

High Throughput Purification Development – Implementation and Case Studies –

Dr.-Ing. Magnus Schroeder

Purification Development
CSL Limited

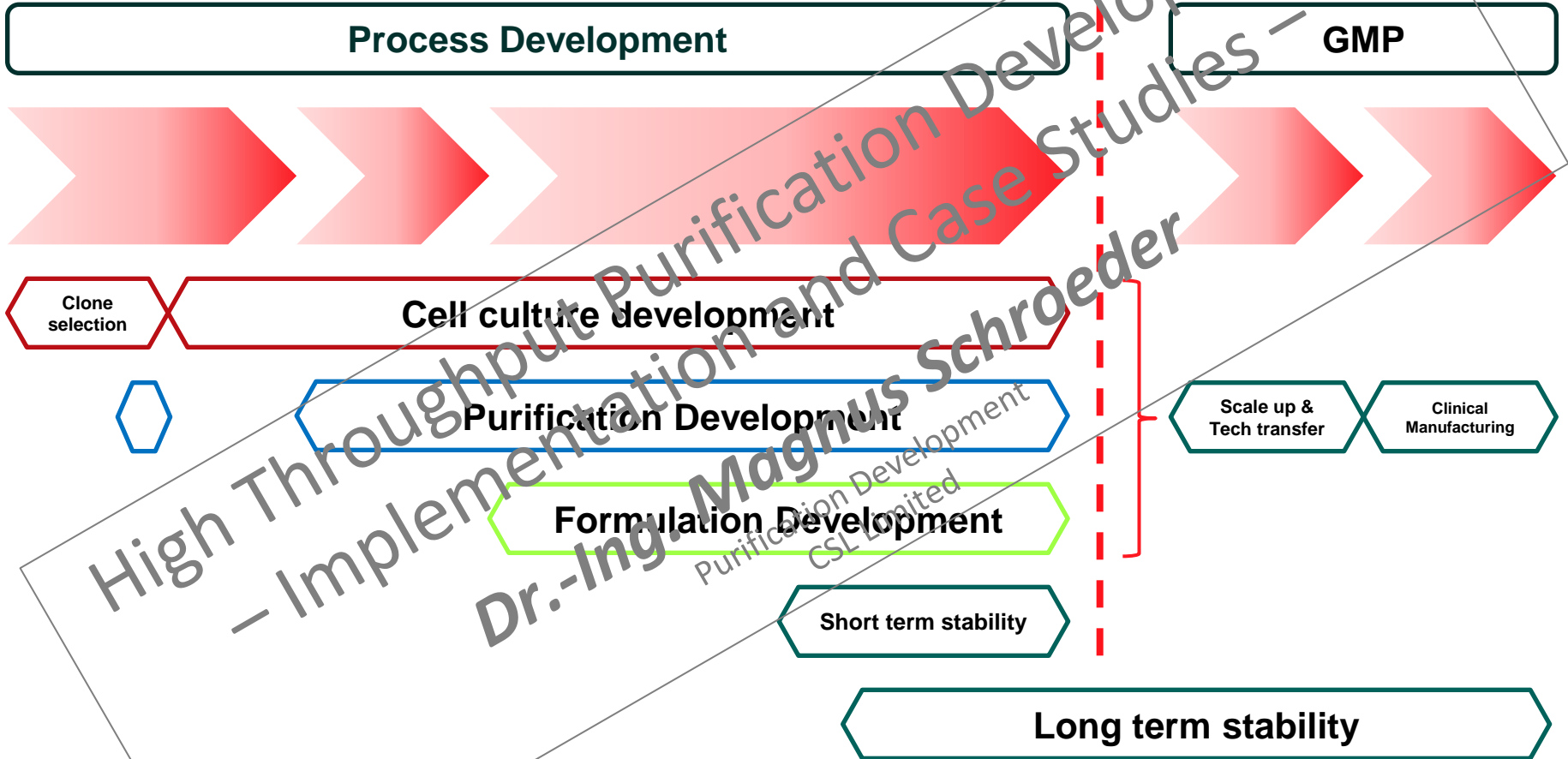
Content

- Introduction to High Throughput Purification Development
- Case Study: Protein A Capture Step Development
- Case Study: Post-Protein A Purification Resin Screen

