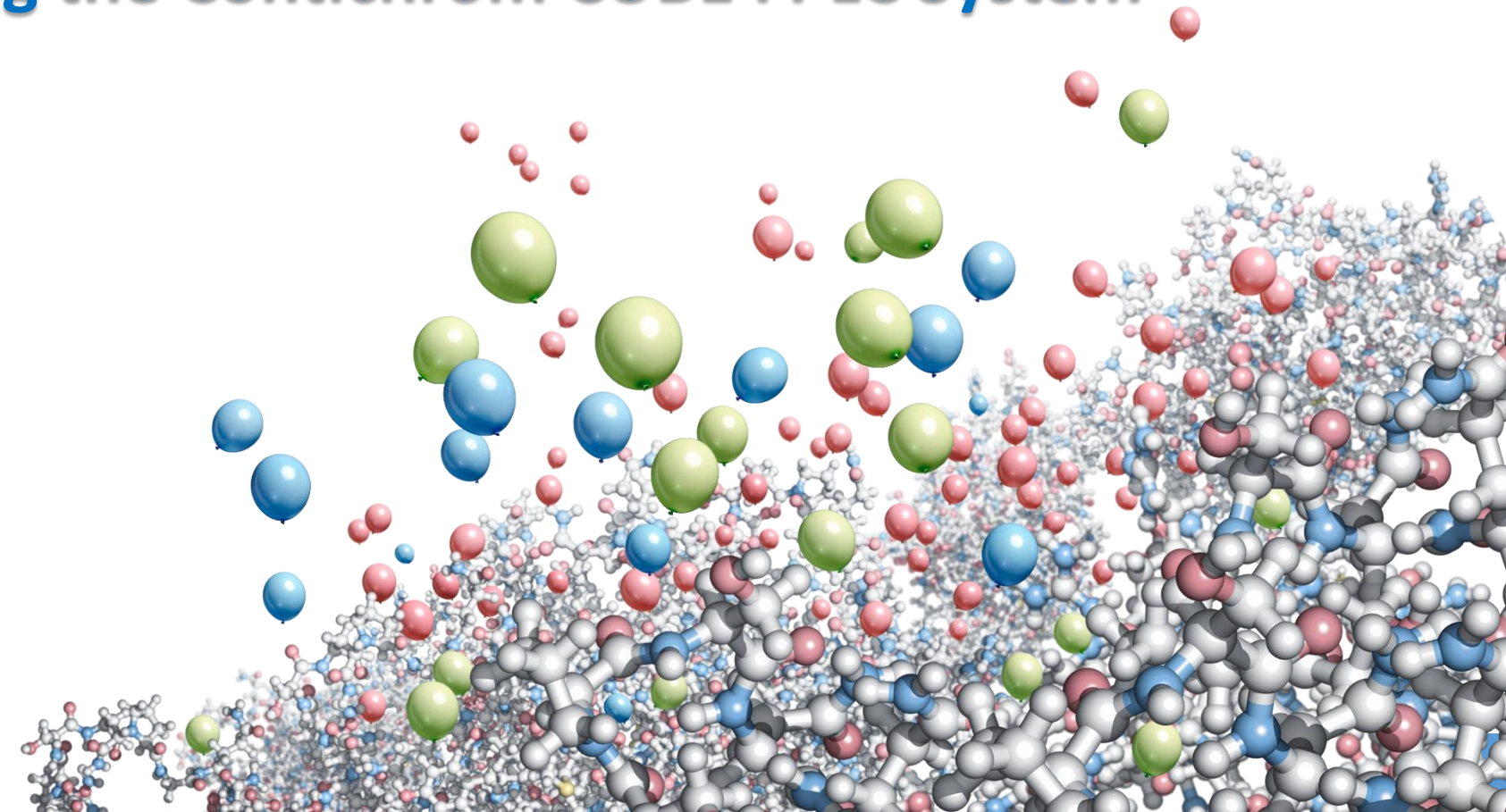
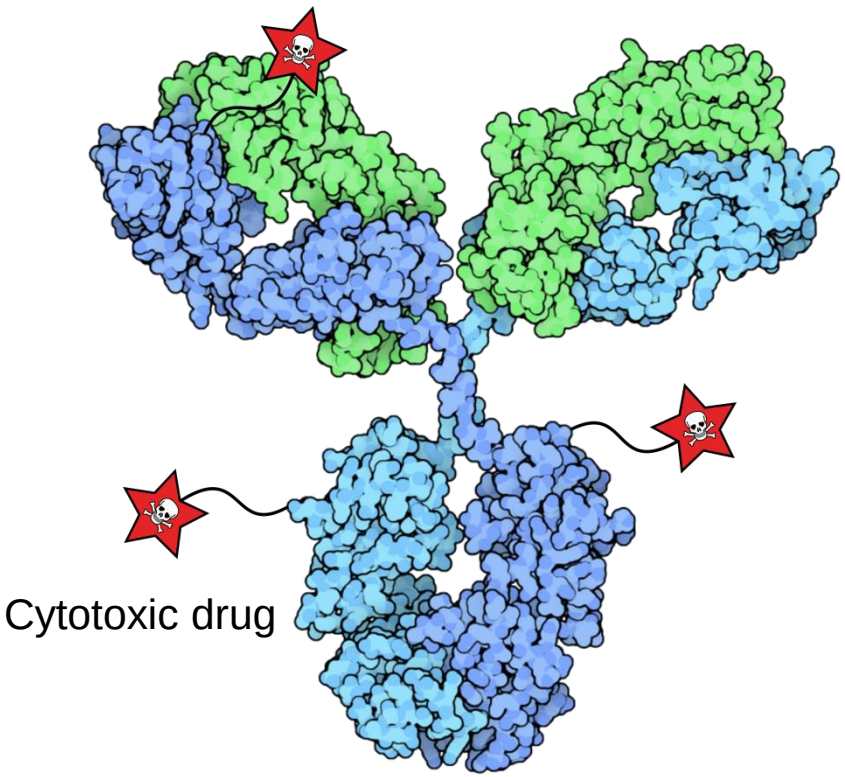
ChromaCon®
A new dimension in purification

