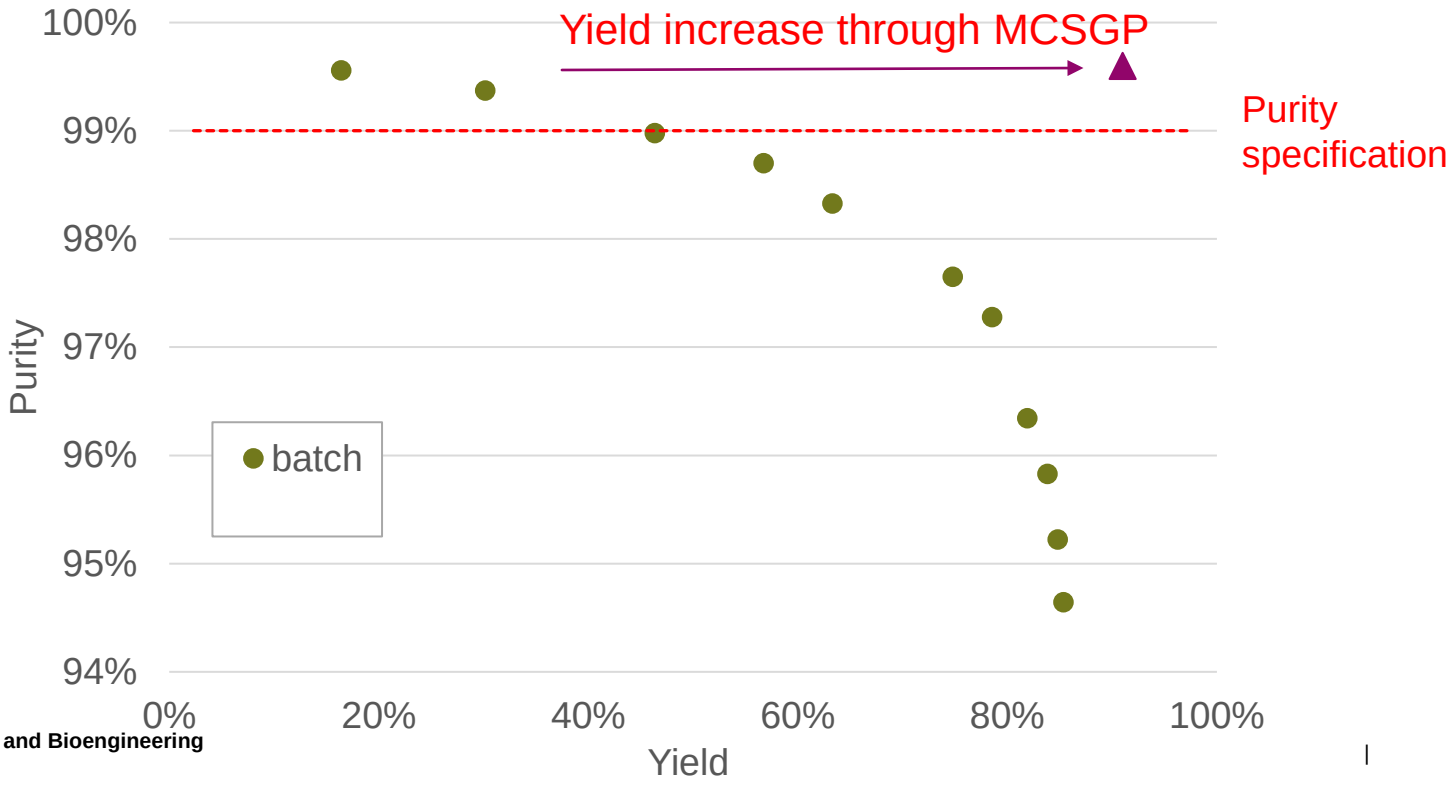


Background – MCSGP process principle

- Single column batch chromatography suffer from a yield-purity trade-off due to overlapping impurities:
- High purity can only be obtained at the cost of yield and vice versa
- Trade-off becomes worse with increasing load and increasing flow rate

→ In batch chromatography: Conflicting aims: purity vs. yield, load, productivity

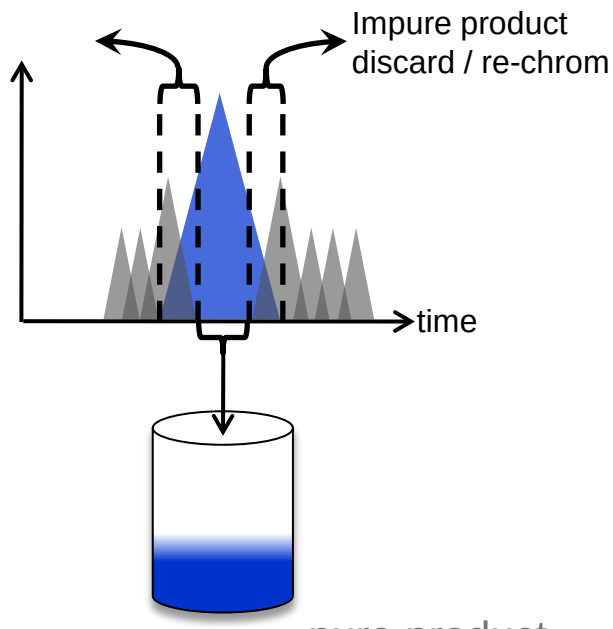
- MCSGP can obtain high purity and yield simultaneously



Background – MCSGP process principle

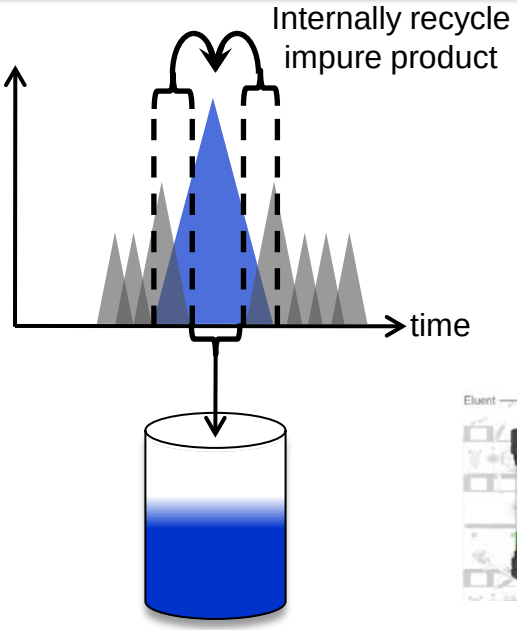
- MCSGP (Multicolumn counter-current solvent gradient purification) is a chromatography process that uses two columns of the same type
- MCSGP uses internal recycling and inline dilution fractions to automatically recover the product from impure side-fractions, eluting only product of high purity at a high yield