Introduction

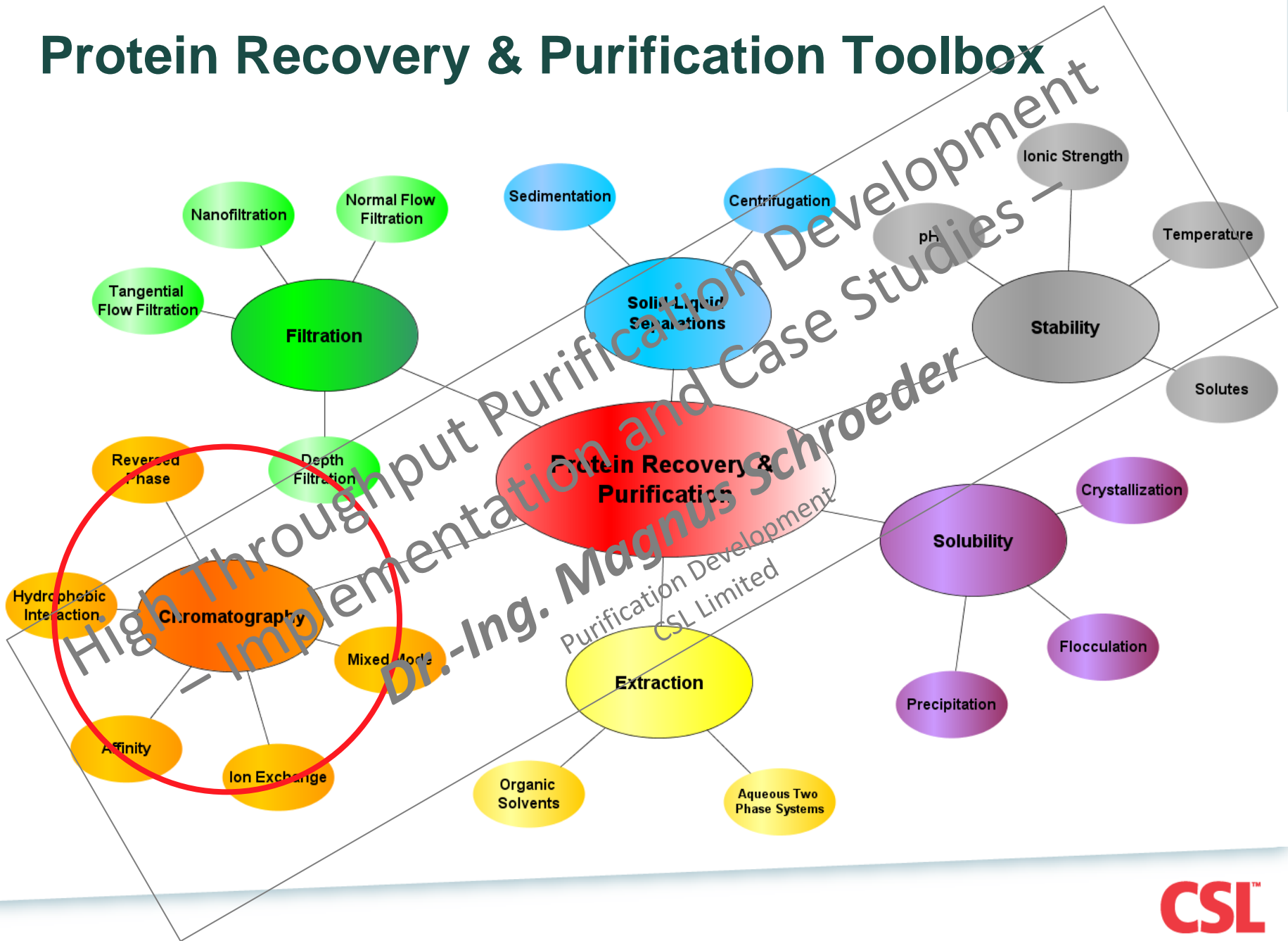
High Throughput Purification Development

Biopharmaceutical Development Project Timelines



High Throughput Purification Development
- Implementation and Case Studies -
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Protein Recovery & Purification Toolbox



Purification Development Challenges

- Product quality
- Removal of impurities
- Cost of goods
- Facility fit
- Process understanding / robustness
- Time-to-clinic / Time-to-market

