Mathematical Modelling of Chromatography as a tool for process understanding and development acceleration
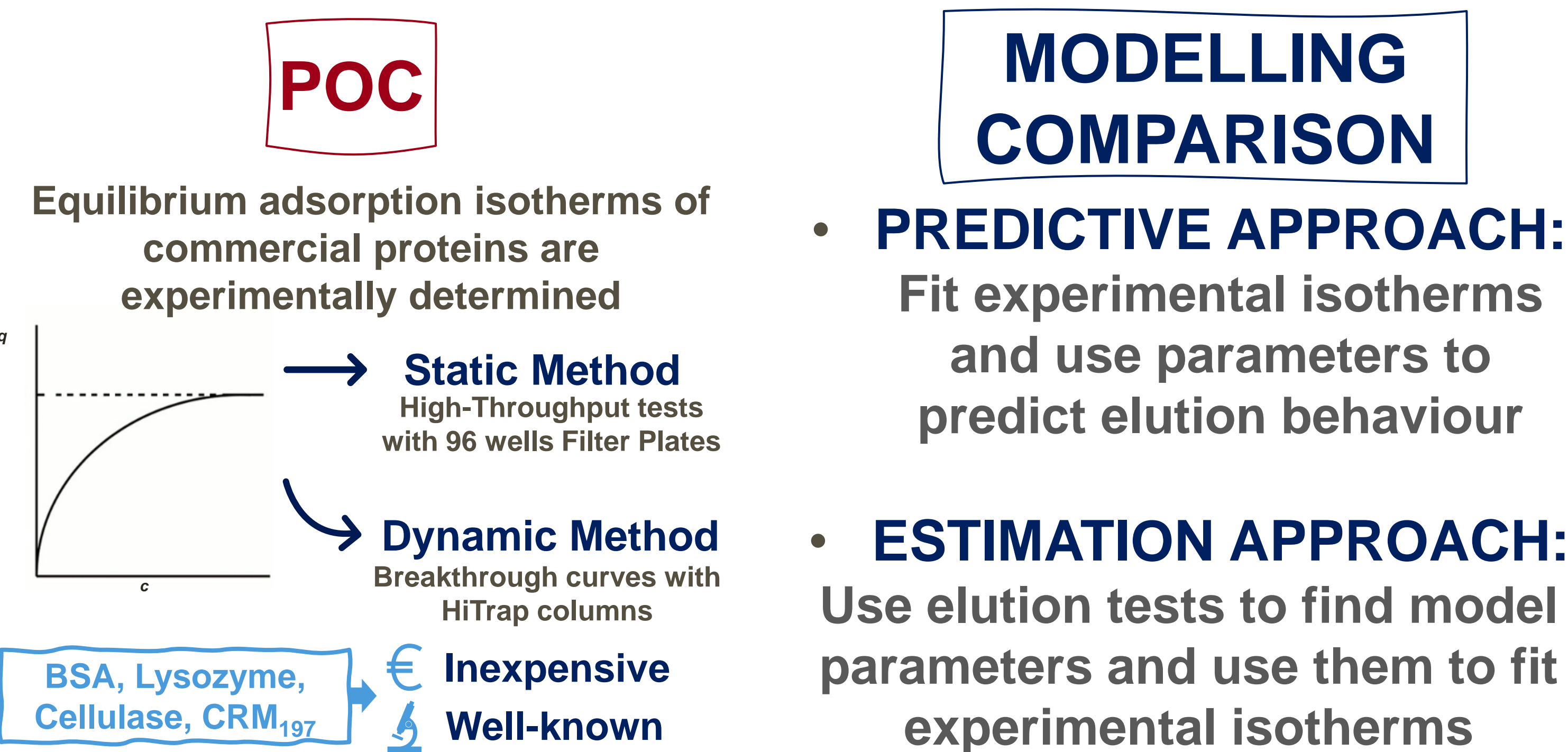