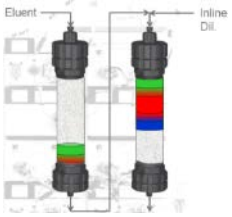
Conventional batch chromatography



pure product

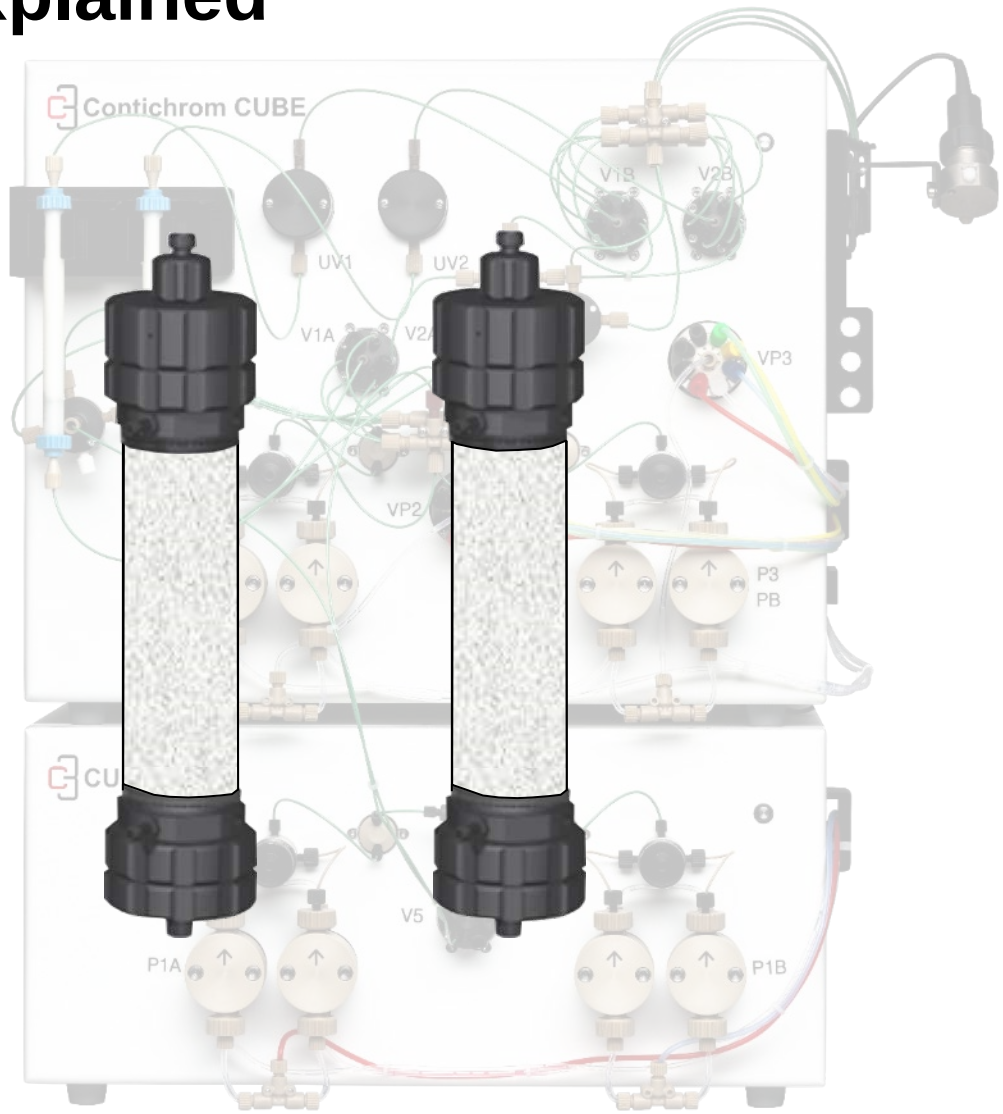
MCSGP chromatography



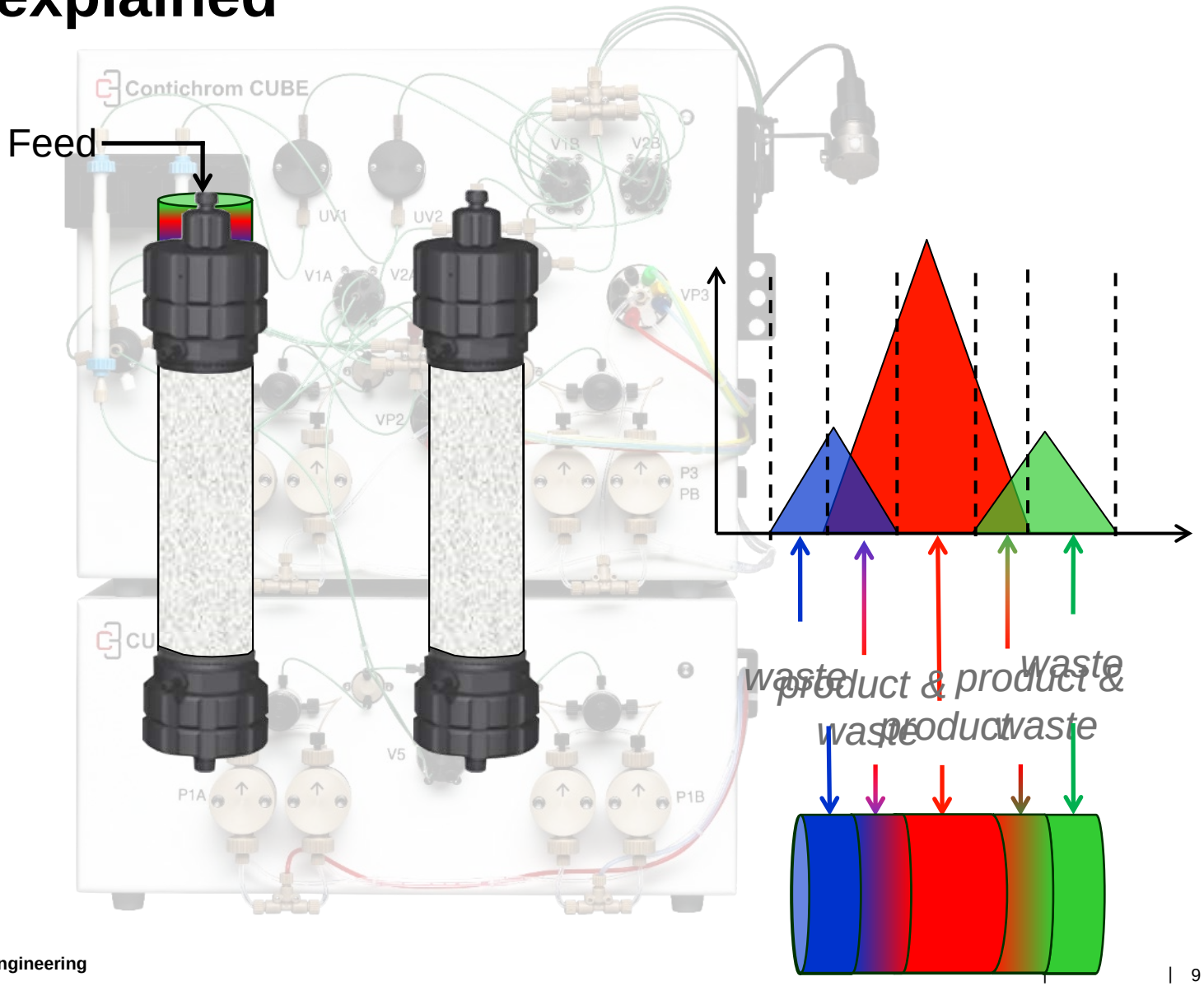


more pure product

MCSGP explained



MCSGP explained



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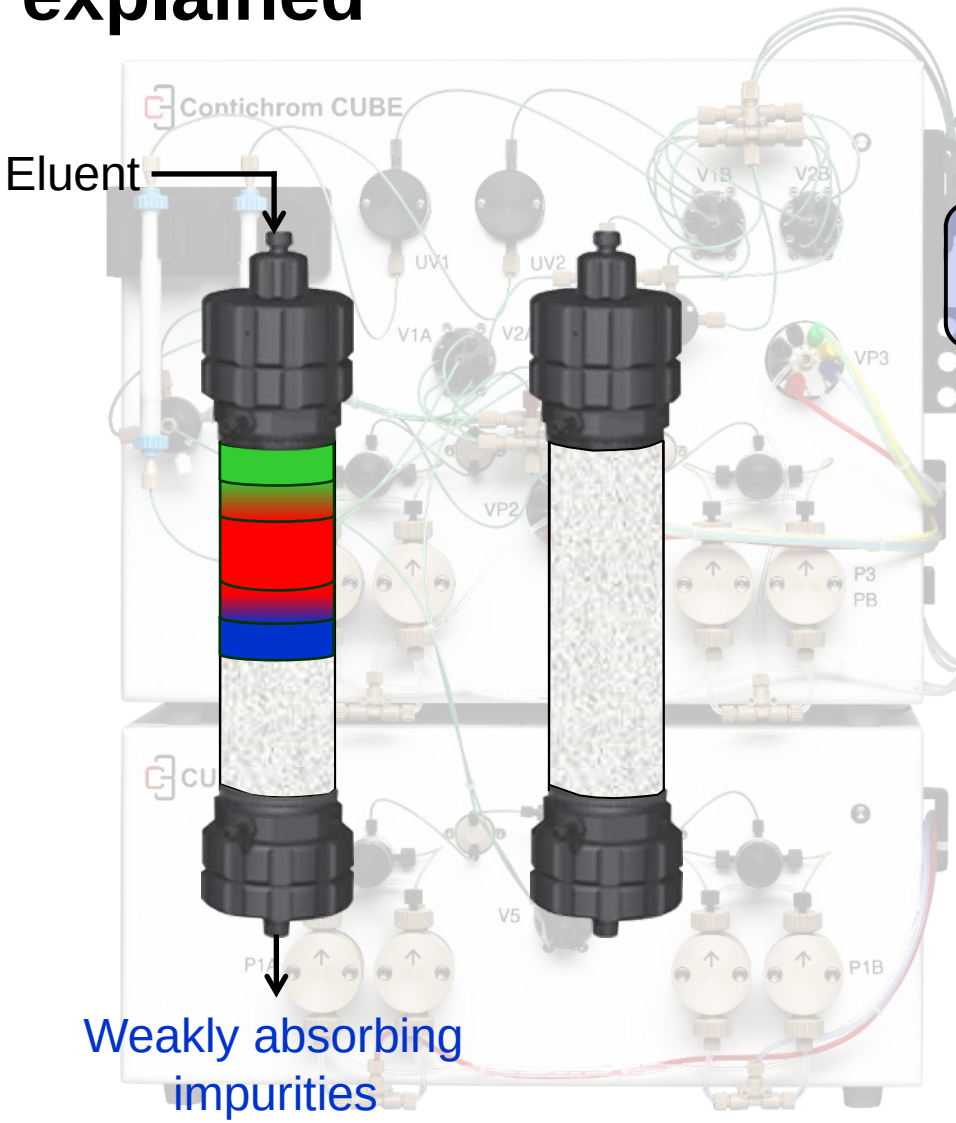


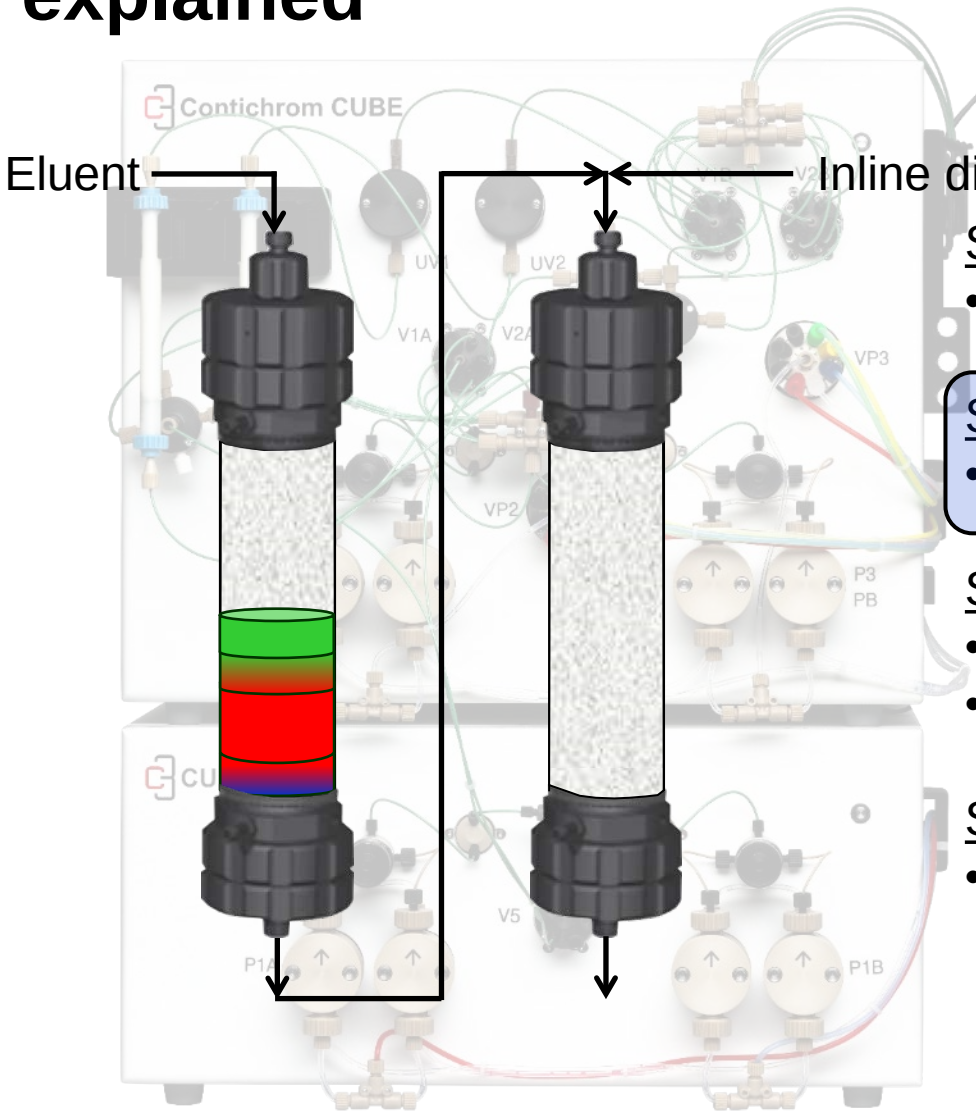
Step 1:
- elute waste

Step 2:
- recycle overlap

Step 3:
- elute product
- feed column

Step 4:
- recycle overlap

MCSGP explained



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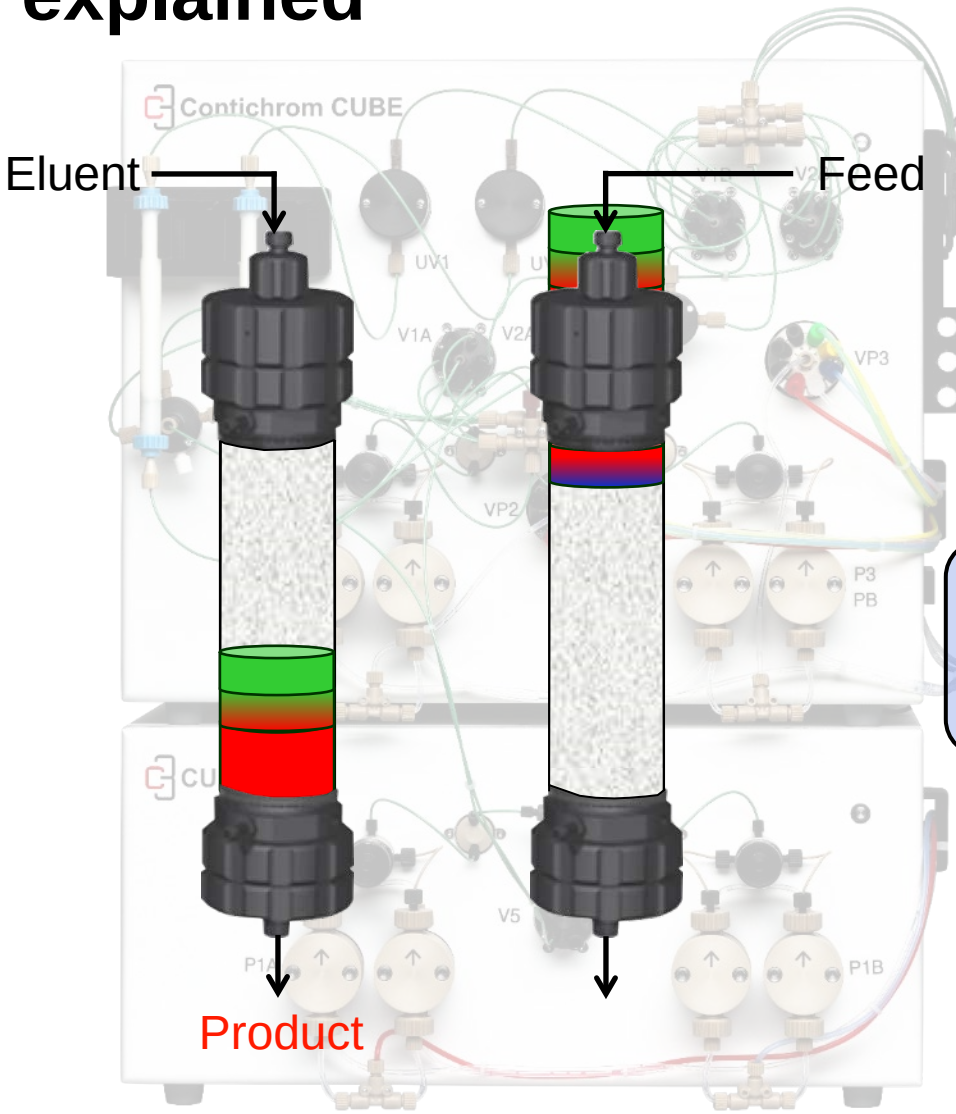