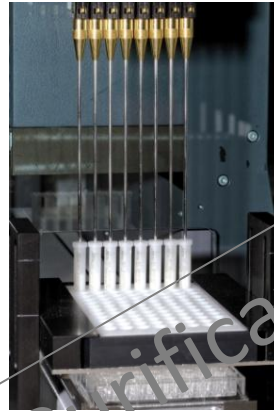


High Throughput Process Development

High Throughput Purification Development



CMO



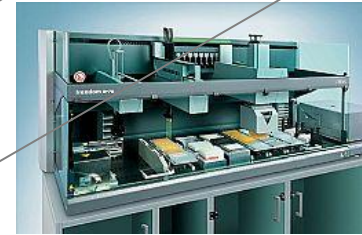
Column screening



Upstream



Batch screening



High Throughput Analytics

High Throughput Purification

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Screening in Batch Mode

- Filter plates for resin equilibration, loading, washing and elution
- Microplate stirrer for agitation during incubation
- Centrifuge / Vacuum manifold for sample collection



Screening in Column Mode

- Screening with pre-packed chromatography columns
- Equilibration, load, wash, elution steps performed by liquid handling system
- 8 columns in parallel with automated fraction collection



Benefits of High Throughput Process Development

- Accelerate Time-to-Market
- Access wider experimental space in shorter time using less material
- Automation of process development processes
- Rational choice of process parameters
- Quality by Design
- Process understanding right from the start

Case Study I

Protein A Capture Step Development

Protein A Capture Step Development

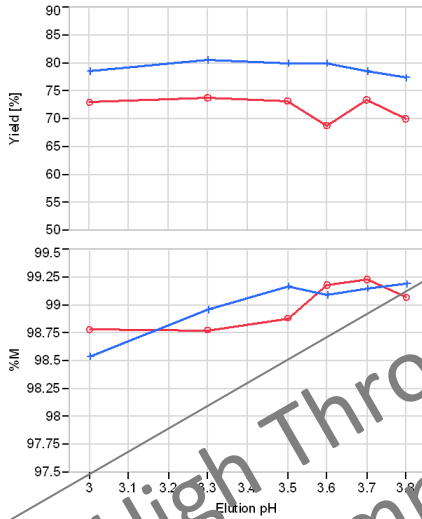
- Protein A capture should enable capture and concentration of product from cell culture harvest while maximizing removal of impurities
- Some choice of Protein A resins available
- Elution conditions are product and resin (ligand) specific
- Wash buffer composition can have a significant impact on impurity removal
- Which resin, elution and wash buffer give best performance?

Protein A Resin Screen

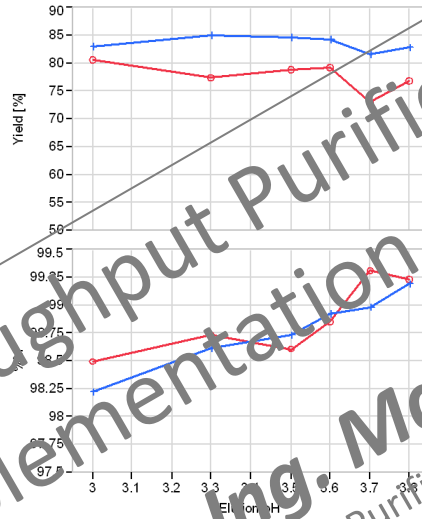
- Evaluate effect of Protein A resin at different pH and buffer concentrations on product yield and aggregate clearance
- 4 Protein A resins
- 6 pH conditions
- 2 buffer concentrations
 - = **48 unique conditions**