# Purification of Antibody-Drug Conjugates using the Contichrom CUBE FPLC System



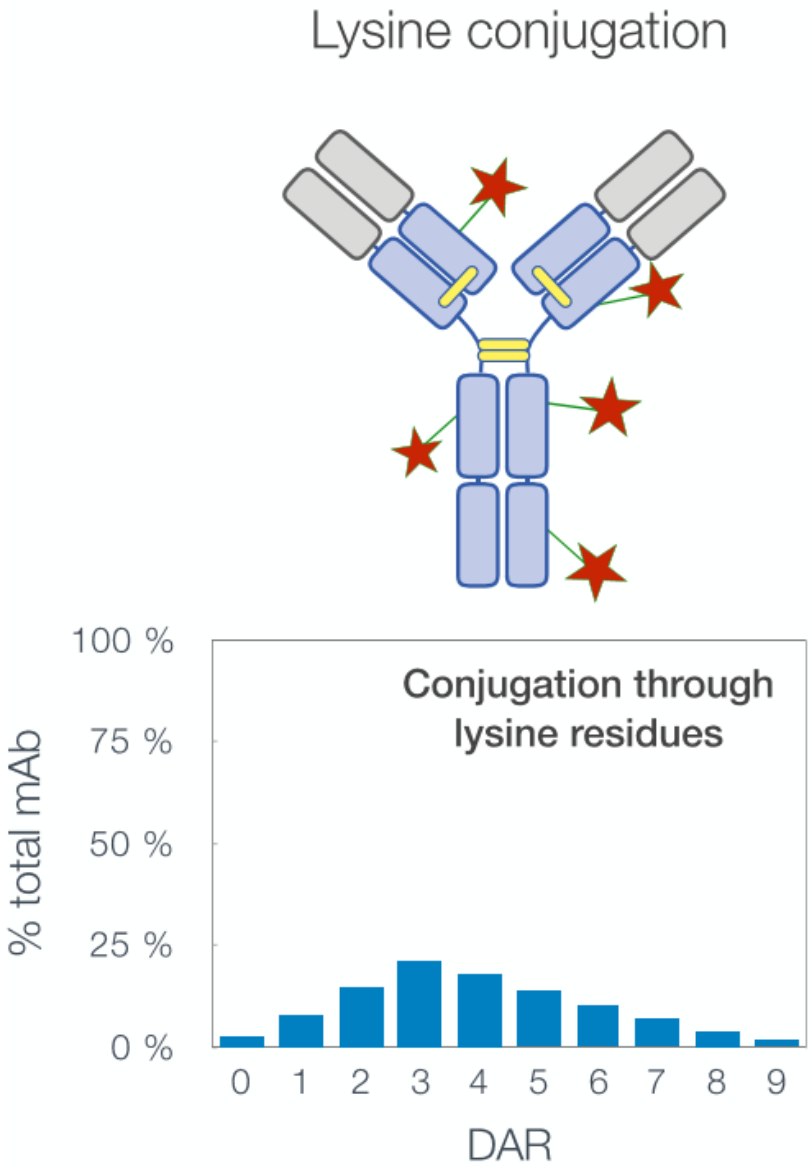
# Antibody-Drug Conjugates




- ADCs: 2 marketed products
- Kadcyla: Lysine linkage, DAR of 3-4
- Adcetris: Thiol Linkage DAR of 3-5
- 56 ADCs in clinical development
- market: 2.8bn USD by 2018

Mode of action:
- Antibody targets cancer cell
- Internalization (endocytosis)
- Drug release and cell killing

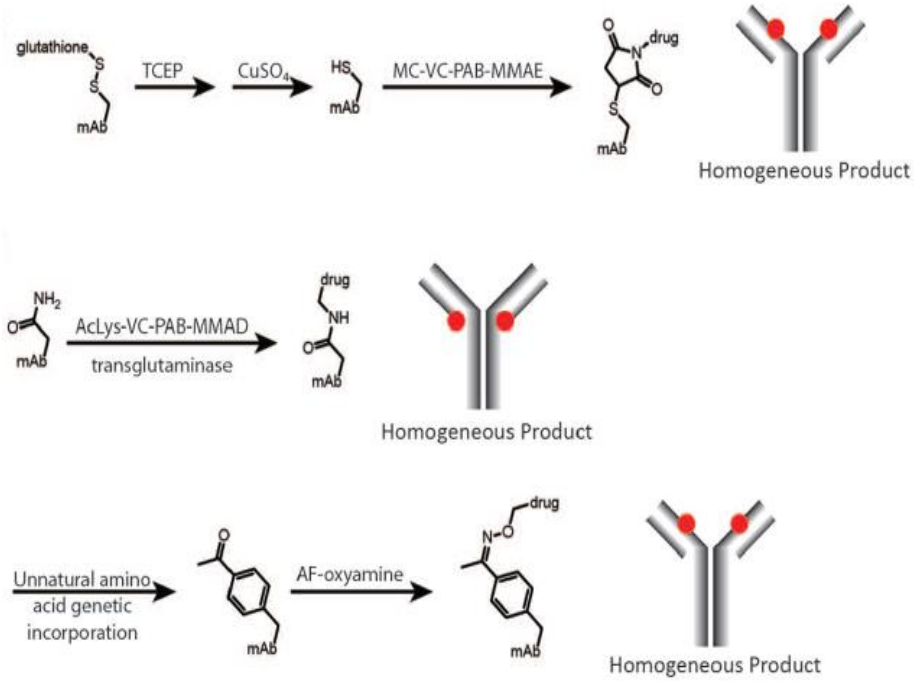
# Introduction Antibody-Drug-Conjugates (ADCs)

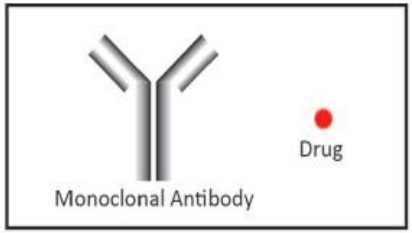



- Non-specific conjugation:
  - at many different sites (>50 Lysines)
  - results in mix of Drug-Antibody Ratios (DAR)
  - DAR of 2-4 most effective
  - need to separate most effective species
  - difficult separation challenge
  - most ADCs in clinical development have been conjugated non-specifically

# Introduction Antibody-Drug-Conjugates (ADCs)

- Site-specific coupling for next-generation ADCs
- Modification of the antibody required in most cases for specific chemistry. Examples:
  - Reduction / coupling (Disulfide bridges)
  - Enzymatic modifications
  - Incorporation of unnatural amino acids