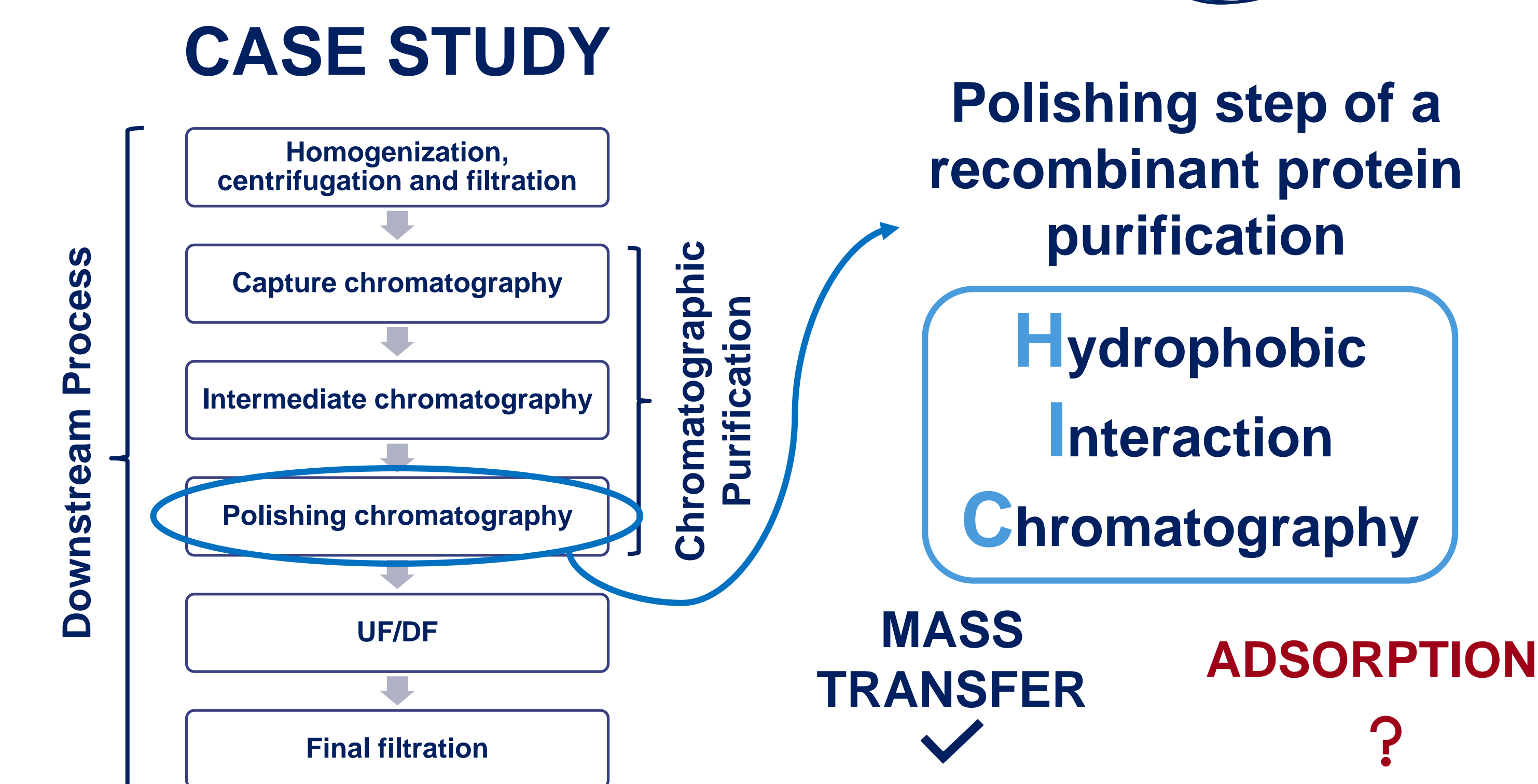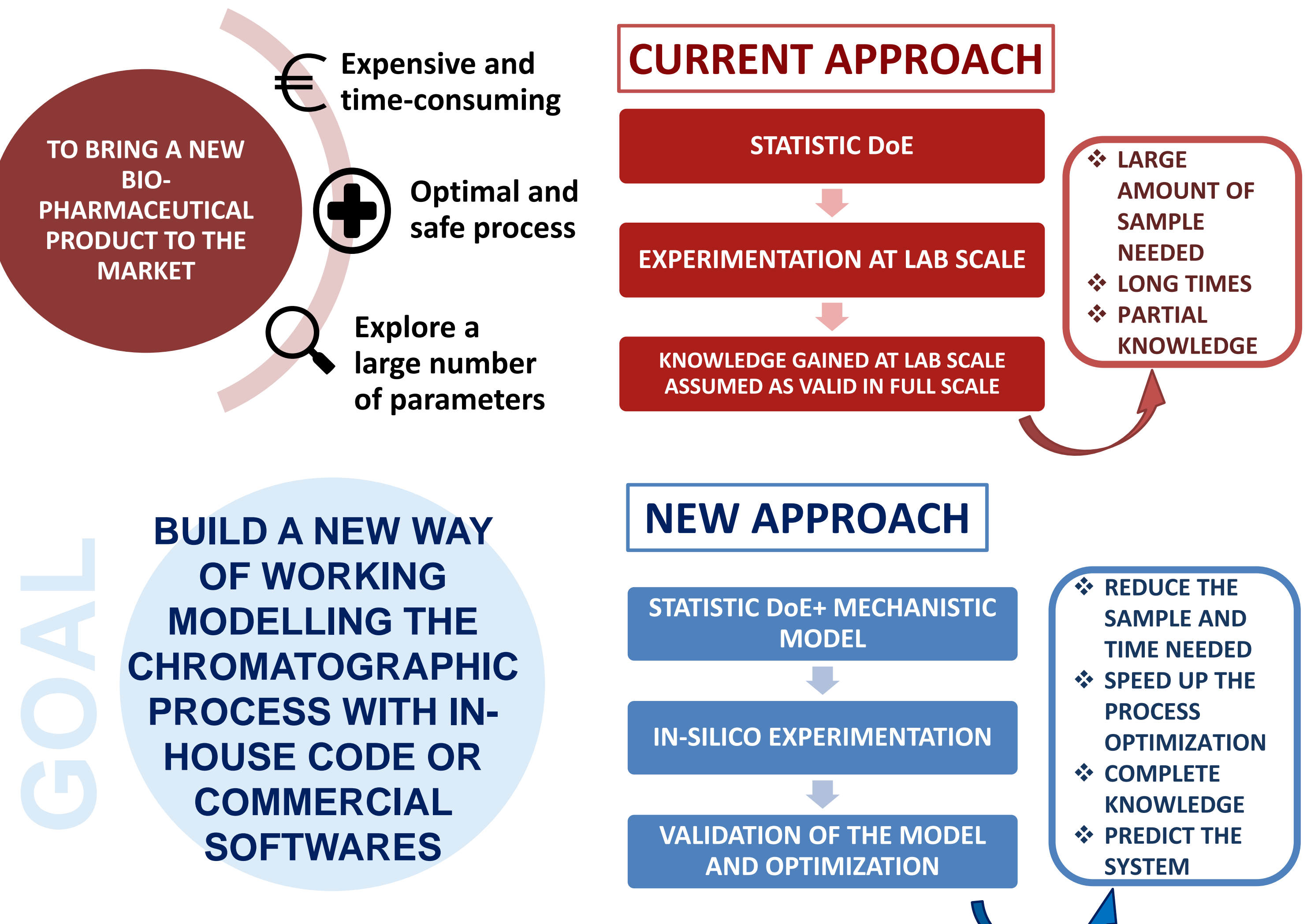
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Introduction