Protein A Resin Screen

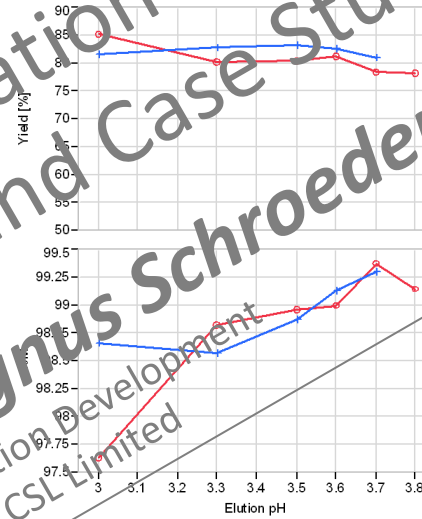
MabSelect SuRe



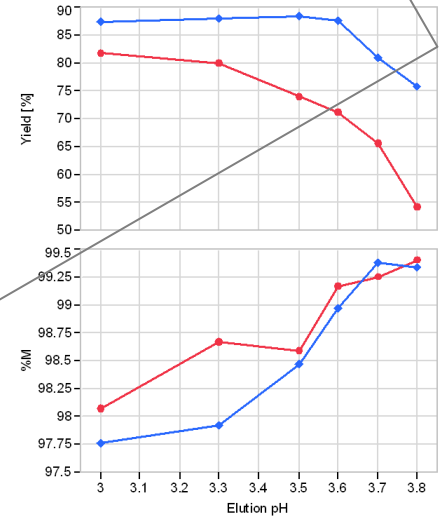
MabSelect SuRe LX



Prosep vA Ultra



Toyopearl rProtein A 650F

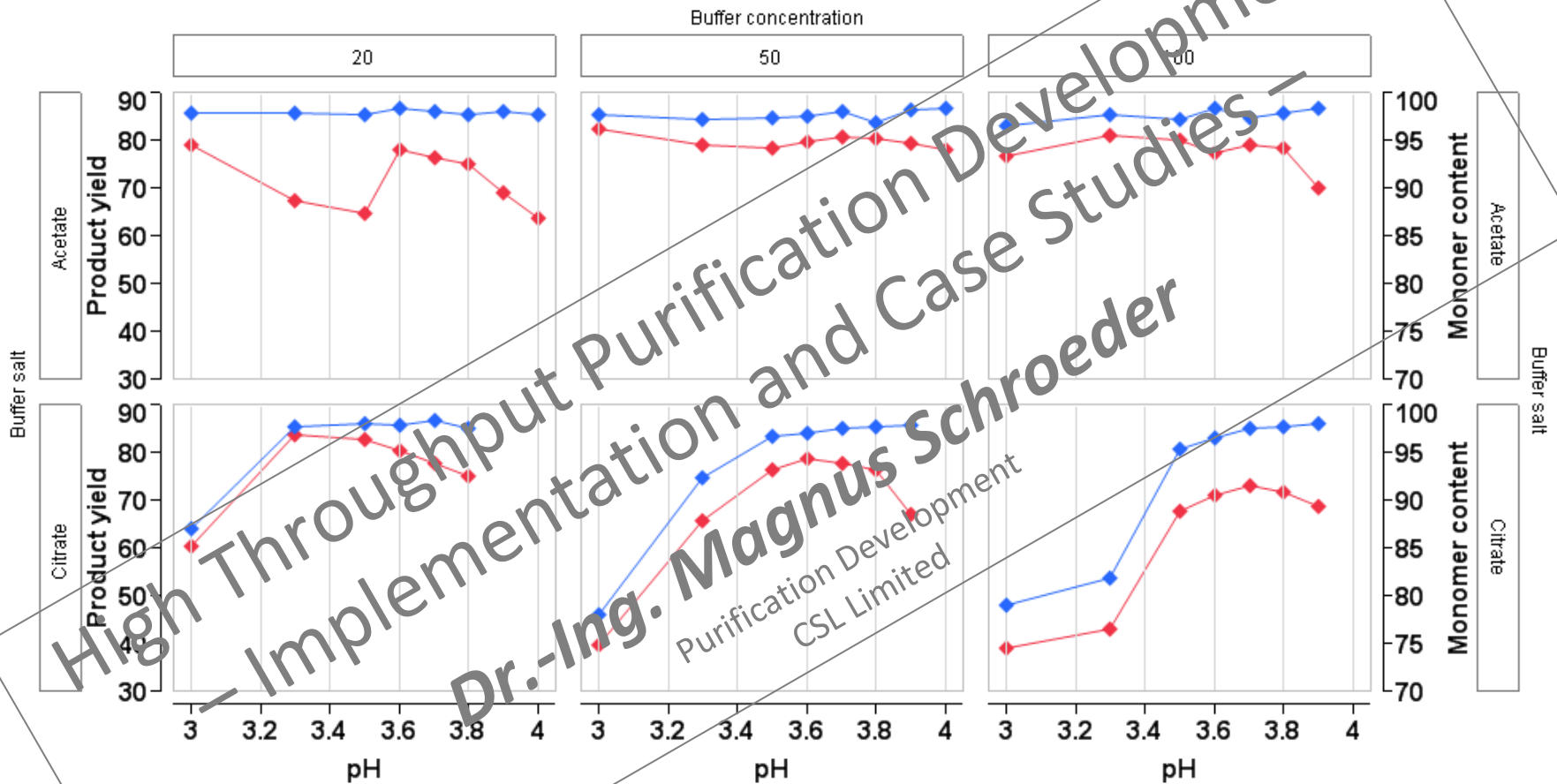


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Protein A Elution Buffer Screen