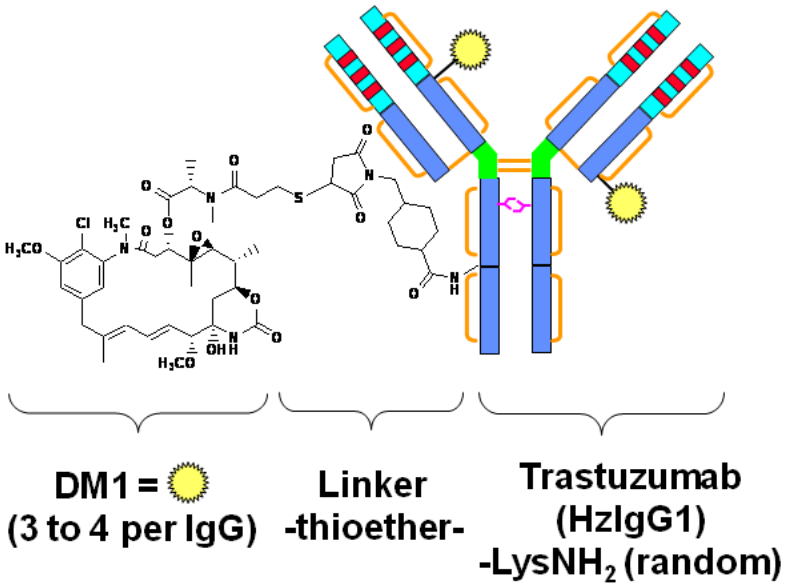

→ Limited to even DAR numbers, mostly DAR 2.0

Figure Source:
Casi, G. and D. Neri (2012).
J Control Release 161(2): 422-428.

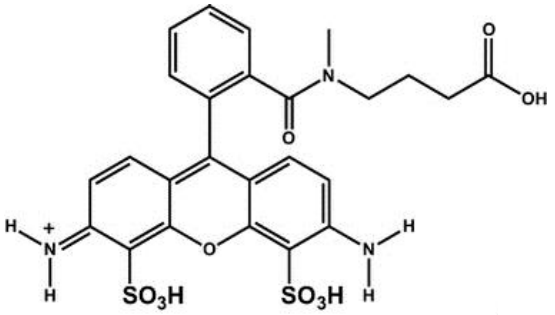
# Case Study - Reference and Model System

- Reference system:
- Kadcyla (Trastuzumab emtasine, Roche):

*Discov Med 10(53):329-339, 2010*




• Model system:
• Trastuzumab conjugated with fluorescent dye Atto-488 using same conjugation chemistry

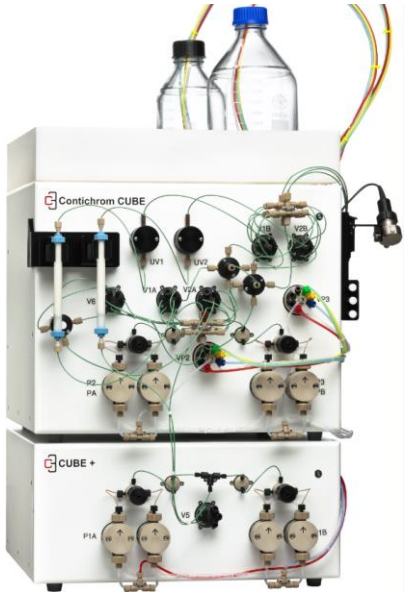


**Atto-488**
(Attotec GmbH)

# Case Study Setup

Contichrom CUBE


Contichrom CUBE Combined




Trastuzumab Conjugation with dye

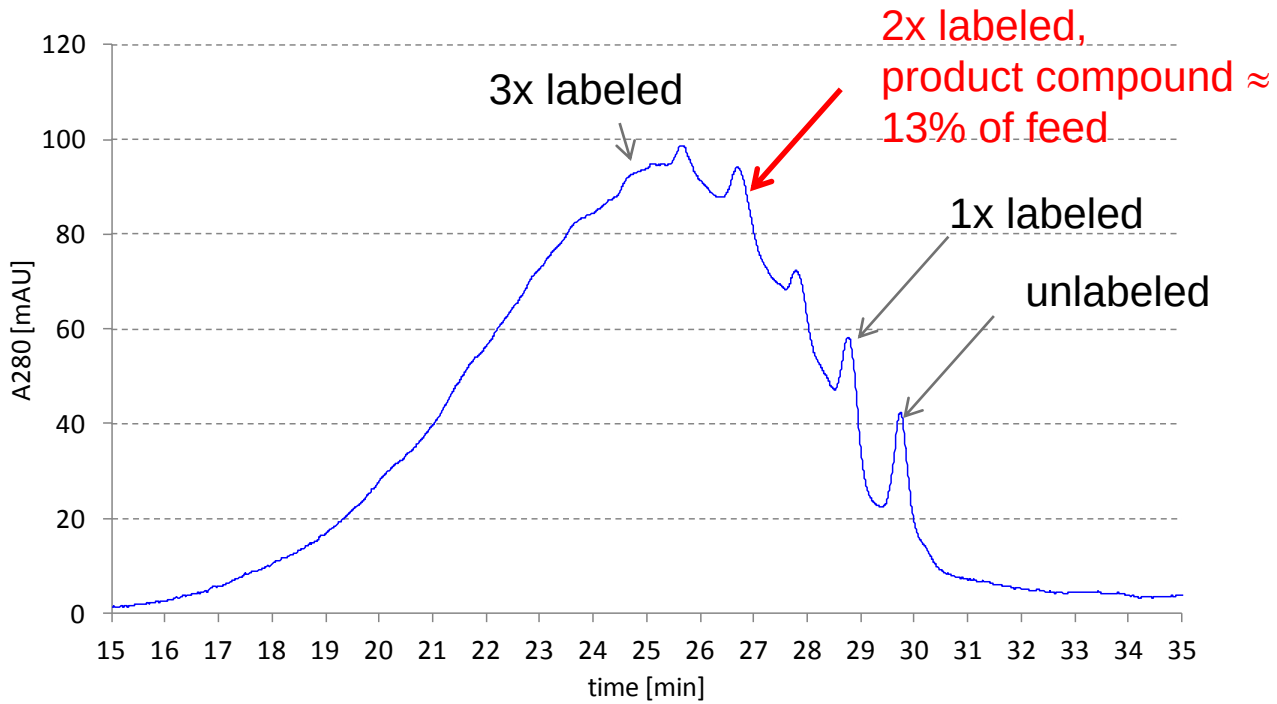
Purify 2-fold labeled form by preparative batch chromatography