€ Expensive and time-consuming

+ Optimal and safe process

🔍 Explore a large number of parameters

TO BRING A NEW BIO-PHARMACEUTICAL PRODUCT TO THE MARKET

GOAL: BUILD A NEW WAY OF WORKING MODELLING THE CHROMATOGRAPHIC PROCESS WITH IN-HOUSE CODE OR COMMERCIAL SOFTWARES

CURRENT APPROACH

STATISTIC DoE → EXPERIMENTATION AT LAB SCALE → KNOWLEDGE GAINED AT LAB SCALE ASSUMED AS VALID IN FULL SCALE

- ❖ LARGE AMOUNT OF SAMPLE NEEDED
- ❖ LONG TIMES
- ❖ PARTIAL KNOWLEDGE

NEW APPROACH

STATISTIC DoE + MECHANISTIC MODEL → IN-SILICO EXPERIMENTATION → VALIDATION OF THE MODEL AND OPTIMIZATION

- ❖ REDUCE THE SAMPLE AND TIME NEEDED
- ❖ SPEED UP THE PROCESS OPTIMIZATION
- ❖ COMPLETE KNOWLEDGE
- ❖ PREDICT THE SYSTEM

CASE STUDY

Downstream Process:
Homogenization, centrifugation and filtration → Capture chromatography → Intermediate chromatography → Polishing chromatography → UF/DF → Final filtration

Chromatographic Purification: Capture, Intermediate, Polishing chromatography

Polishing step of a recombinant protein purification

Hydrophobic **I**nteraction **C**hromatography

MASS TRANSFER ✓

ADSORPTION ?