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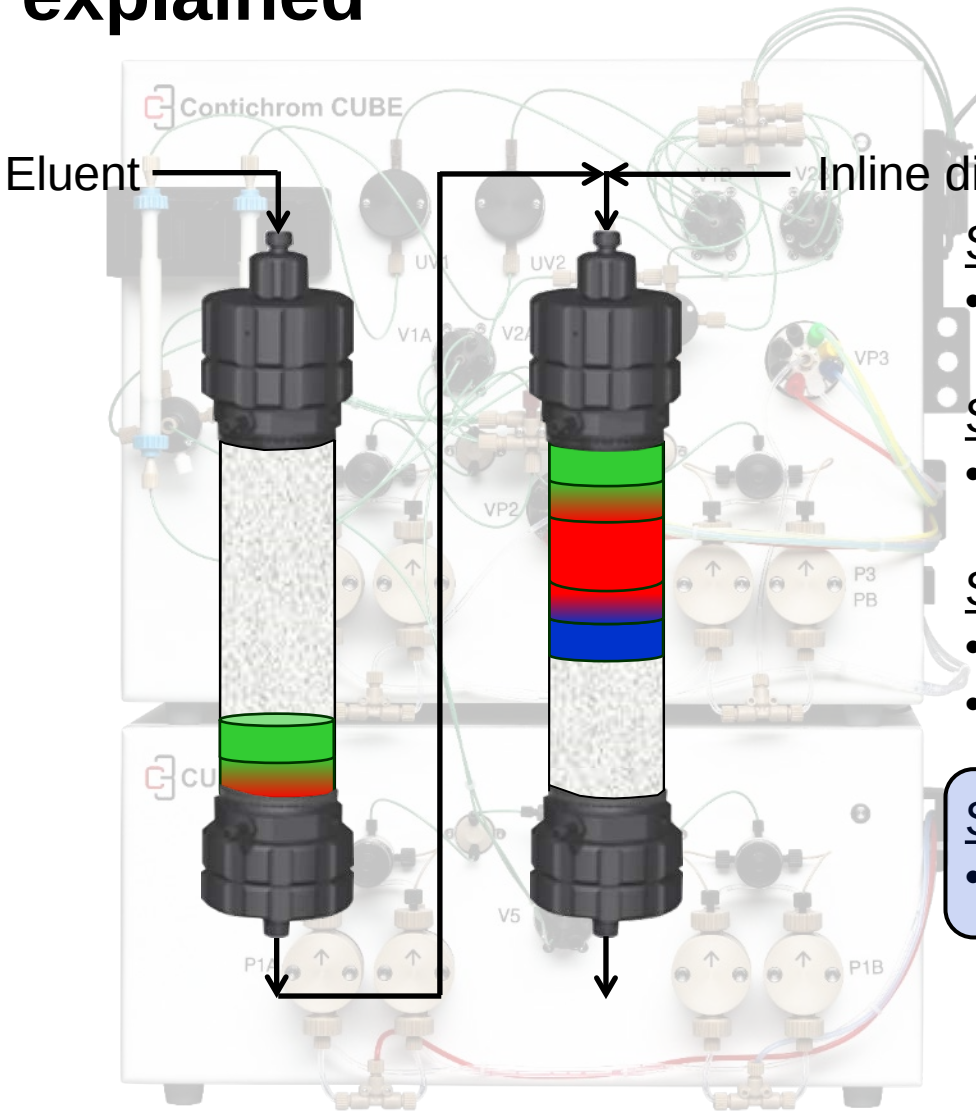

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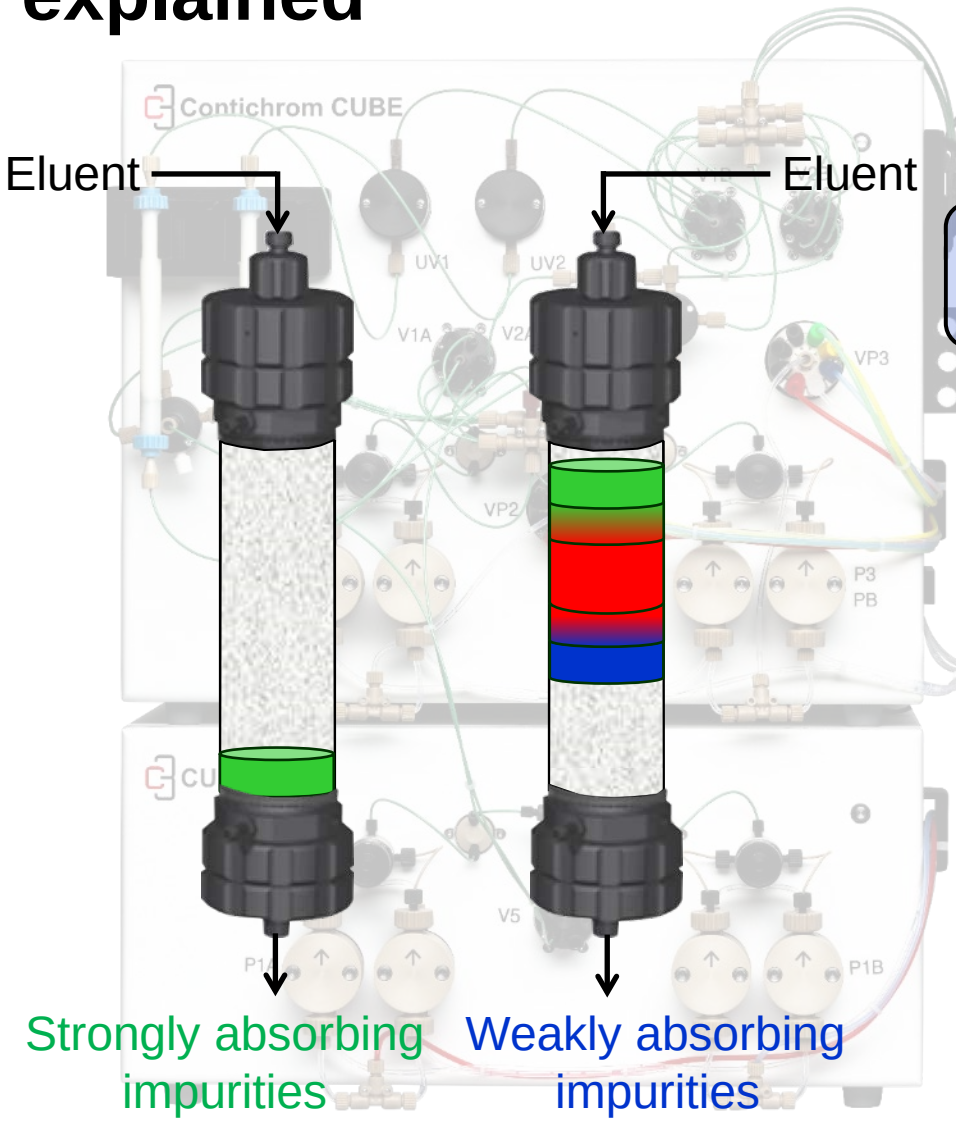

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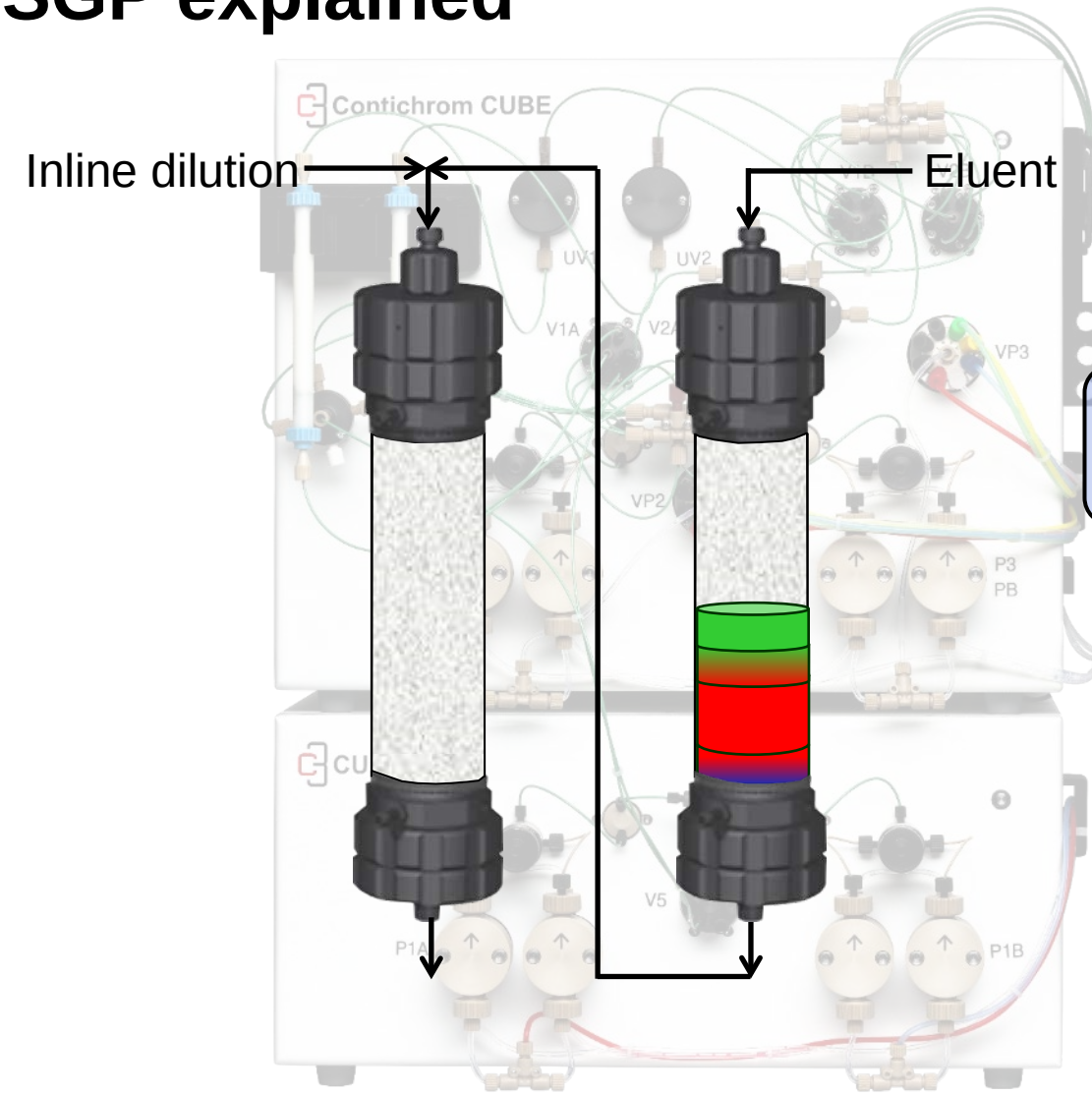