Purify 2-fold labeled form by MCSGP

Analyze product pools by HPLC and compare results

# Conjugation

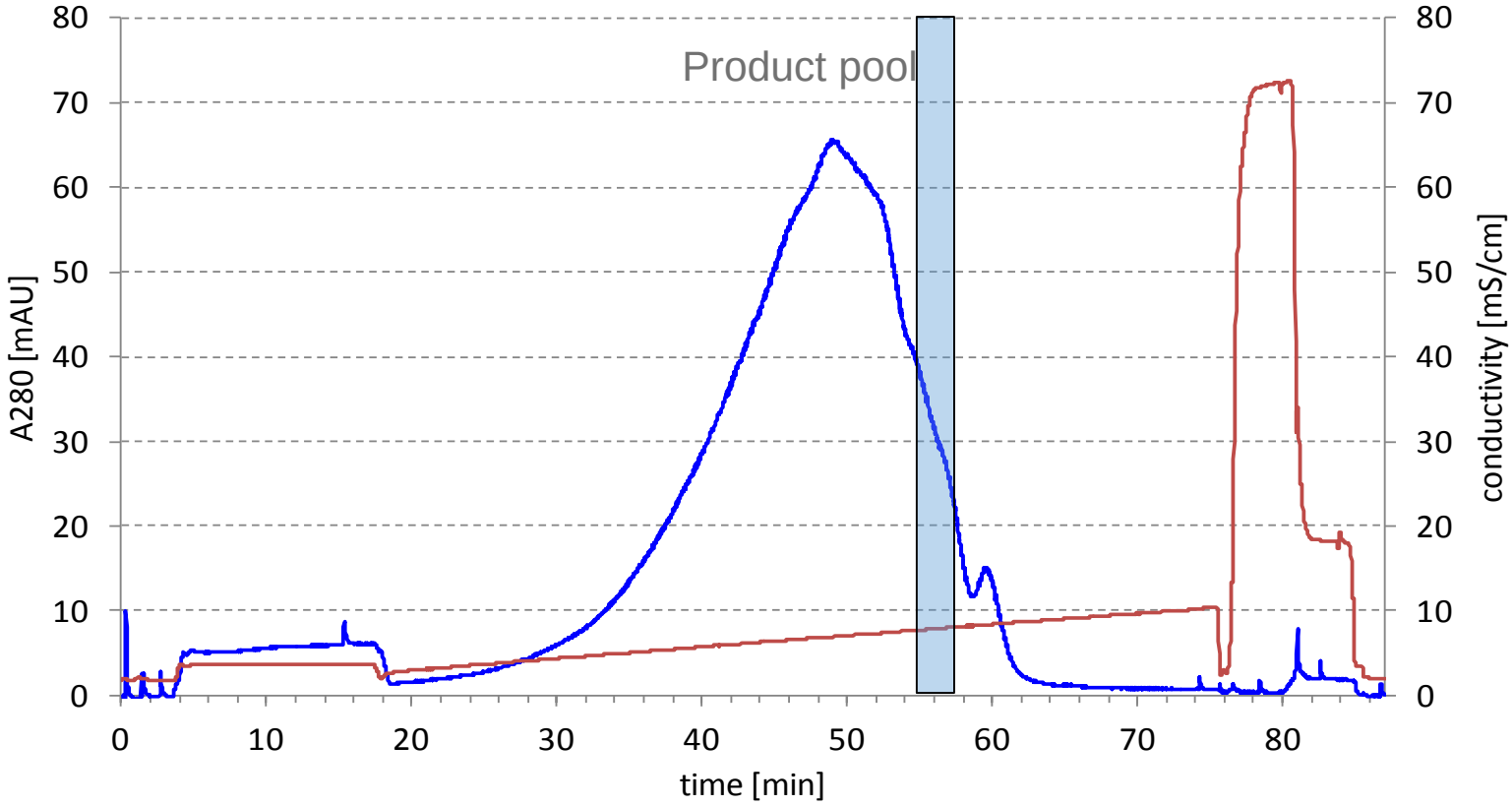
- Unspecific conjugation to Lys residues leads to strong ADC heterogeneity
- Analytical Cation Exchange chromatogram of coupling reaction product (feed for preparative chromatography):




Analytical column: Propac wCX-10, 4.6 x 250 mm

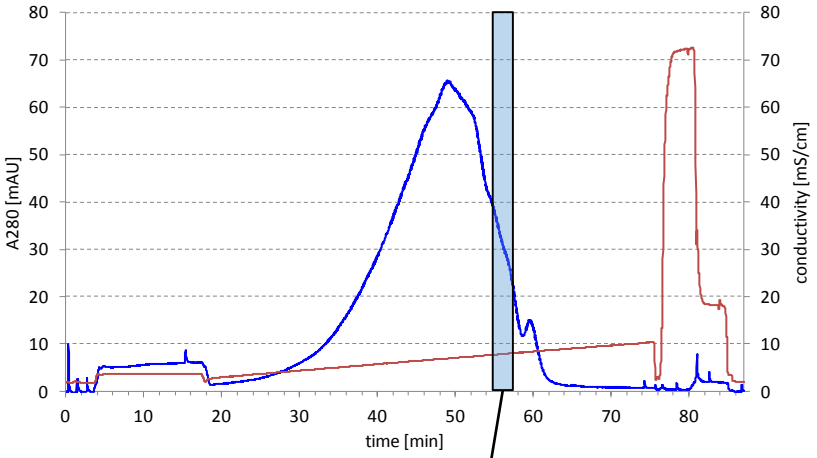
# Preparative Batch Gradient Purification

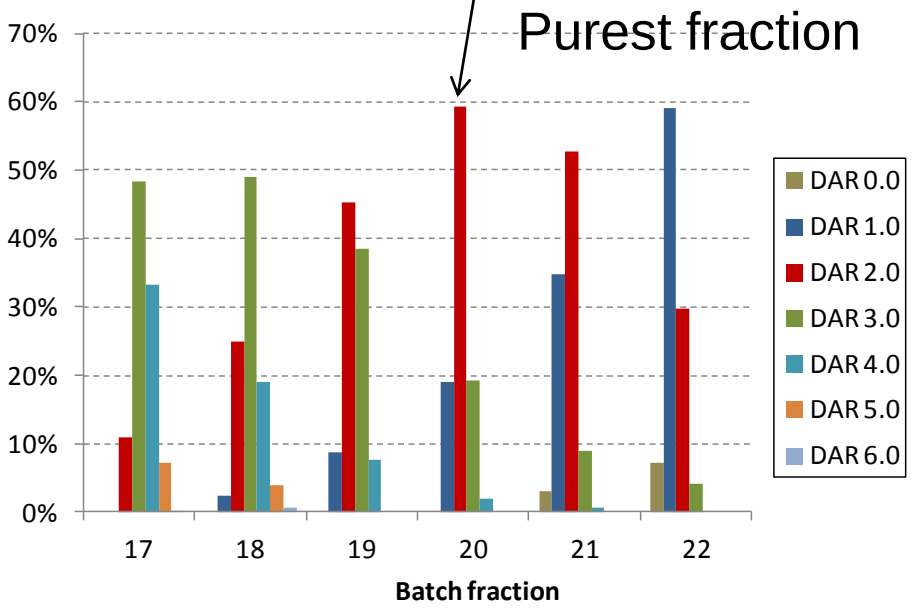
- Run conditions: Load 4.1 g labeled mAb / L, linear salt gradient elution
- 0.5 x 10 cm column, packed with YMC BioPro SP 10
- Preparative chromatogram (batch – single column):