MODELLING COMPARISON

- **PREDICTIVE APPROACH:** Fit experimental isotherms and use parameters to predict elution behaviour

- **ESTIMATION APPROACH:** Use elution tests to find model parameters and use them to fit experimental isotherms

POC

Equilibrium adsorption isotherms of commercial proteins are experimentally determined

→ **Static Method** High-Throughput tests with 96 wells Filter Plates

→ **Dynamic Method** Breakthrough curves with HiTrap columns

BSA, Lysozyme, Cellulase, CRM$_{197}$ → € Inexpensive / Well-known
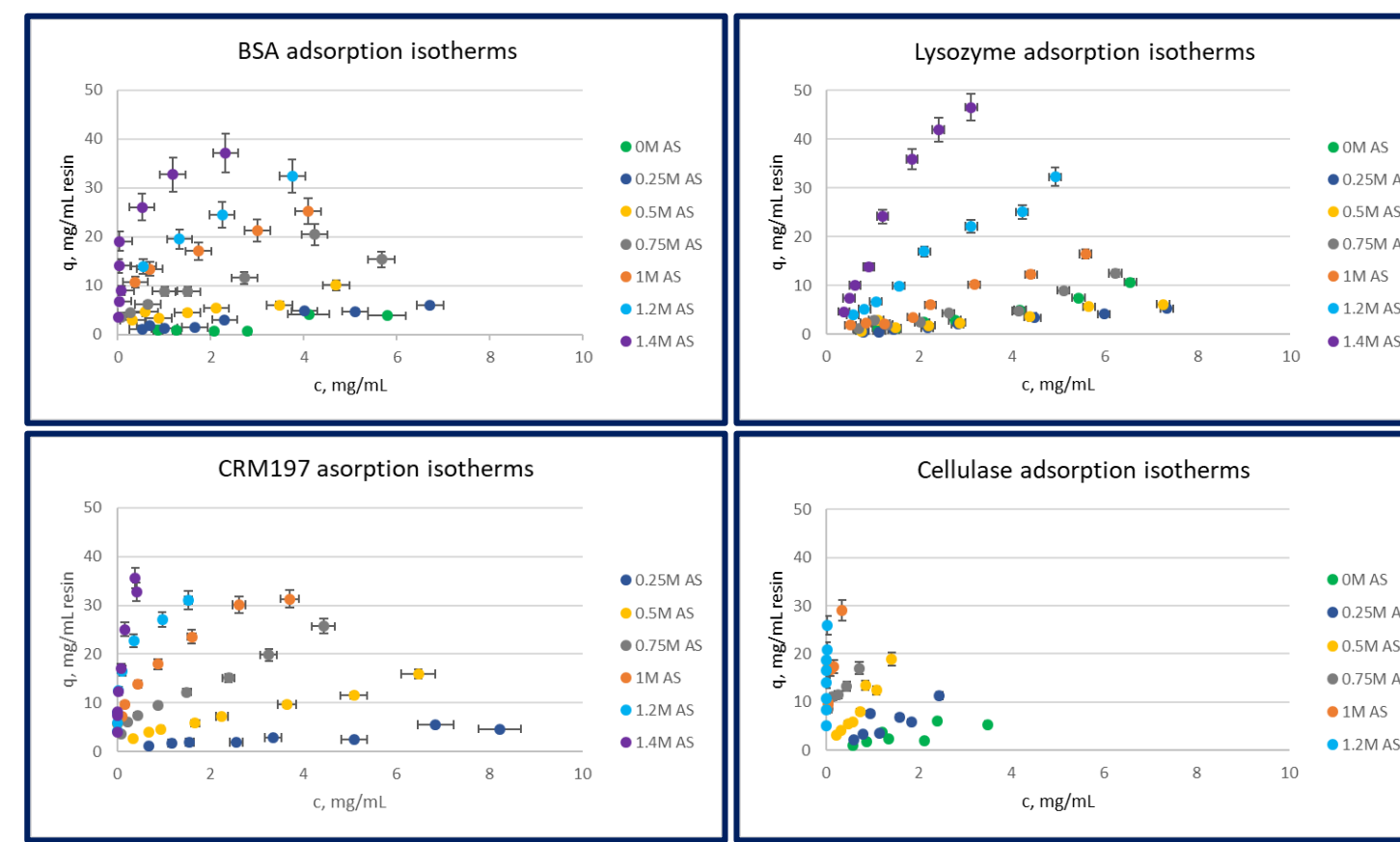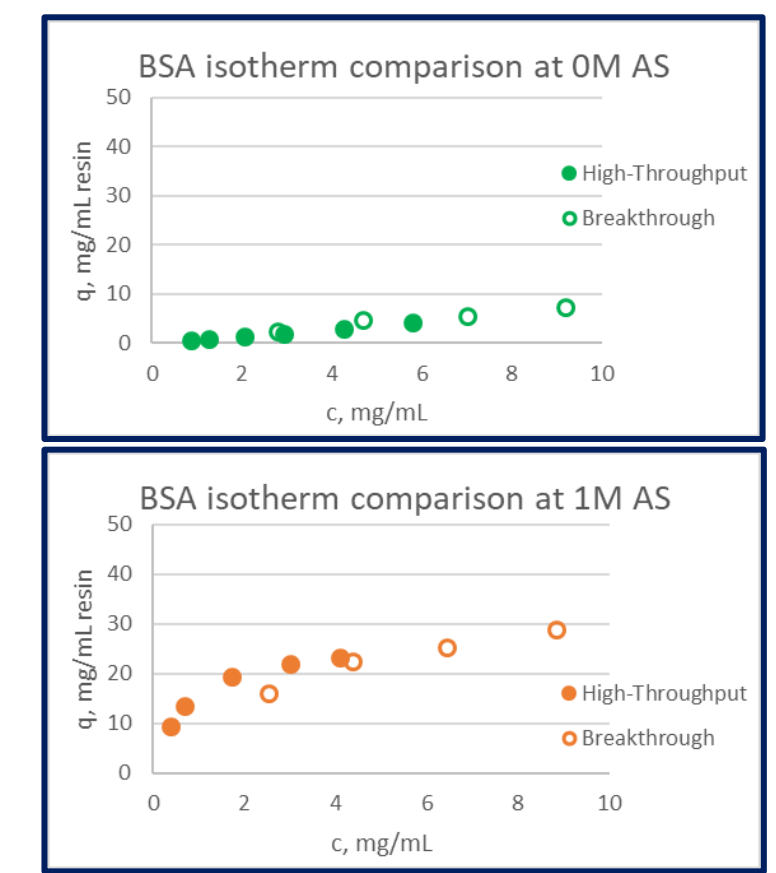
Results