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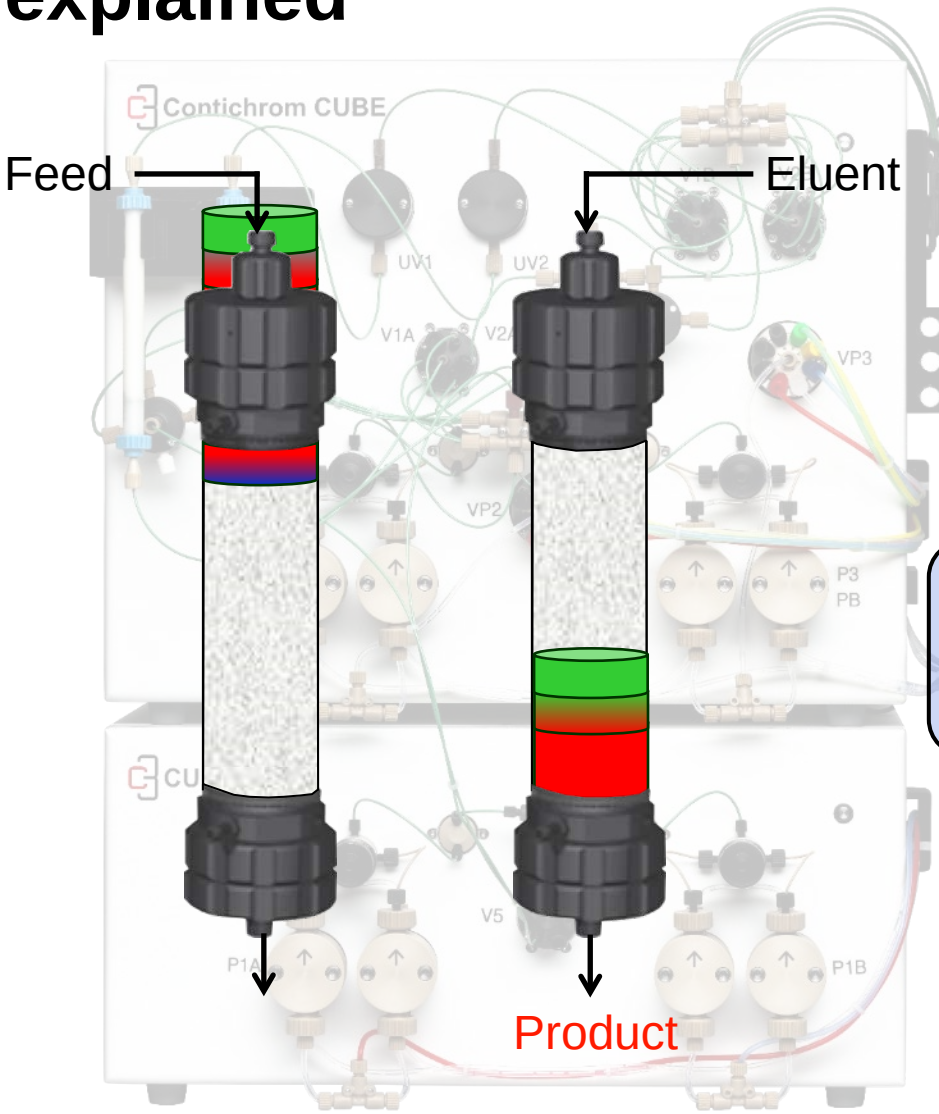
MCSGP explained



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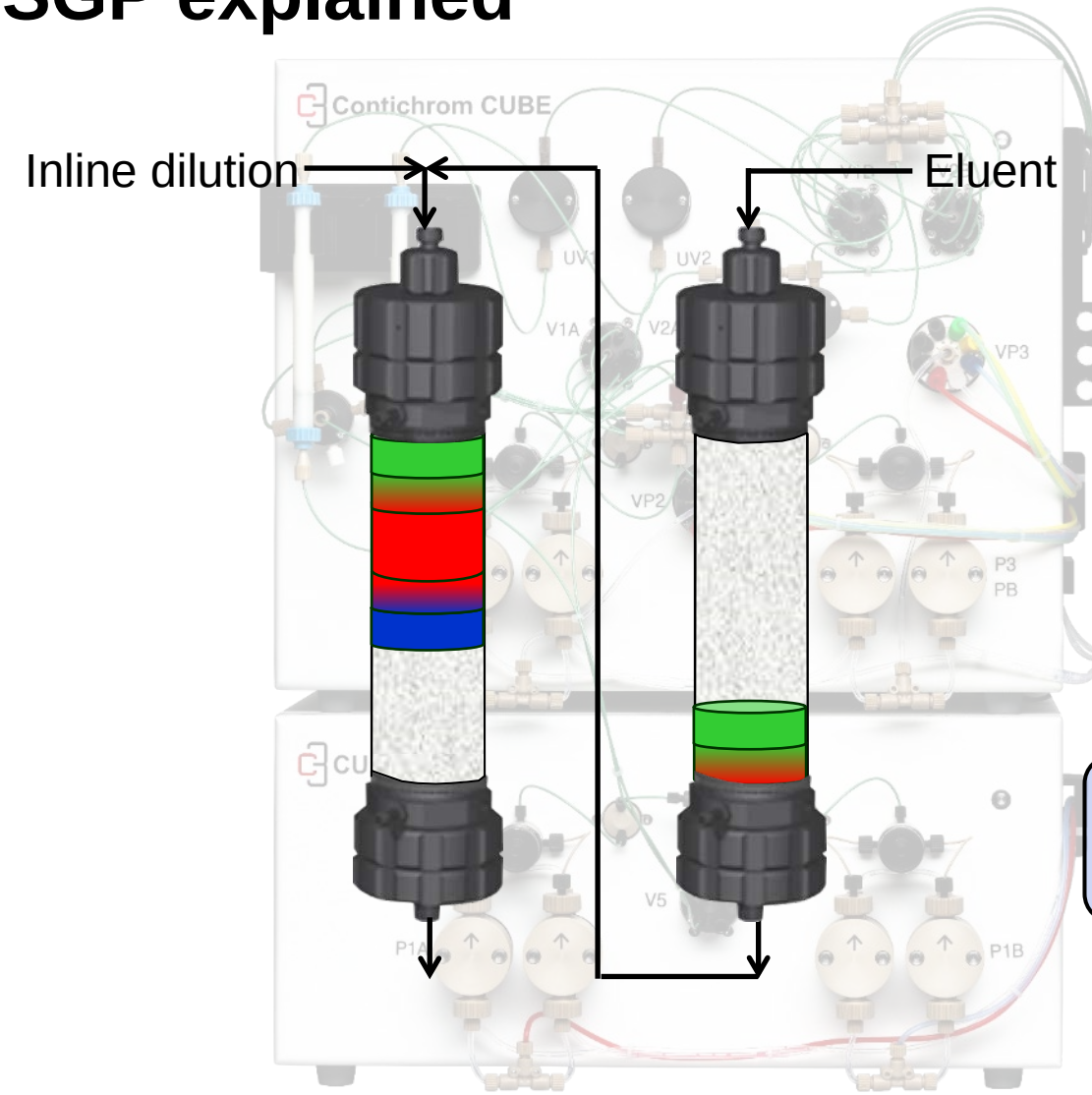

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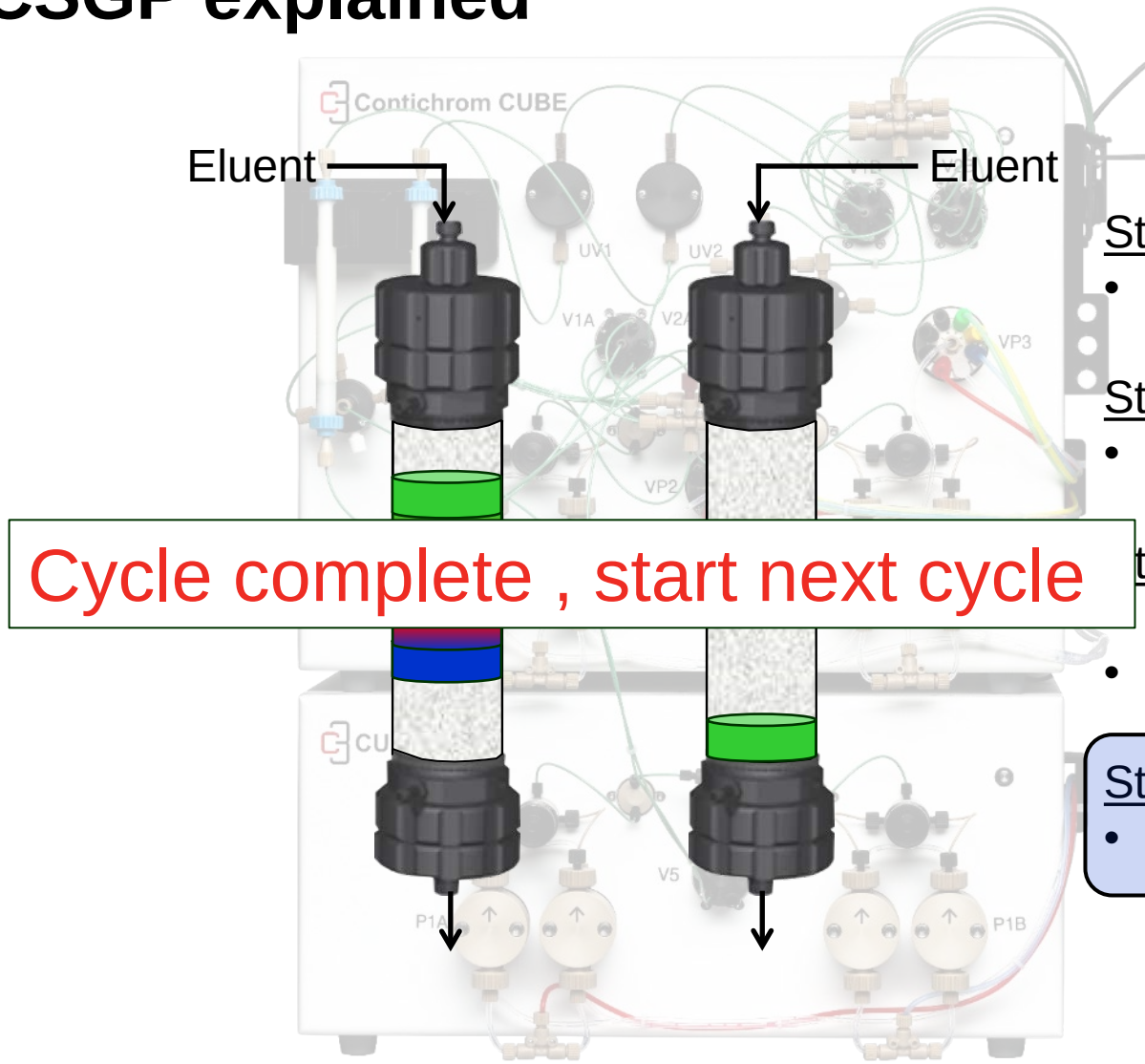


Cycle complete , start next cycle

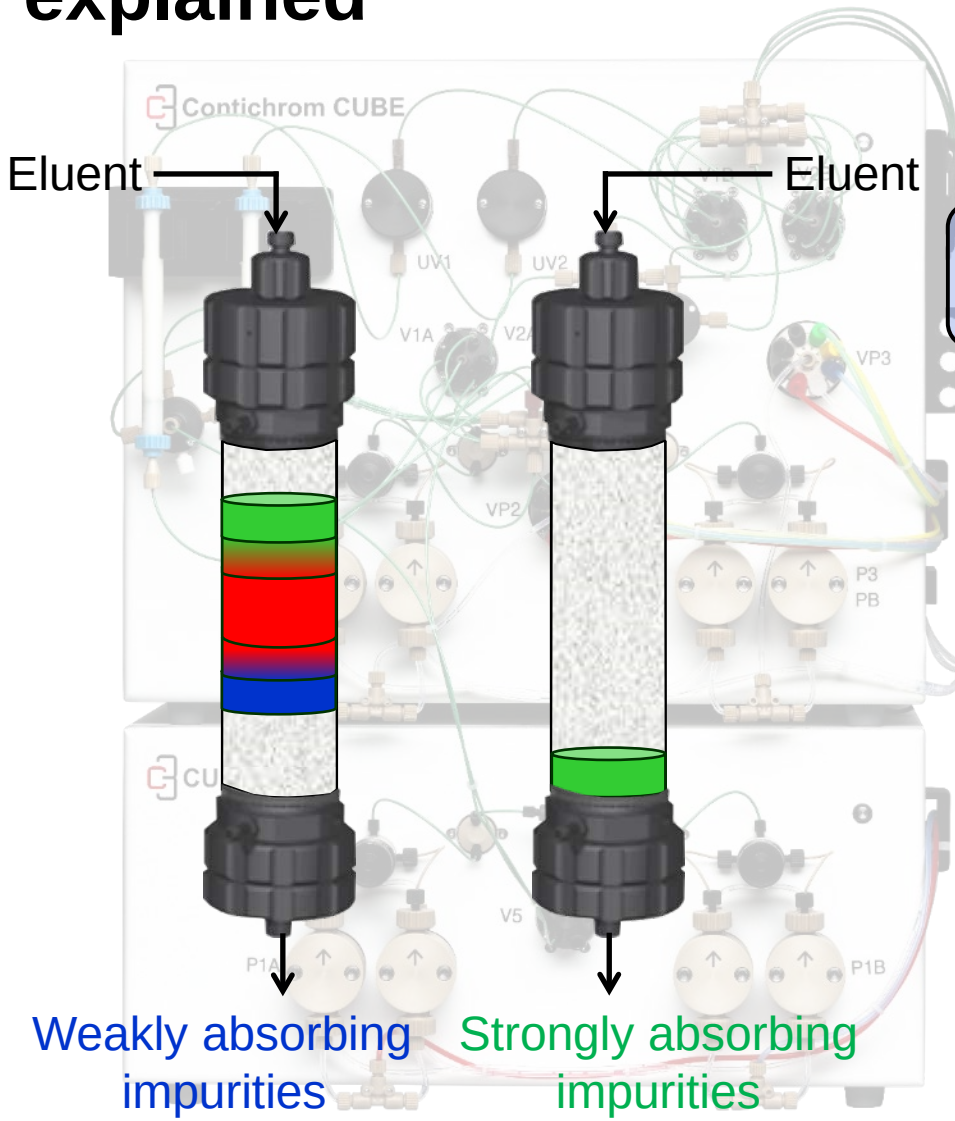
Step 1:
- elute waste

Step 2:
- recycle overlap

tep 3:
- elute product
- feed column

Step 4:
- recycle overlap

MCSGP explained



Start over

Step 1:
- elute waste

Step 2:
- recycle overlap

Step 3:
- elute product
- feed column

Step 4:
- recycle overlap

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MControl - Dynamic process control for MCSGP