# Preparative Batch Gradient Purification

## Fraction analysis of batch run by mass spectrometry:

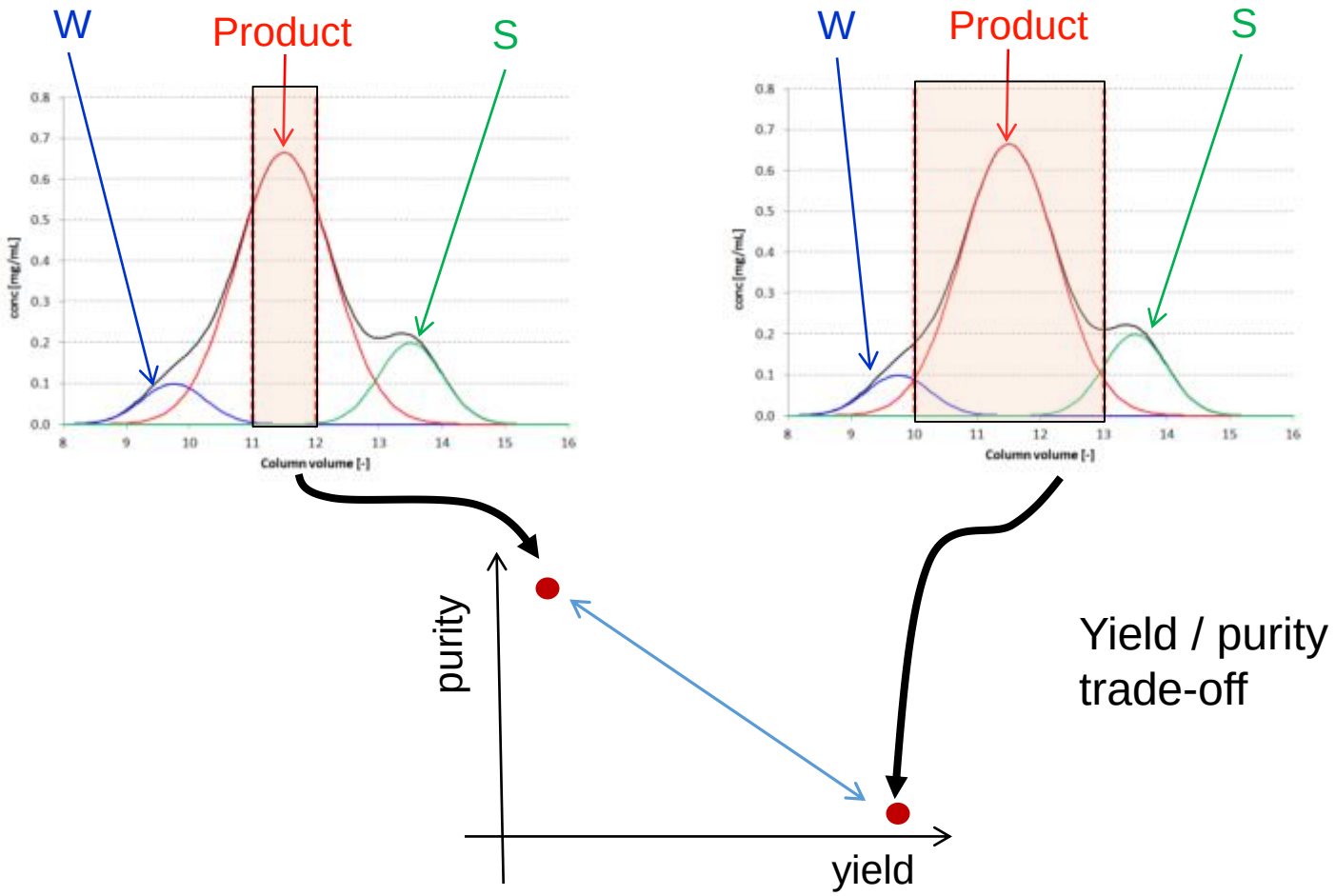



Maximum content of desired DAR 2.0 species was 59%. obtained with a yield of 34%

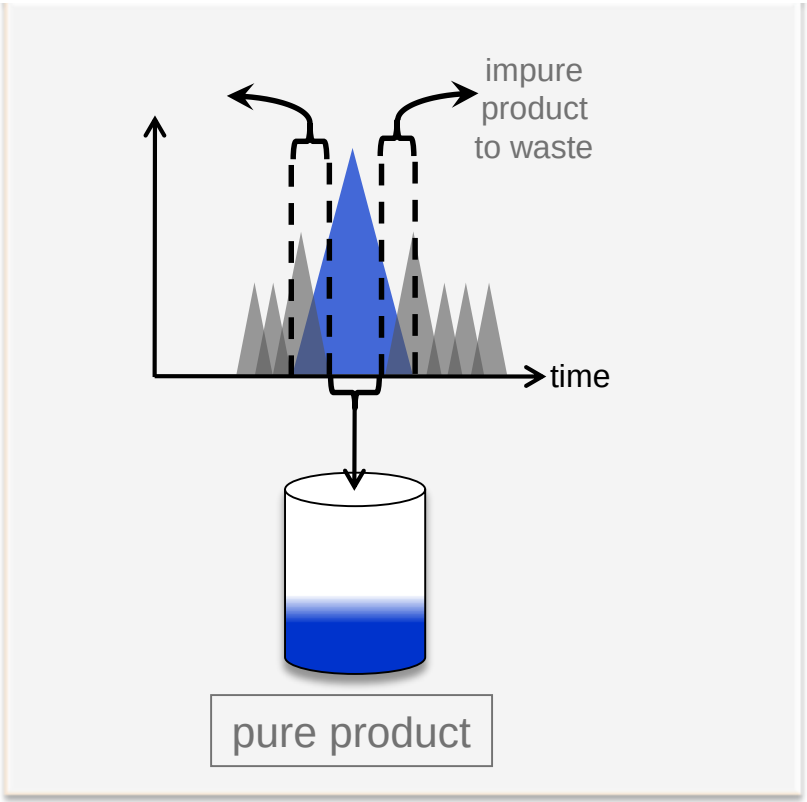
# Batch Chromatography: Trade-off between Yield/Purity