EXPERIMENTAL ISOTHERM DETERMINATION

HIGH-THROUGHPUT experimentations in 96-wells filter plates, protein concentration increases from 1 to 10 mg/mL for six different salt concentrations in a butylic resin.

BREAKTHROUGH experimentations in 1 mL column with butylic resin at four different protein concentrations and three salt conditions.

Breakthrough isotherm points compared to high-throughput isotherms seem follow the isotherm shape reaching higher protein concentrations
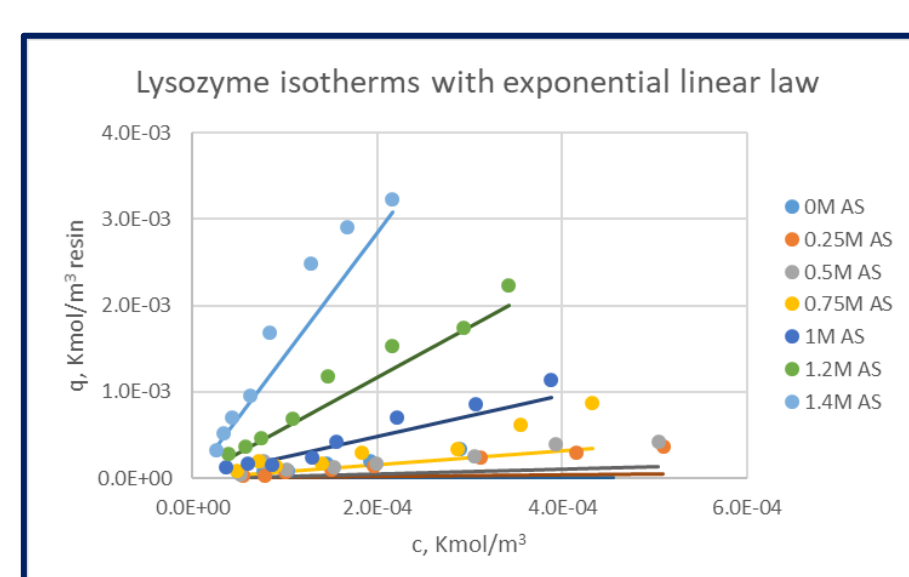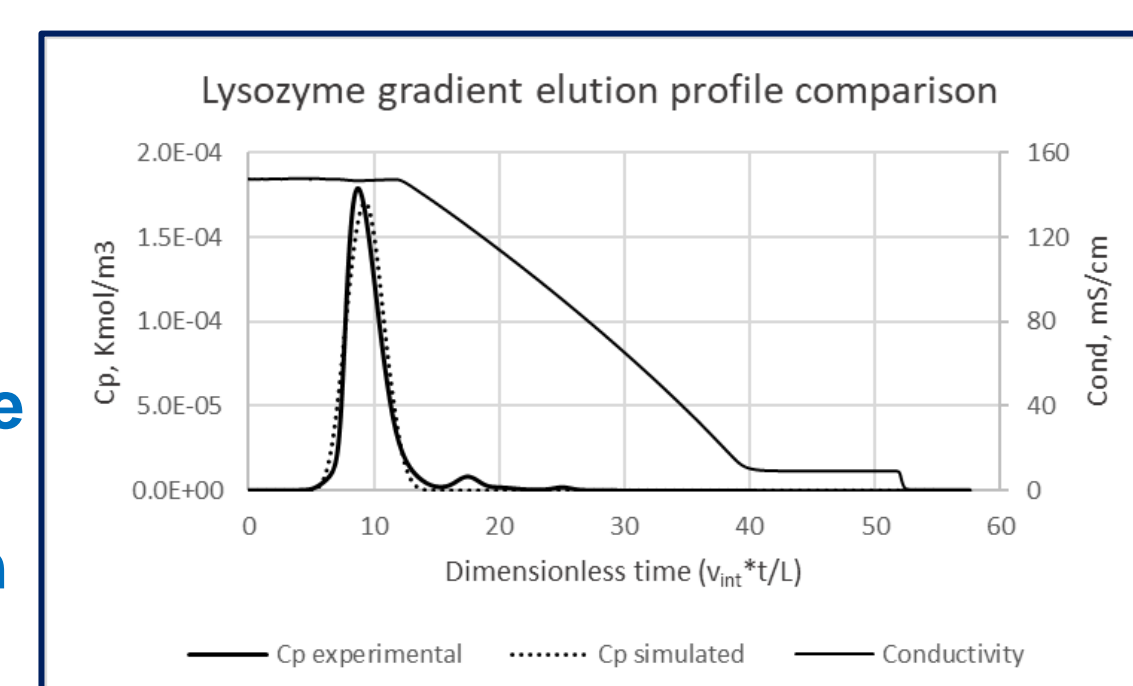
PREDICTIVE APPROACH