- Evaluate effect of elution buffer on product yield and aggregate clearance
- 1 Protein A resin
- 3 buffer systems
- 3 buffer concentrations
- 8 pH conditions
 - = **72 unique conditions**

Protein A Elution Buffer Screen



Left Scale: ◆ Product yield
 Right Scale: ◆ Monomer content

Third buffer not shown – Data filtered for high yield

Protein A Wash Buffer Screen

- Evaluate effect of intermediate wash buffer on product yield, aggregate removal and HCP clearance
- 1 Protein A resin
- 1 elution buffer
- 48 wash buffers
 - **48 unique conditions**

Protein A Wash Buffer Screen



Data filtered for high yield

Conclusions

- Protein A resin with desired properties can be chosen
- Elution buffer for maximized product yield, impurity removal and stability can be selected and behavior of the system is characterized
- Wash buffer for maximized impurity removal with minimal product loss can be chosen covering a diverse chemical space

Case Study II

Post-Protein A Purification Resin Screen

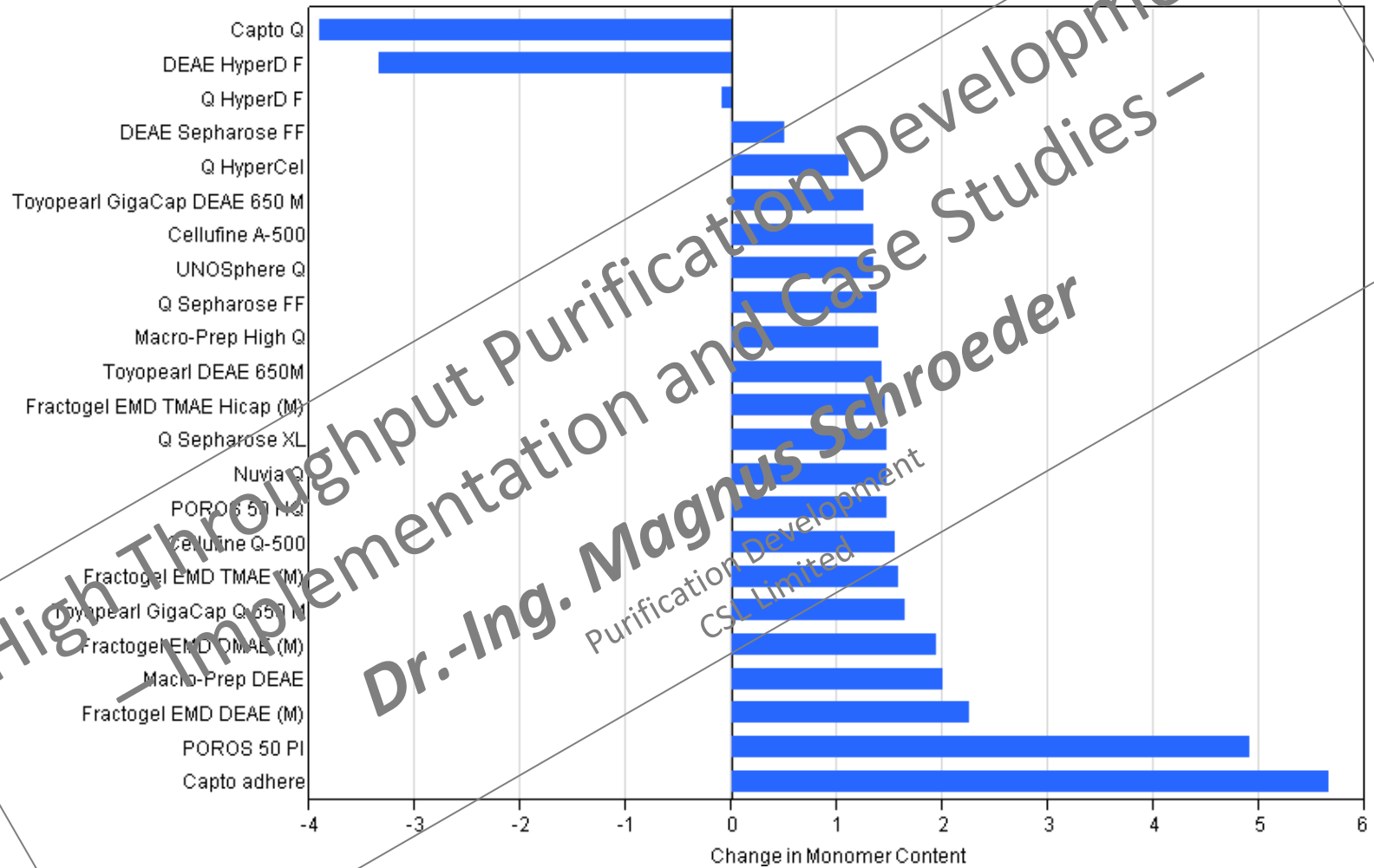
Anion & Cation Exchange Resin Screen

- Post-Protein A purification steps should enable high purity at acceptable yields and processing times
- Ion exchange is the preferred Post Protein A purification step
- Large choice of ion exchange resins available
- Resin performance is often product specific and hard to predict
- Which resins have the greatest potential for purification task?

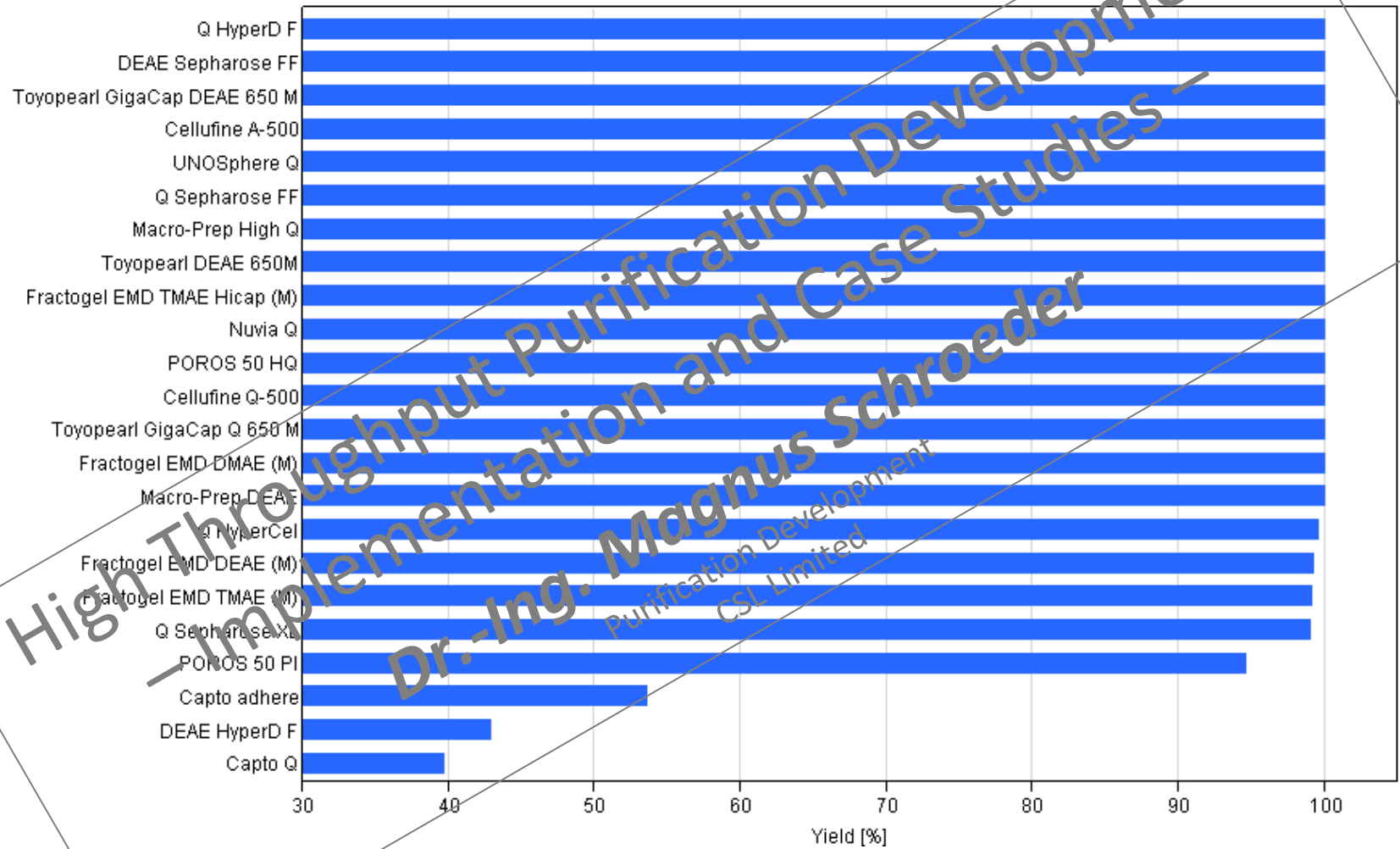
Anion & Cation Exchange Resin Screen

- 16 cation exchange / cationic mixed-mode resins
- 23 anion exchange / anionic mixed-mode resins
- High throughput batch screen
- Anion exchange in flowthrough mode
- Cation exchange in bind&elute mode
- Equal load per resin volume