- Yield-purity trade-off for ternary separations

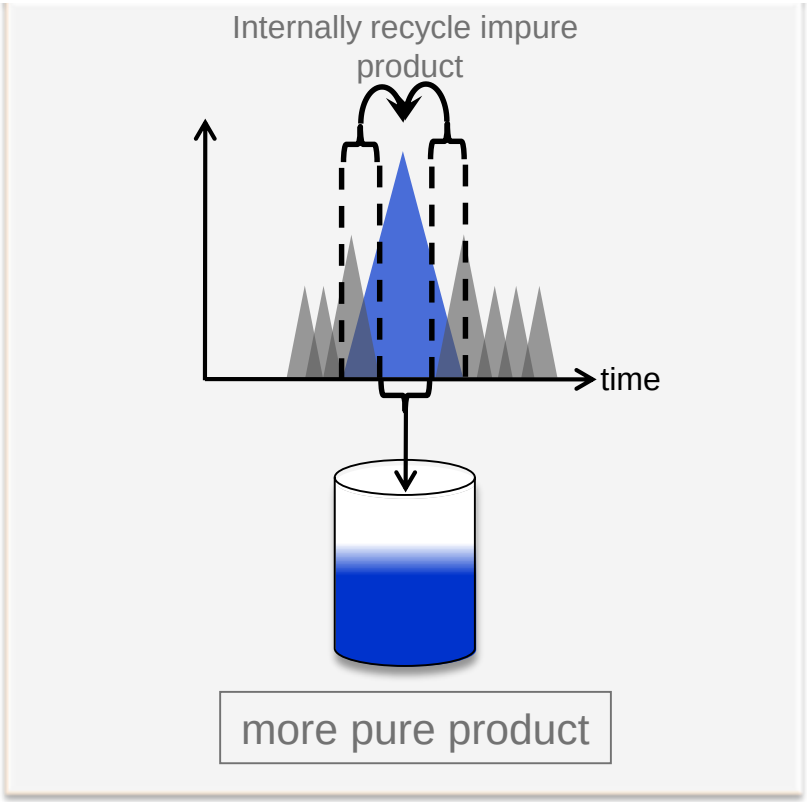


# MCSGP Principle: Recover product in side fractions

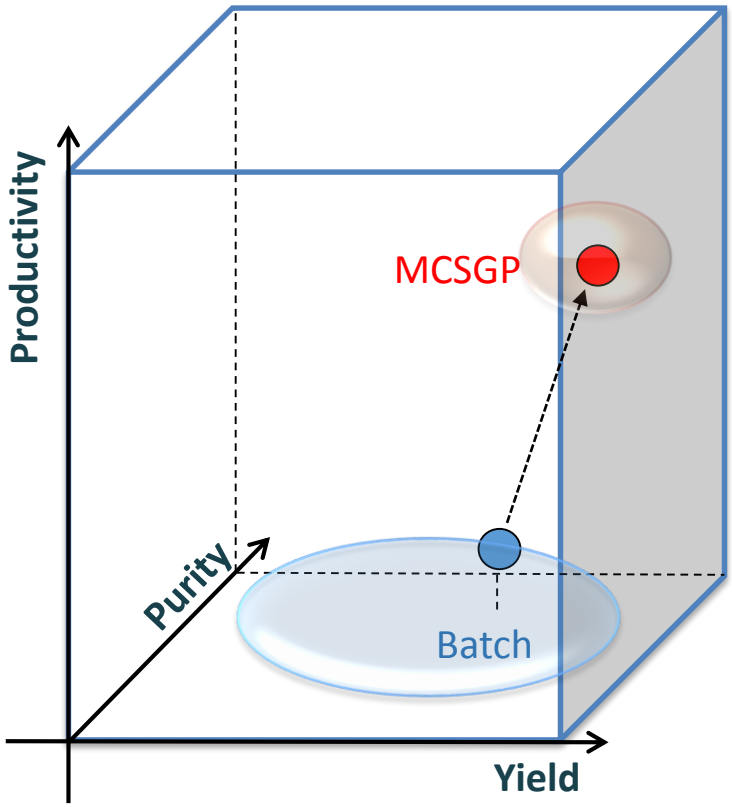
## Conventional single column batch chromatography