SIMULATION WITH A IN-HOUSE CODE

PDE System:
- Mass balance in the interstitial liquid
- Mass balance in the pores of the beads
- Equilibrium between solid and liquid

Bind-elute tests with both isochratic and gradient elution are performed and then simulated. Elution profiles are compared to validate the model.

Test fitting equations that describes adsorption behaviour:

$$q = \frac{\lambda b \, e^{kC_{salt}} C_p}{1 + b e^{kC_{salt}} C_p}$$

$$q = \frac{\lambda b C_{salt}^{\alpha} C_p}{1 + b C_{salt}^{\alpha} C_p}$$
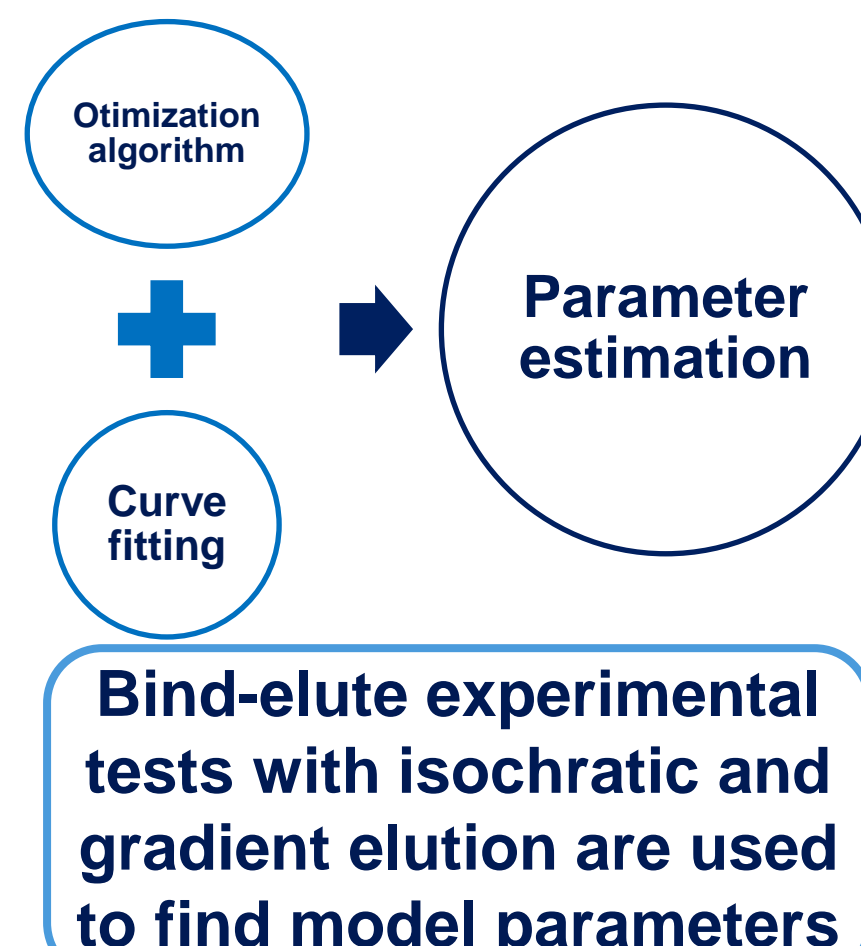
$$q = a \, e^{kC_{salt}} C_p$$

Isotherms parameters from experimental data
- ➤ V_d, ε_p, ε_c and D_{ax} are found experimentally with Acetone and Dextran injections.
- ➤ k_f, D_m and D_p are found with empirical correlations.