MControl – Robust MCSGP operation

In MCSGP, operation with MControl significantly reduces effects of the following parameters on product quality:

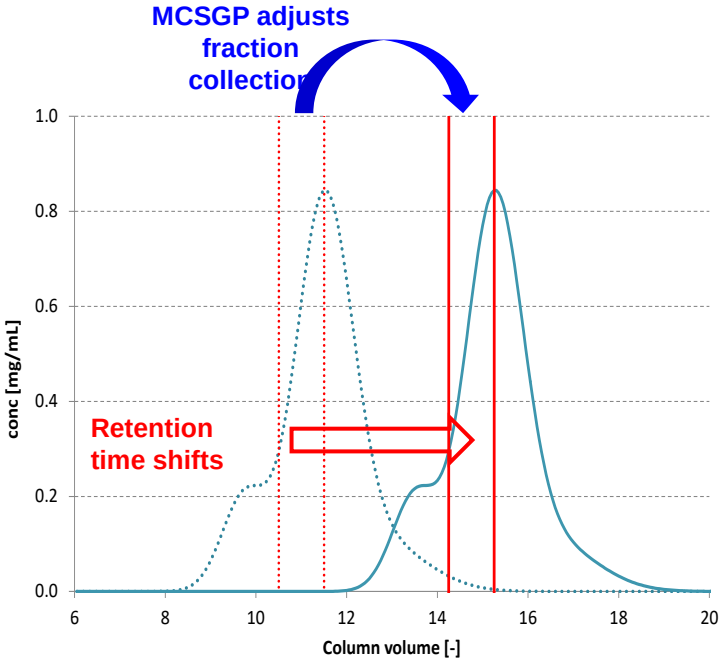
- temperature
- solvent quality
- conductivity, pH
- column variability (bed height, aging, packing quality)

MControl compensates for peak shifts by adjusting the fractionation start:

→Same product fraction position

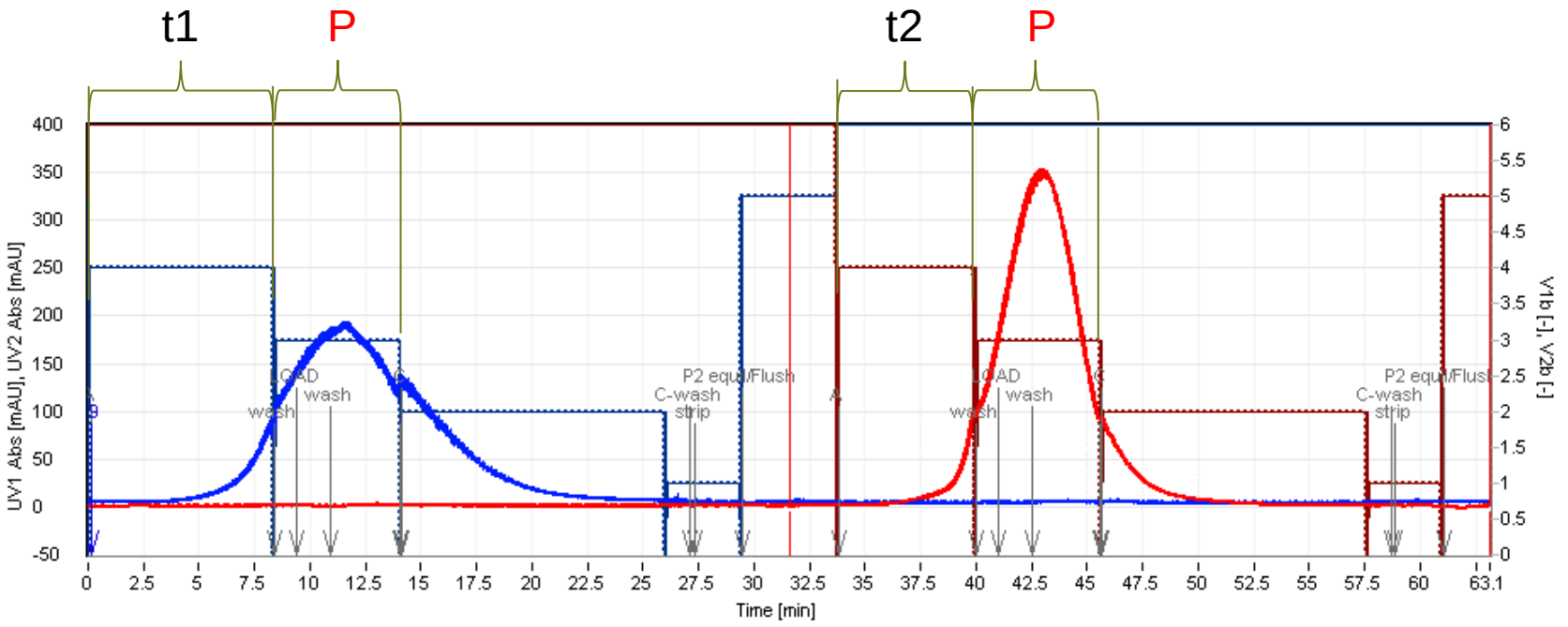
→ Same product quality

→ Increased robustness of continuous process operation



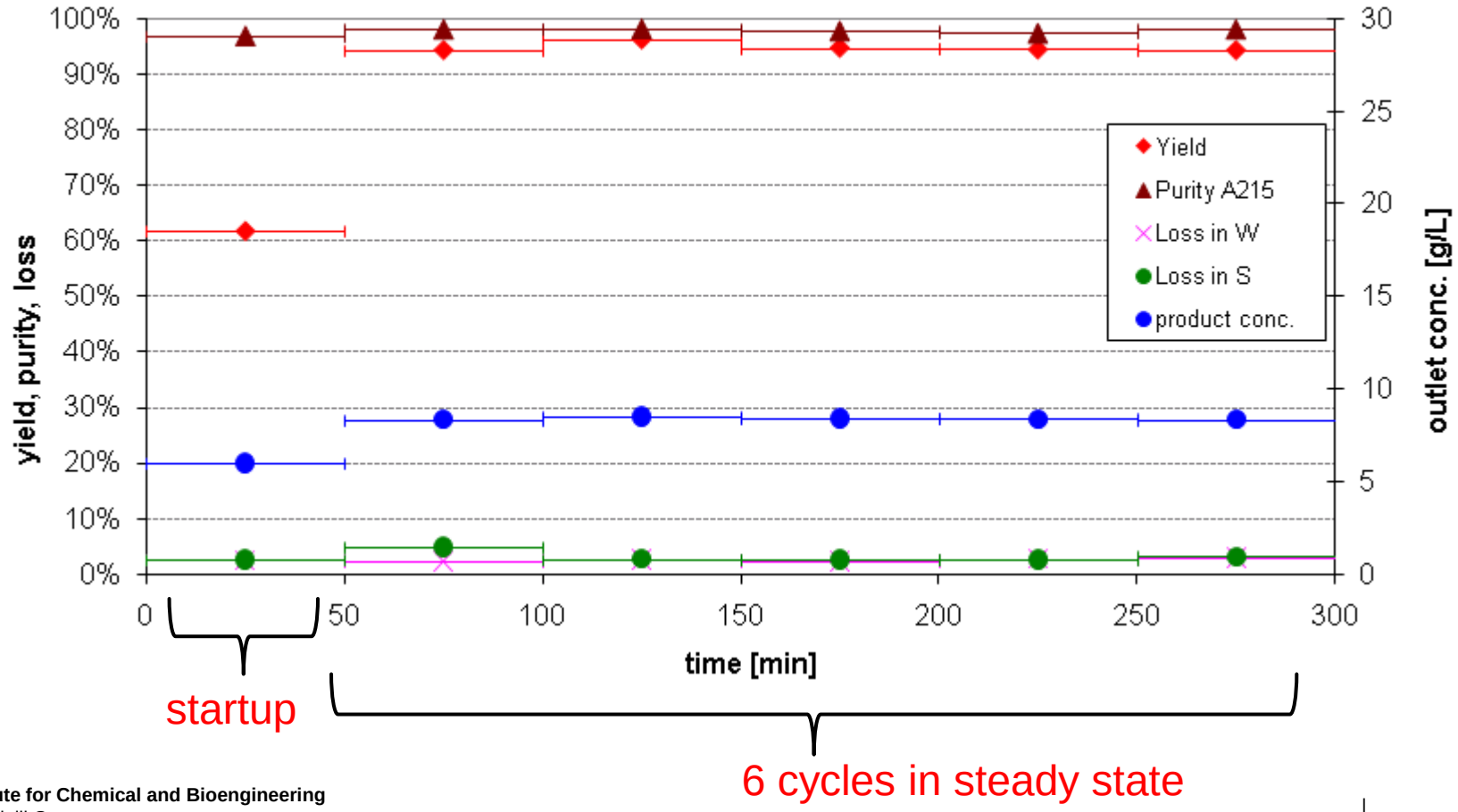
MControl: Dynamic MCSGP Process Control

- Example: MCSGP run on Contichrom with two different columns
- Chromatograms show 6 cycles superimposed, small protein model system, cation-exchange, linear gradient elution
  - MControl runs the linear gradient at the same slope, prolonging the elution (t1, t2) until the UV threshold is reached and product collection starts (P).



MCSGP operation

- Cyclic steady state of MCSGP: Constant product concentration and purity
- MControl supports robust operation



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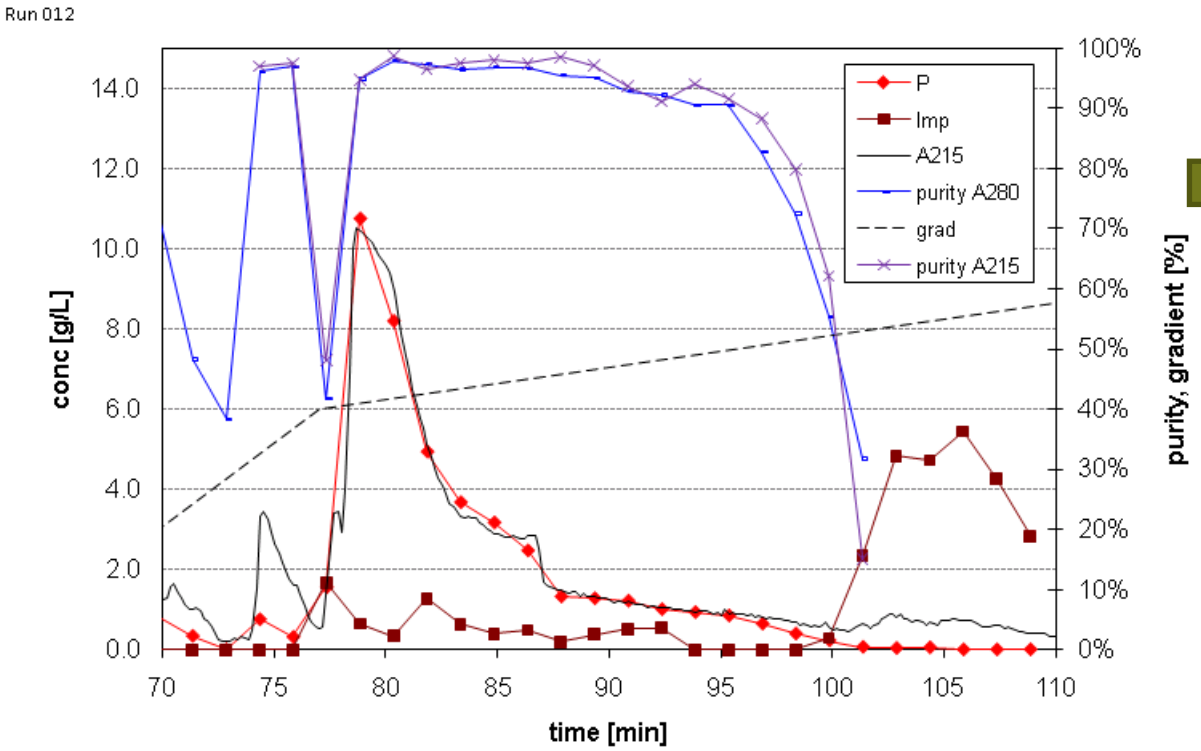
MCSGP for peptide purification – case studies