## MCSGP chromatography



# MCSGP Principle: Recover product in side fractions



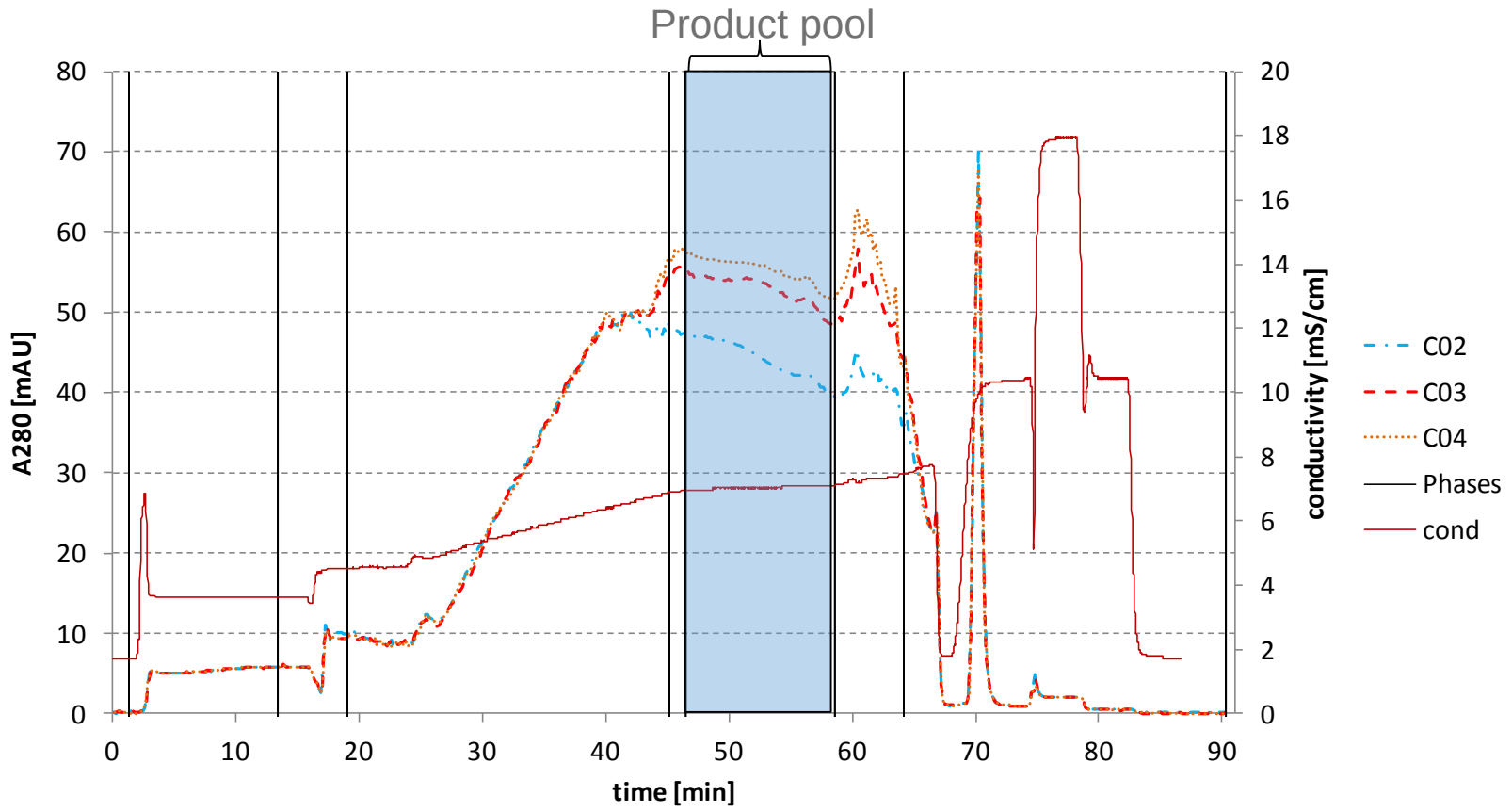

- Conventional single column batch chromatography operates largely 2-dimensional: tradeoff between yield and purity

- MCSGP is more effective than batch chromatography due to its counter-current mode of operation, allowing production at high yield and high purity simultaneously.

- MCSGP improves chromatography in a 3rd dimension, productivity: In addition to operating at high yield and purity, the process improves productivity

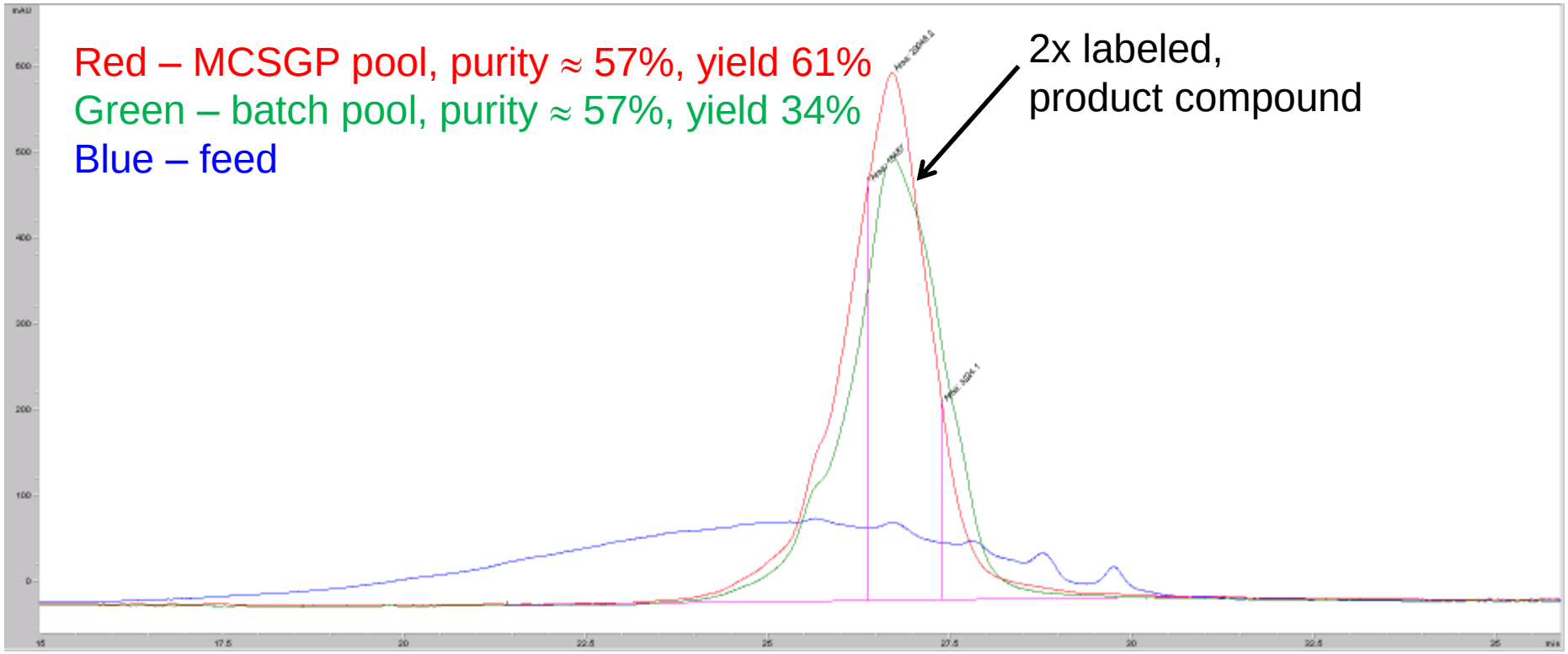
# MCSGP Purification

- Overlay of preparative chromatograms of subsequent cycles: Similar profiles indicate that cyclic steady state has been reached:



# Comparison Batch versus MCSGP

- Comparison of analytical chromatograms of batch and MCSGP product pools of comparable purity, corresponding to 2-fold labeled Trastuzumab (DAR = 2). The Feed chromatogram is also shown.