ESTIMATION APPROACH

A commercial software is used to find a suitable model

CHROM **X** & DSP **X** from GoSilico

Optimization algorithm + Curve fitting → Parameter estimation

Bind-elute experimental tests with isochratic and gradient elution are used to find model parameters

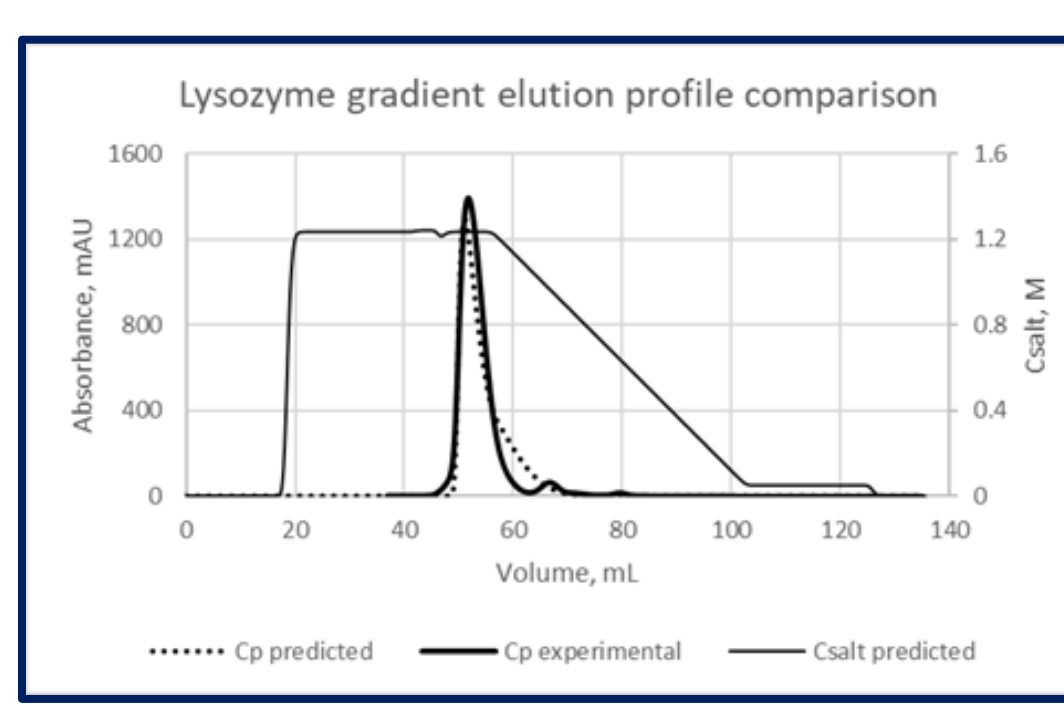Isotherm laws used from the software to model HIC are complex.