Peptide Case study*

Bristol-Myers Squibb ChromaCon® A new dimension in purification KAI PHARMACEUTICALS

Batch reference run



Difficult, center-cut purification

... Good for MCSGP

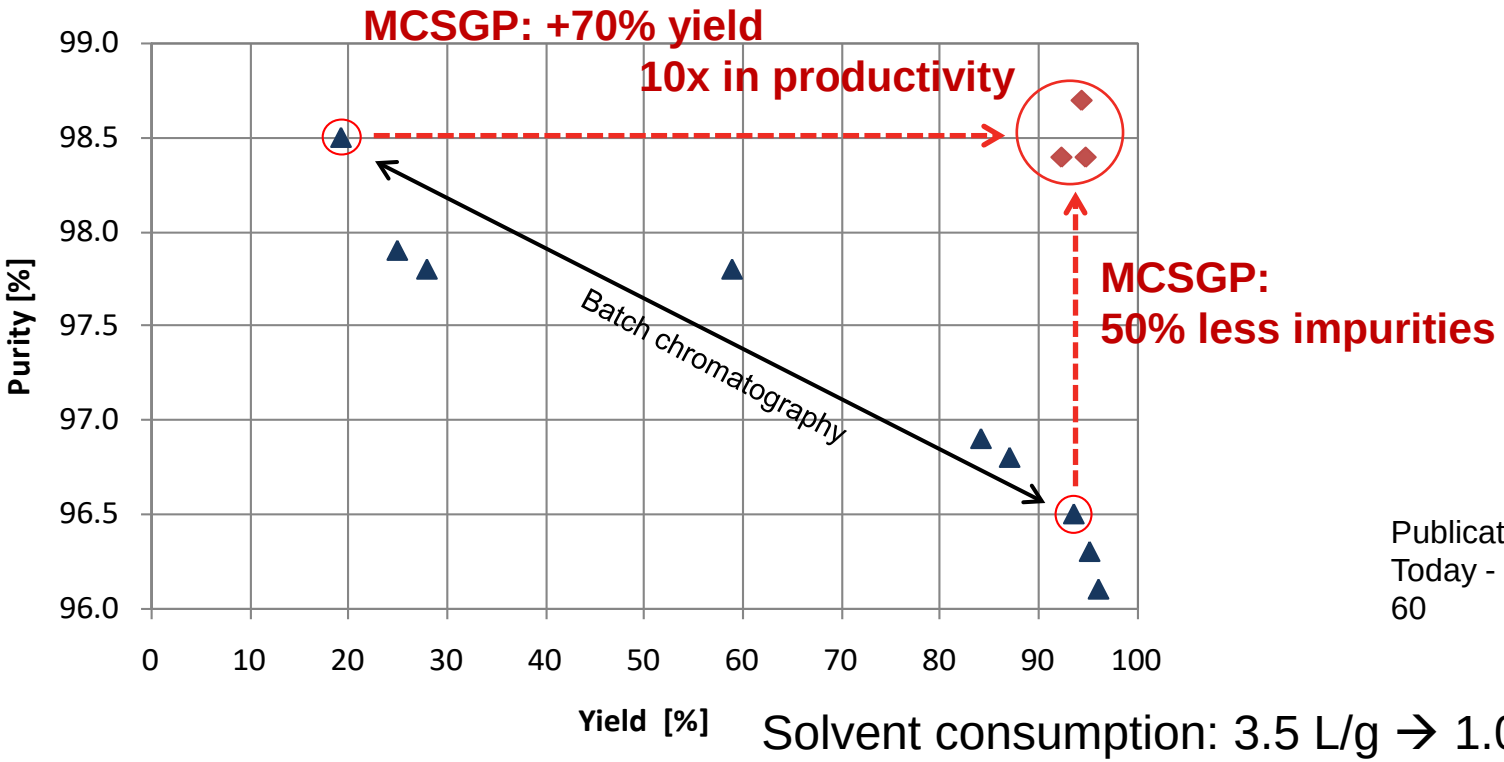
*Publication: Chemistry Today - vol. 31(5), pp. 56-60

Gradient elution on Kromasil C-18. 10 um, 0.46 x 25 cm, solvents: water, ACN

Peptide Case study results

MCSGP resolved the yield/purity trade-off problem of batch:

- ✓ 70% yield increase at target purity
- ✓ 10x productivity improvement
- ✓ 70% decrease in solvent consumption (S.C.) at target purity



Publication: Chemistry Today - vol. 31(5), pp. 56-60

Solvent consumption: 3.5 L/g → 1.0 L/g
Productivity: 3 g/L/h → 30 g/L/h

Case: Therapeutic peptide purification by MCSGP (Liraglutide)

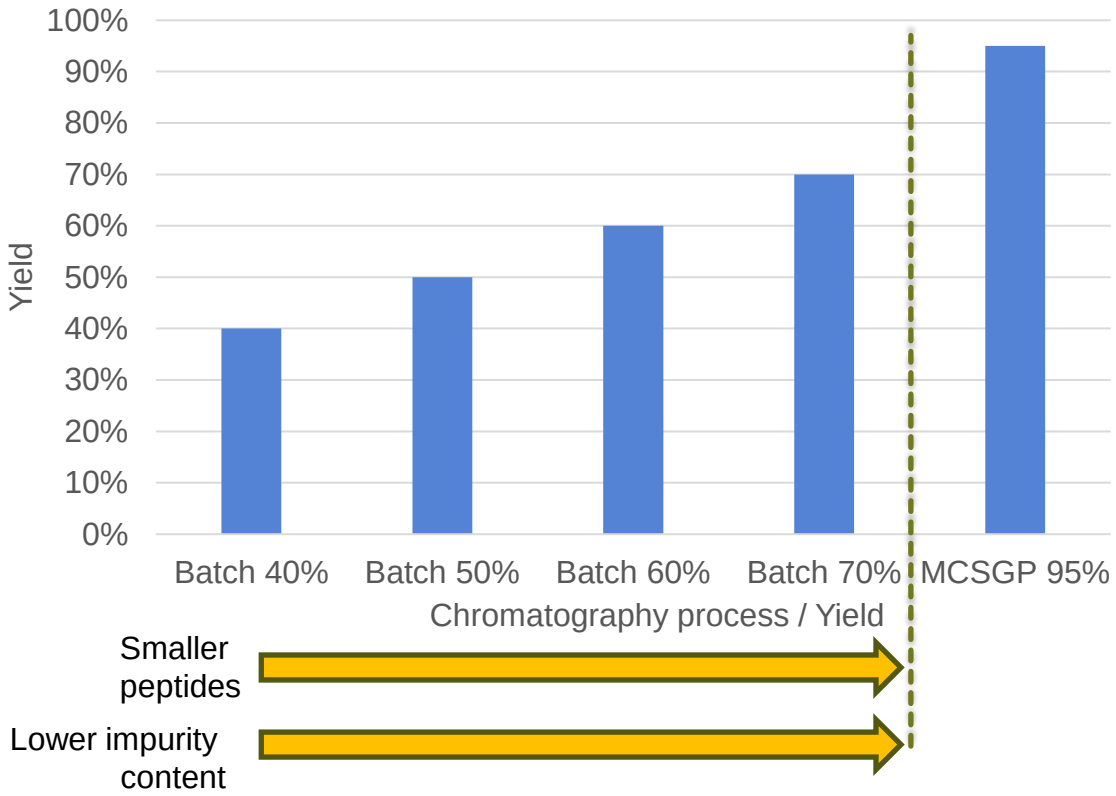
novo nordisk®

- Collaboration between University of Lund (Sweden) and Novo Nordisk
- Detailed results are confidential
- Outcome: At high purity (98%, 99%), MCSGP was more favorable than batch chromatography
- Productivity was improved through MCSGP

Economic evaluation of MCSGP

Assumptions for economic evaluation

- Batch chromatography has varying yields of 40, 50, 60, 70% representing varying impurity content / purifications of peptides with different sizes (15, 20, 25-mer)
- Rationale: shorter peptides → less complex synthesis → fewer impurities → higher yield in batch chromatography
- Yield of MCSGP is 95%, independent of difficulty of separation / peptide size
- The load for all batch runs and MCSGP was assumed to be 10 g crude/L of stationary phase



Further Assumptions

- Parameters for simulation of production and purification of 10 kg peptide:

		Batch	MCSGP
Column bed height	[cm]	25.0	10.0
Replacement of stationary phase	[%/year]	30	30
Stat. phase costs	[US$/kg]	7000	7000
Synthesis batch size	[kg]	1	1
Synthesis costs / g	[US$/g]	200	200
Synthesis costs / batch	[US$]	200,000	200,000
Solvent costs	[US$/L]	6	6
Chrom. system costs	[US$]	500,000	1,500,000
Depreciation period	[a]	10	10
number of samples to be analyzed per cycle	[-]	10	1
QA/QC costs per sample	[US$]	200	200
Plant operating costs	[US$/day]	8,000	5,000
Max. time permitted for chromatography	[hrs]	16	16

- Further assumption for batch chromatography: Use of re-chromatography: 25% of yield loss can be recovered

Further assumptions

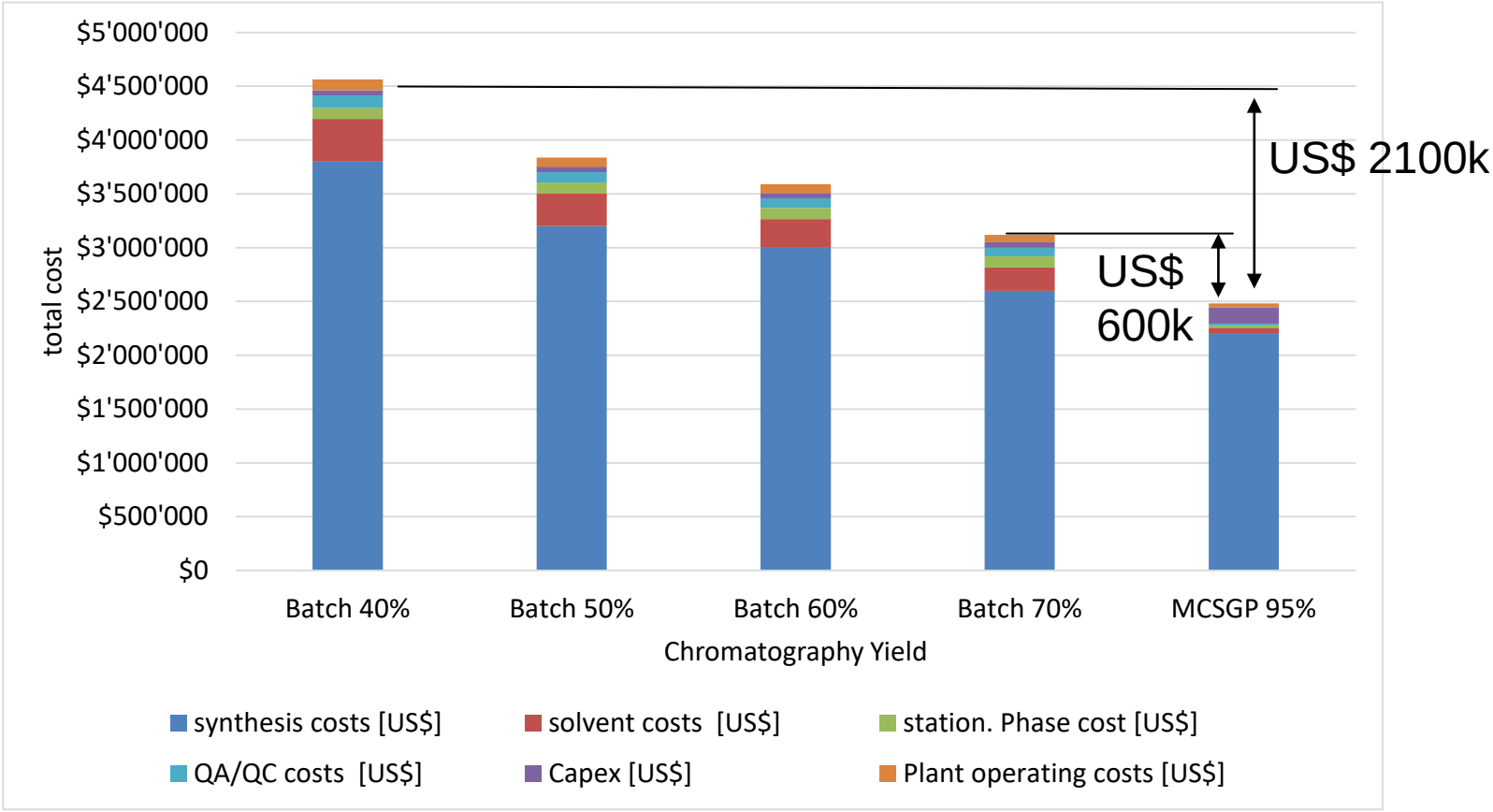
- Parameters for simulation of production and purification of 10 kg peptide:

Parameter	Unit	Batch 1	Batch 2	Batch 3	Batch 4	MCSGP
Yield	[%]	40	50	60	70	95
Flow rate	[cm/h]	181	181	181	181	271
Cycle time	[min]	232.7	232.7	232.7	232.7	80

- Assumed linear flow rate in MCSGP 50% higher than batch flow rate:
  - MCSGP can achieve high product yield in spite of a high flow rate which causes larger overlaps of product and impurities, due to its internal recycling capabilities.
  - the shorter bed height of MCSGP allows larger linear flow rates due to reduced backpressure.

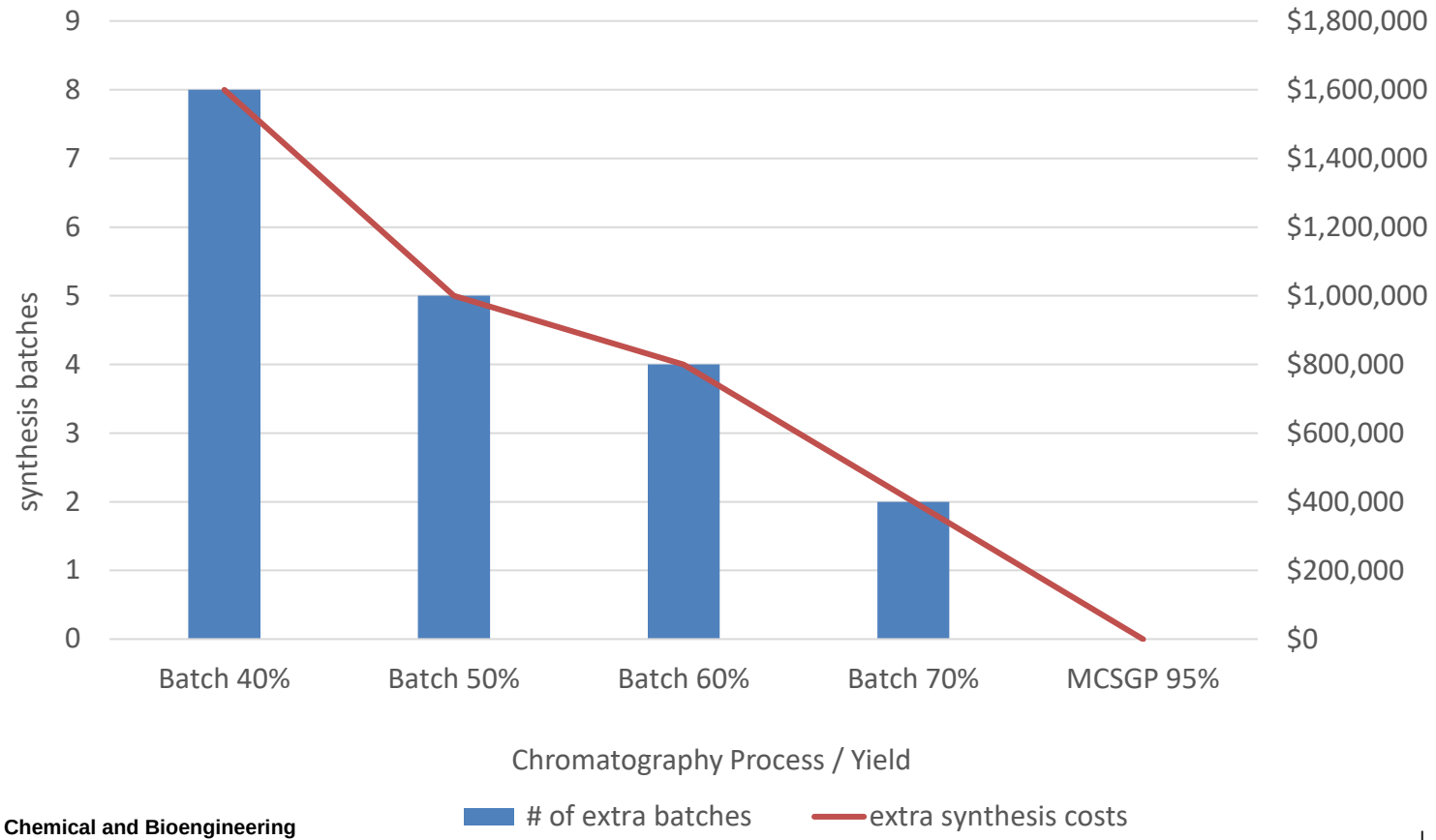
Results: Total costs including synthesis and re-chromatography

- Cost benefit of MCSGP: US$ 0.6 million to US$ 2.1 million / 10 kg peptide



Results: Cost difference batch - MCSGP

- Due to low yield of batch chromatography, additional synthesis batches need to be produced, driving up costs compared to MCSGP.
- With increasing chromatography yield the number of required extra batches decreases, improving overall costs

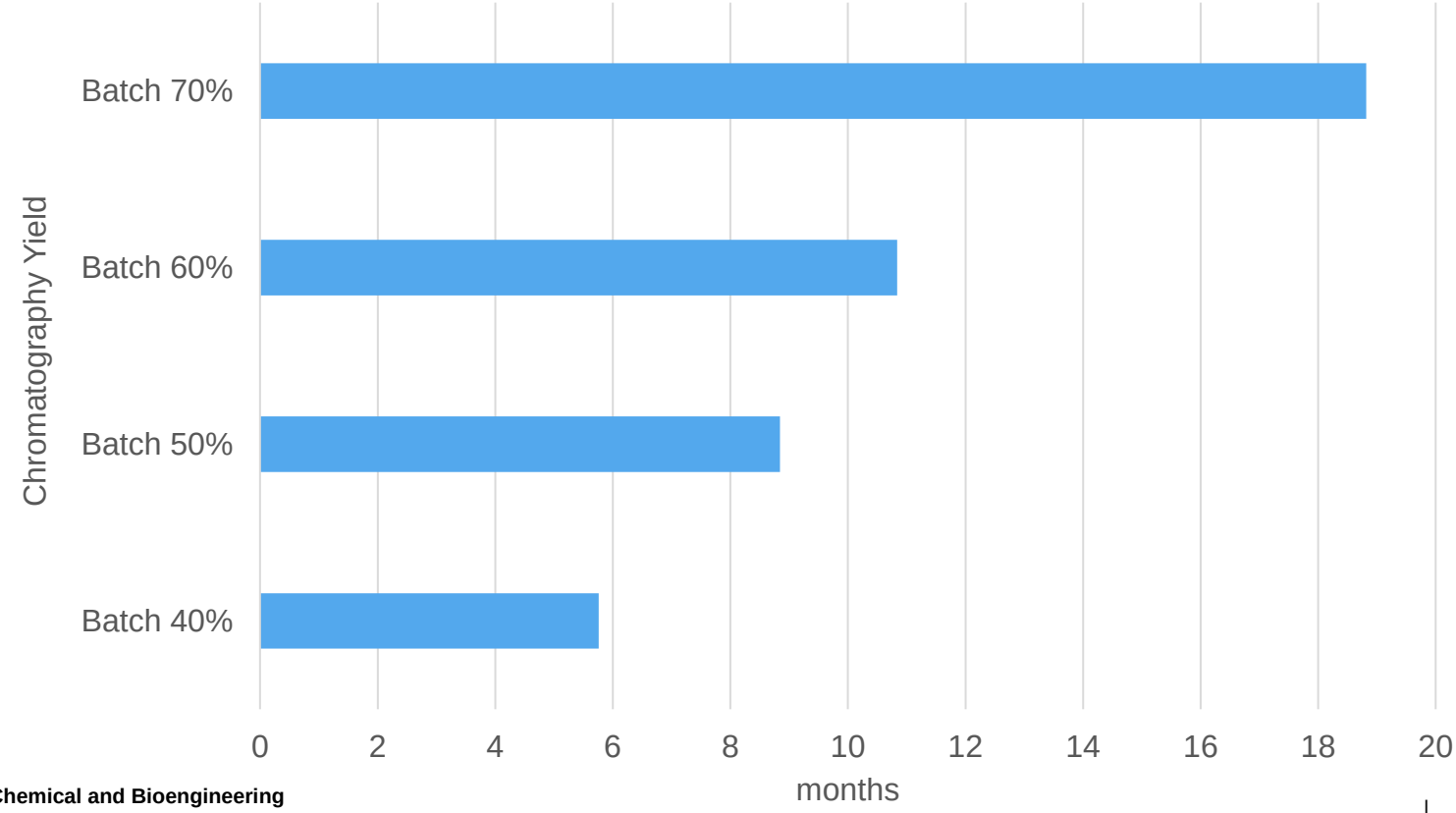


Results: Payback period of MCSGP

- Payback period of MCSGP is calculated relative to the batch runs with 40%, 50%, 60% and 70% yield respectively (10 kg of peptide per year)
- Payback = (CAPEX difference batch-MCGSP)/(savings through MCSGP per year)

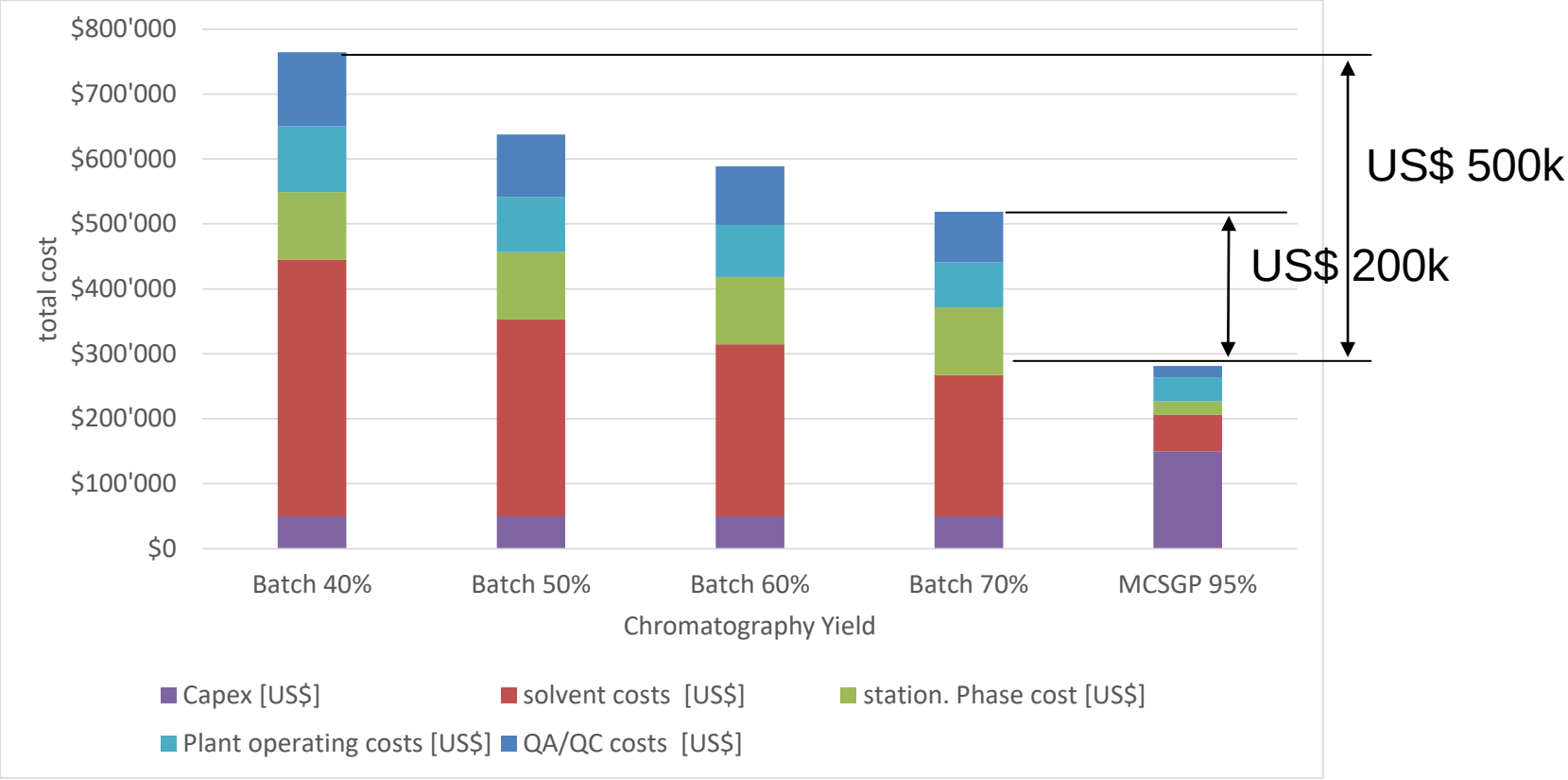
→ In all cases the payback period of MCSGP is equal or less than 19 months