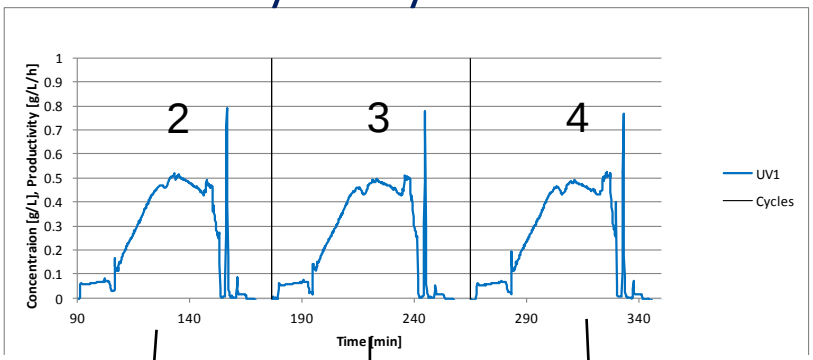


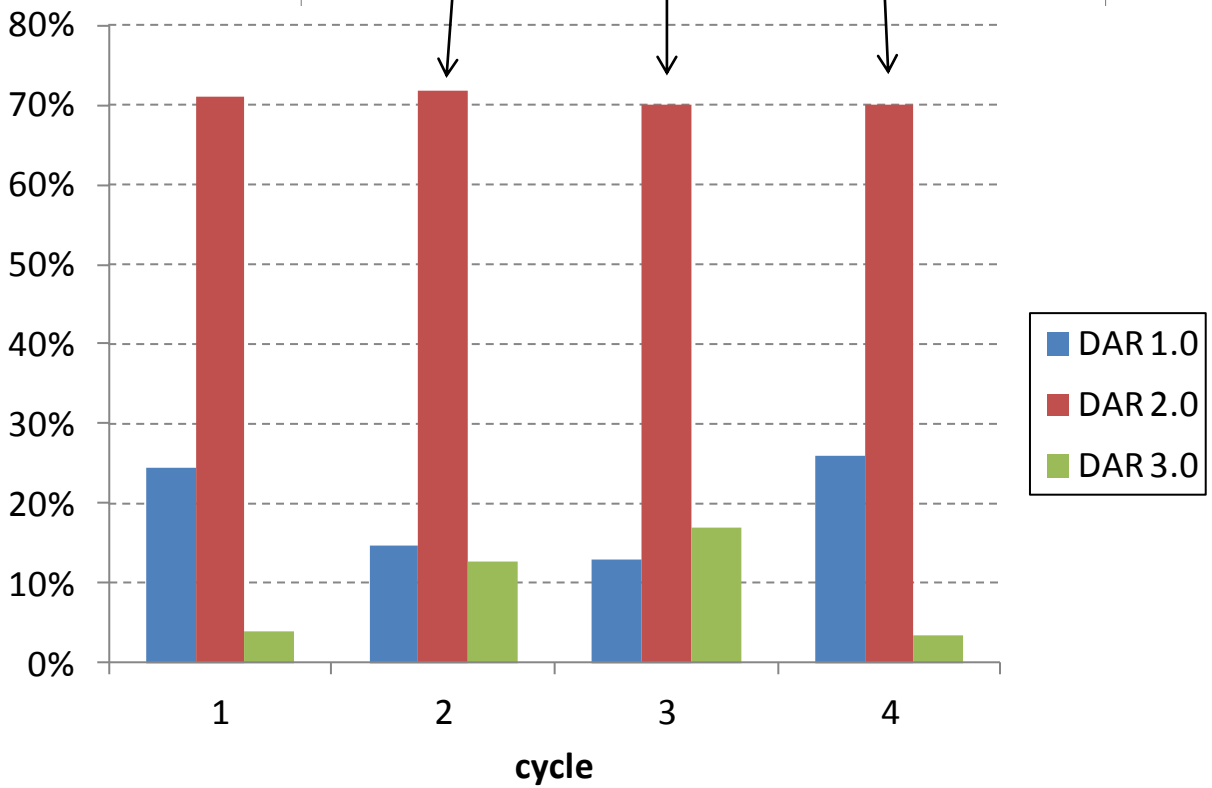

Details Purity determination: Valleys 26.37 and 27.37 min, DAR2 peak 26.69 → Δearly 0.319 min, Δlate 0.684 min

# MCSGP Purification

- product from individual MCSGP cycles was analyzed by MS

High content of 70% of desired DAR 2.0 species maintained over the cycles of MCSGP process
With yield of 61%



# Comparison Batch versus MCSGP

- Performance data:

| Process | Purity [%] | Yield [%] | Product conc [g/L] | Load [g/L] | Produc-tivity [g/L/h] | Buffer cons. [L/g] |
|---|---|---|---|---|---|---|
| Batch | 59 | 34 | 0.5 | 0.5 | 0.11 | 142 |
| MCSGP | 70 | 61 | 0.5 | 0.5 | 0.20 | 64 |
| Improve ment | / | + 80% | / | / | + 80% | - 55% |

- Performance improvement over batch chromatography:
  - → Purity improvement from 59% to 70%
  - → Yield increase from 34 to 61% (80% improvement)
  - → 80% Productivity increase
  - → 55% Reduction in buffer consumption

# Summary

- MCSGP is a scalable process to purify 1st generation ADCs
  - with defined DAR
  - with high yield
  - continuously with minimal handling effort
- Performance improvement over batch chromatography:
  - → Purity improvement from 59% to 70%
  - → Yield increase from 34 to 61% (80% improvement)
  - → 80% Productivity increase
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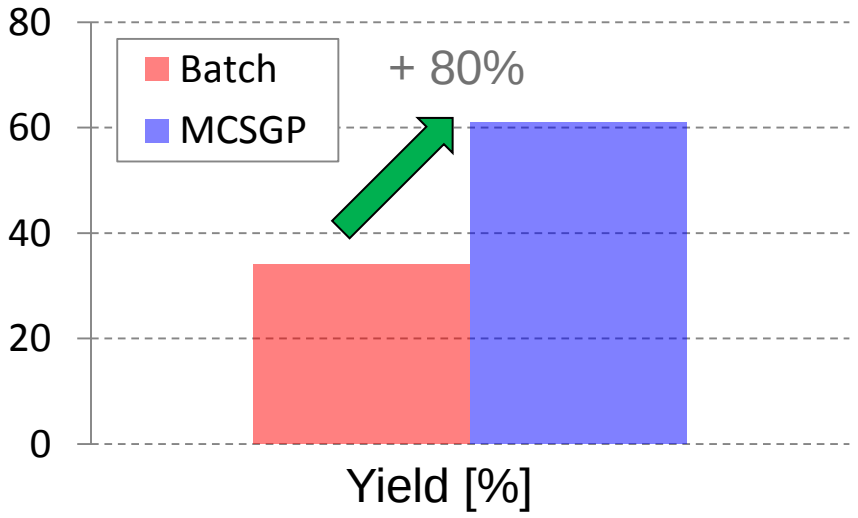

# Acknowledgements


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