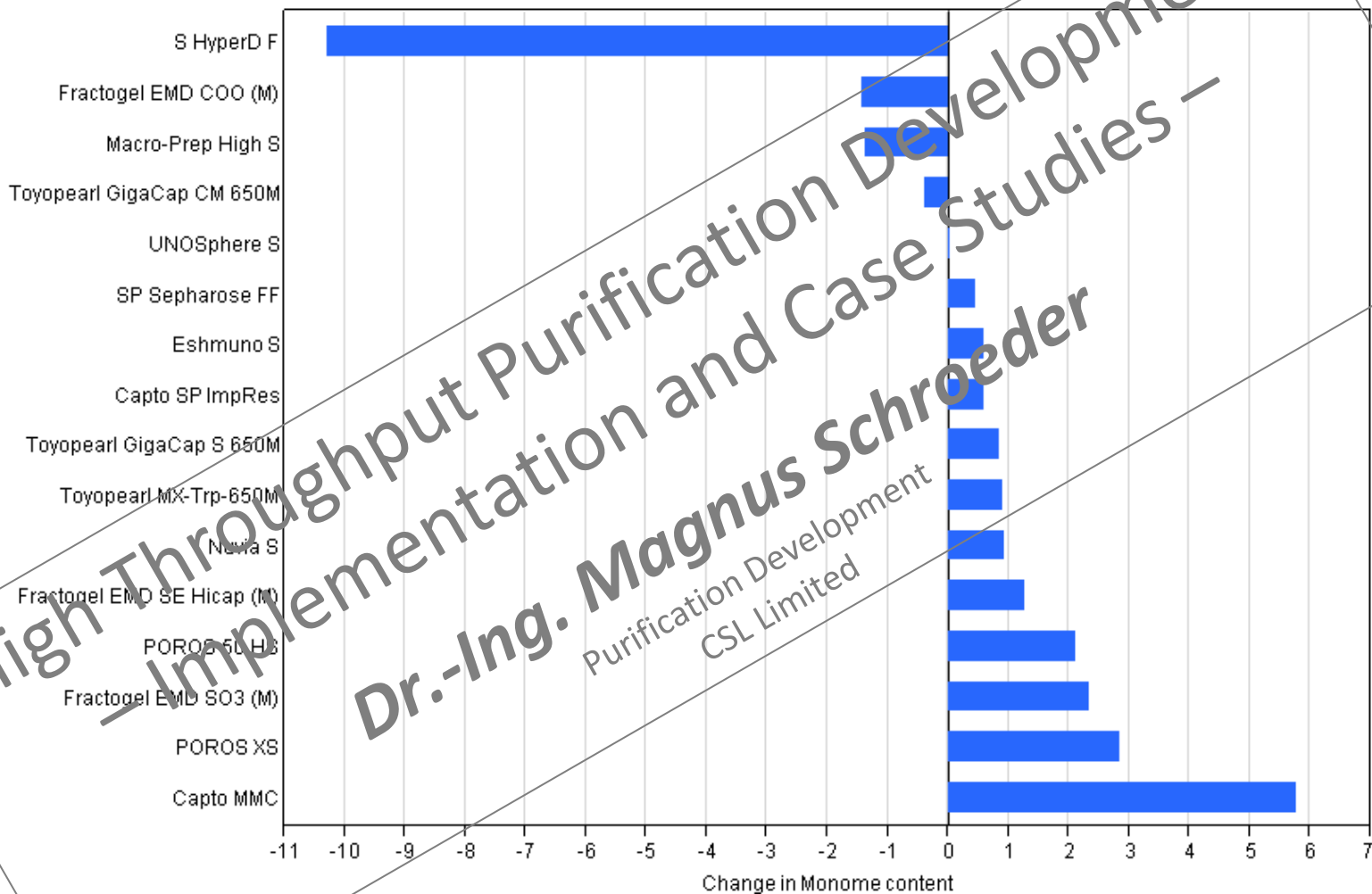
Anion Exchange Aggregate Clearance



Anion Exchange Product Yield

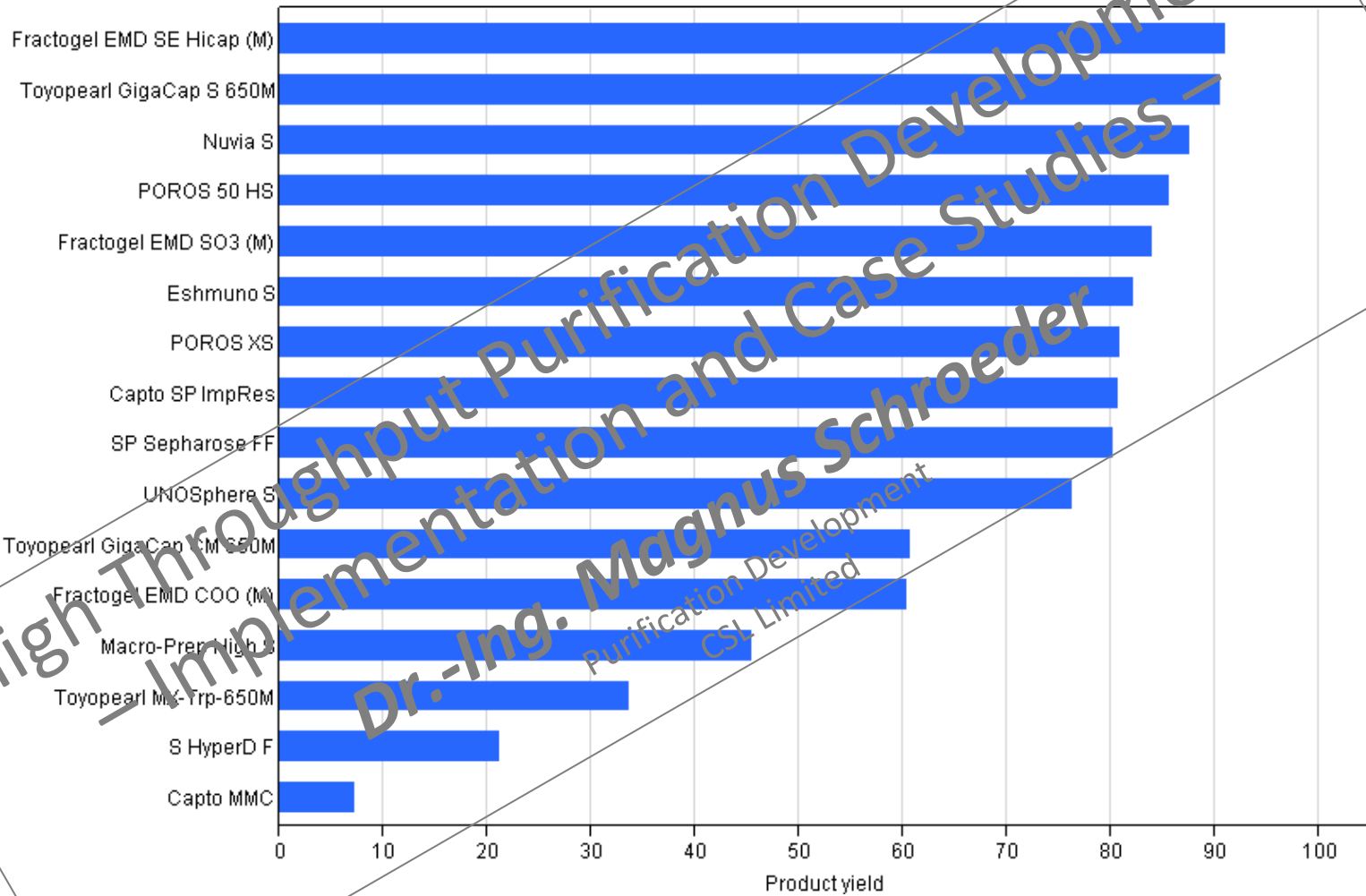


Cation Exchange Aggregate Clearance



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Cation Exchange Product Yield



Conclusions

- Large differences in yields and aggregate clearance for different resins
- Aggregate clearance at acceptable yields higher for anion exchange resins
- Resins for post-Protein A purification with high likelihood of success can be selected based on initial screen within 24 h

Summary

- High Throughput Purification Development platform established
- Enabling screening of up to 5000 conditions per week
- Chromatography development modules for
 - Resin screen
 - Elution conditions
 - Wash buffer composition

Acknowledgements

Purification Development

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