→ Payback period can be even shorter if more than 10 kg peptide is produced per year



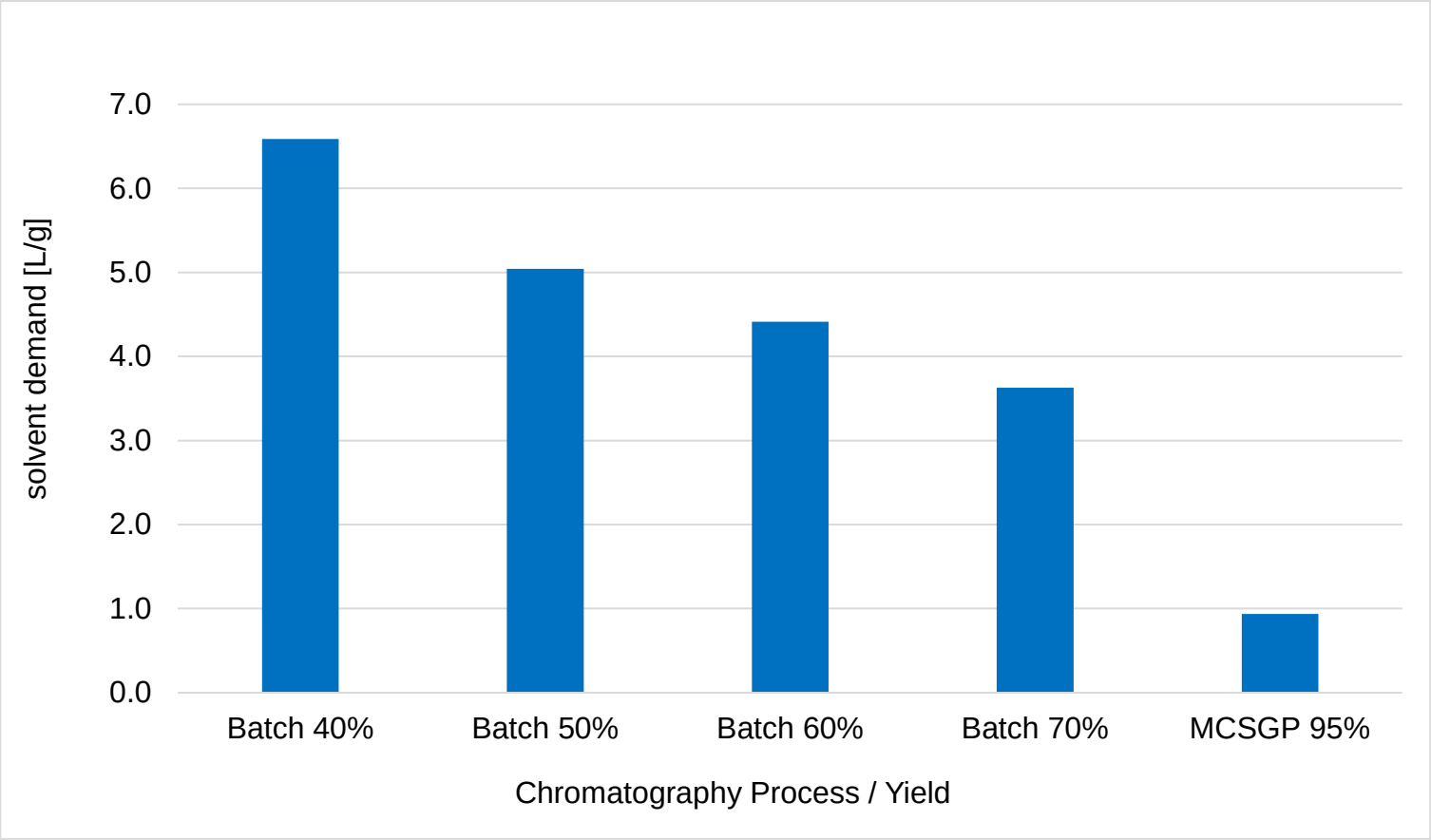
Results: Total costs including synthesis and re-chromatography

- Only chromatography cost shown (10 kg peptide):
  - Solvent cost dominating for batch chromatography
  - CAPEX dominating for MCSGP
- Savings through MCSGP (chromatography costs only) : US$ 200k - US$ 500k



Results: Solvent consumption

- MCSGP cuts solvent consumption by up to 85%, corresponding to 56,000 L p.a.
- Additional cost savings through reduced solvent handling/storage/disposal /recycling … not included in evaluation but in favor of MCSGP



Results: Column and pump sizes

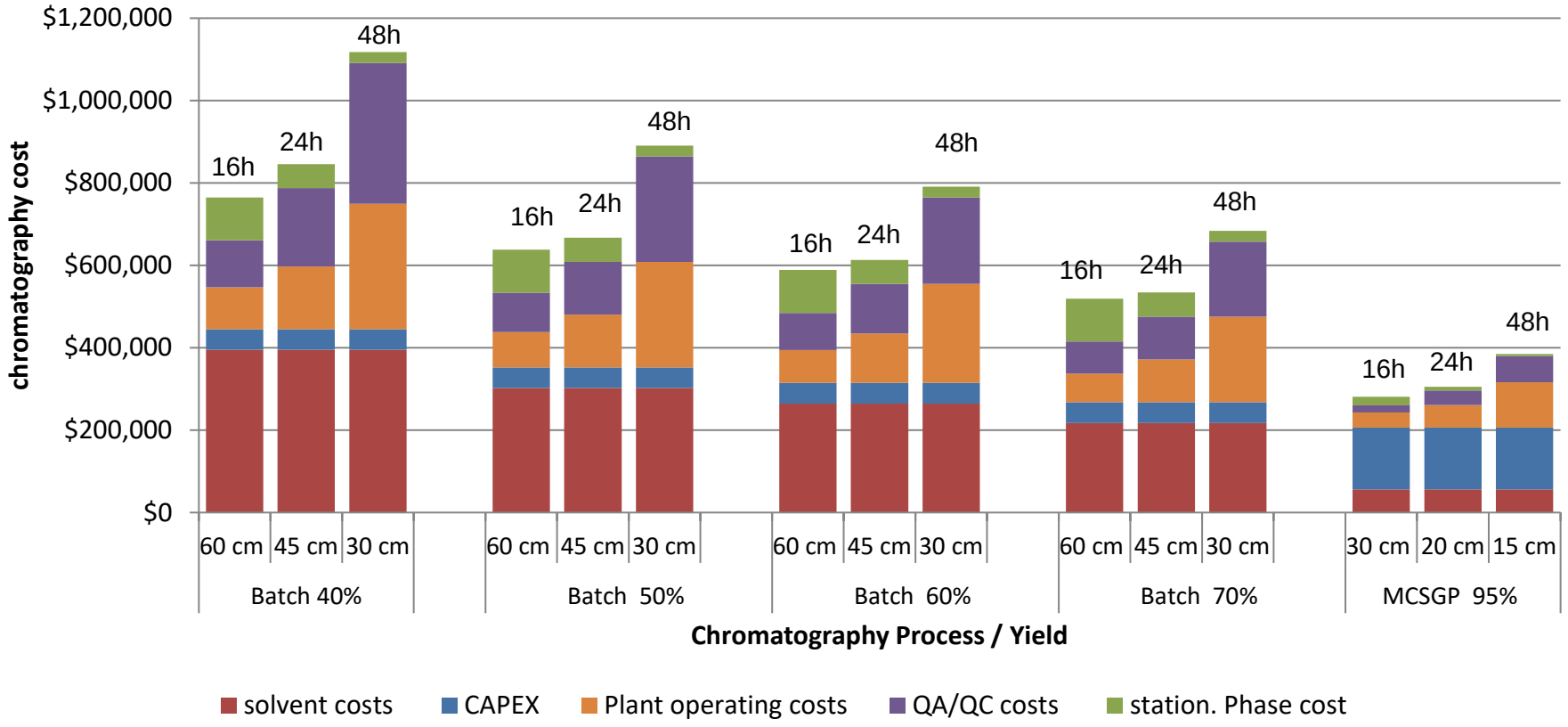
- Comparison of Batch and MCSGP:

		Batch 1	Batch 2	Batch 3	Batch 4	MCSGP
Yield	[%]	40	50	60	70	95
Column inner diameter	[cm]	60	60	60	60	30
Column volume	[L]	70.7	70.7	70.7	70.7	2x 7.1
Required pump size on skid	[L/min]	8.5	8.5	8.5	8.5	3.2

- Column diameter reduced from 60 cm i.d. to 2x 30 cm i.d.
- Total column packing volume reduced from 70.7 L to 14.2 L
- Pump flow rate on skid reduced from 8.5 L/min (510 L/h) to 3.2 L/min (190 L/h)

Results: Chromatography costs

- Sensitivity analysis: use of smaller columns.
- Use of smaller columns reduces stationary phase costs but drives up QA/QC costs and plant operating cost through the increased processing time → increased chromatography costs



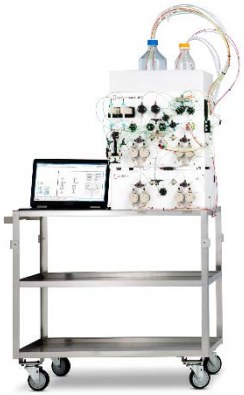
MCSGP scalability

Lab Scale systems
- Cost-competitive, all-in-one process capabilities

Pilot / Production-scale (GMP)
- High throughput, reduced costs

ChromaCon
A YMC Company

Contichrom CUBE 30/100



Flow rates:
36 mL/min
100 mL/min

YMC

Contichrom TWIN MCSGP HPLC GMP systems

LEWA

Flow rates: up to 20 L/min

Conclusions

- MCSGP simultaneously achieves high yield and purity in difficult peptide purifications
- The increased yield of MCSGP …
  - ✓ allows downscaling of the upstream chemical synthesis steps
  - ✓ improves productivity of the downstream process leading to smaller columns required
  - ✓ reduces solvent consumption
  - ✓ eliminates the need for re-chromatography
  - ✓ avoids generation of side-fractions to be stored and analyzed (reduction of analytical burden)
- All abovementioned points lead to massive cost savings compared to single column batch chromatography
- Economic analysis: Savings for an annual production amount of 10 kg peptide (synthesis, chromatography and re-chromatography) from US$ 0.6 million to US$ 2.1 million expected, in comparison to the single column reference process

Thank you for attending … any questions?

Contact: Thomas Müller-Späth
thomas.mueller-spaeth@chem.ethz.ch