The laws are (in equilibrium conditions):
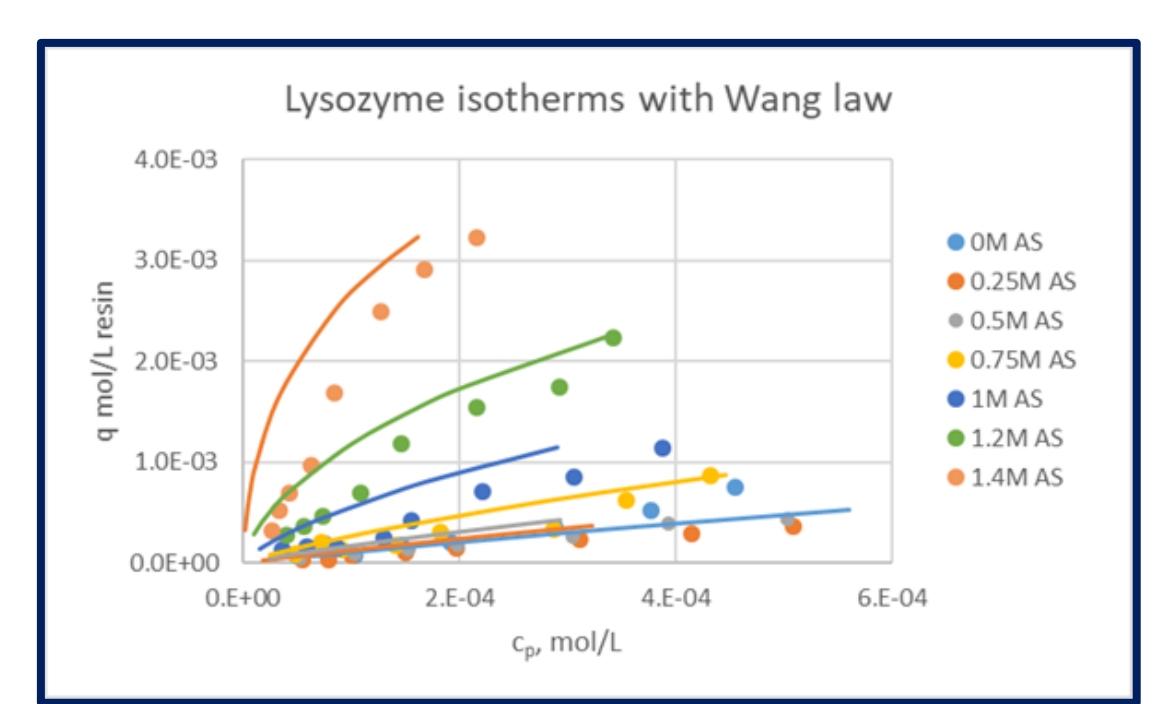- the law developed by Mollerup et al.

$$q = k_{eq}\left(1 - \frac{q}{q_{max}}\right)^n \exp\left(k_s C_{salt} + k_p C_p\right) C_p$$

- the law developed by Wang et al.

$$q^{1+n\beta} = k_{eq}\left(1 - \frac{q}{q_{max}}\right)^n C_p$$

Isotherm parameters found from chromatograms fitting are used, as a validation, to fit experimental isotherms

Conclusions

EXPERIMENTAL ISOTHERM DETERMINATION

- ❖ High-Throughput method needs small amount of product but requires high experimental effort
- ❖ Breakthrough tests are experimentally simple but require a large amount of sample
- ❖ Isotherm points obtained with two methods are on the same isotherm

PREDICTIVE APPROACH

Experiments for parameter determination → Simulations → Experiments to validate the model
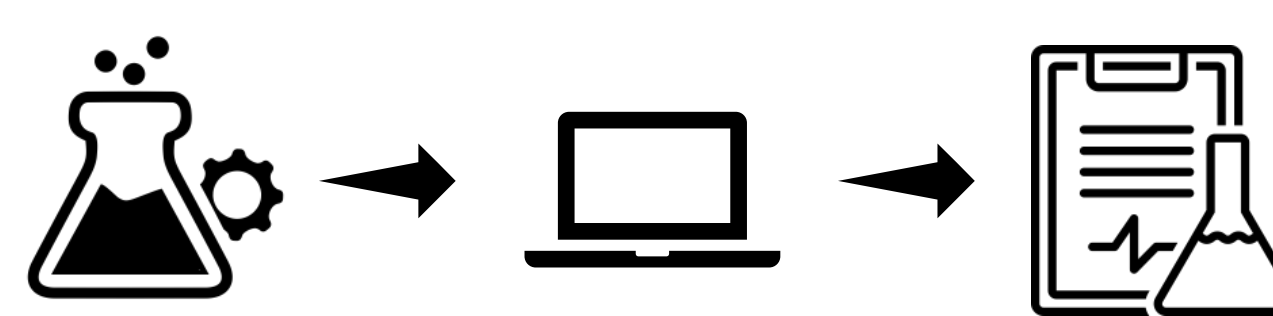
- ❖ Feasible for simple systems and pure proteins
- ❖ High experimental effort

ESTIMATION APPROACH

Experiments in different operative conditions → Curve fitting → Parameter estimation

- ❖ Good for complex systems
- ❖ Licence costs and advanced knowledge required

Schmidt-Traub H., 2005, "Preparative Chromatography of Fine Chemicals", *Henner Schmidt-Traub*. [ISBN: 3-527-30643-9].
Chen J., Sun Y., *Journal of Chromatography A*, 992 (2003) 29–40.
Wang G., Hann T., Hubbuch J., *Journal of Chromatography A*, 1465 (2016) 71-78.
Mollerup J. M., Hansen T. B., Kidal S., Staby A., *Journal of Chromatography A*, 1177 (2008) 200-206.