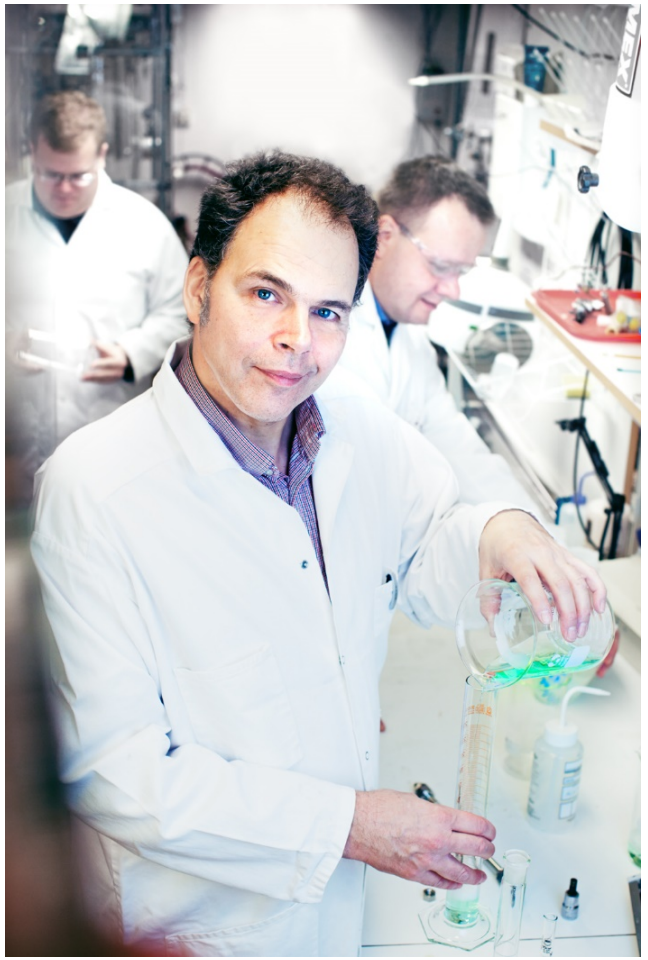
The Fundamental Separation Science Group

www.separationscience.se
www.FSSG.se/publications

Torgny Fornstedt

Professor in Analytical Chemistry



Department of Engineering and Chemical Sciences, Karlstad, Sweden

Who Are We?

- **Torgny Fornstedt**,
  Prof. Analytical Chemistry
- **Jörgen Samuelsson**,
  Assoc. Prof in Surface Biotechnology
- **Patrik Forssén**,
  Research Engineer in Scientific Computing
- **Martin Enmark**,
  PhD in Chemistry
- **Marek Lésko**,
  Postdoc in Chemical engineering
- **Emelie Glenne**,
  PhD-student
- **Joakim Bagge**,
  Researcher

Associates:

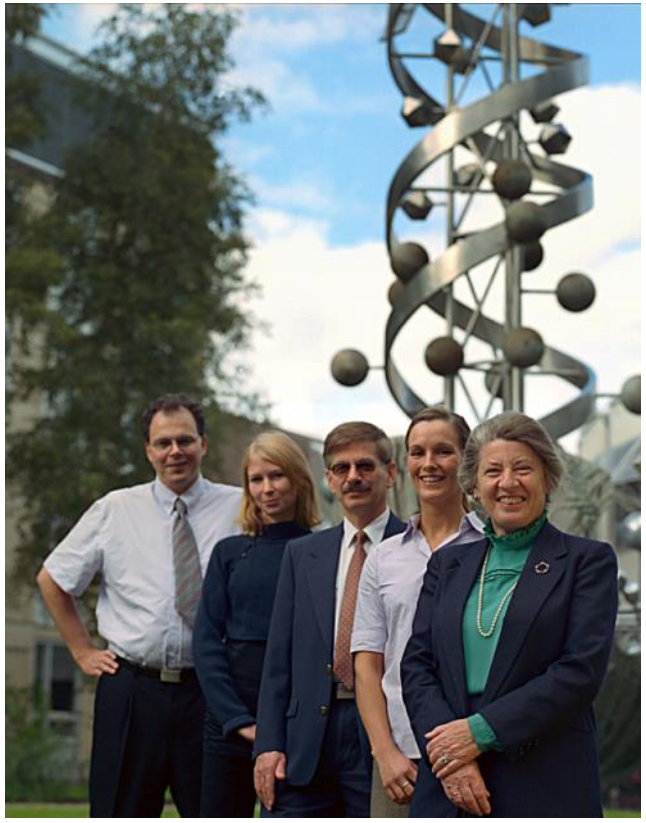
- **Karol Lacki**,
  soon-to-be Adjunct Professor
- **Maria Rova**,
  PhD in Biochemistry
- **Marek Szymański** (Örebro University),
  Postdoctoral fellow in Scientific Computing

We focus on combining experiments and theory, in order to understand how molecules interact with separation phases and biosensors chips etc.

Our Background at Uppsala University

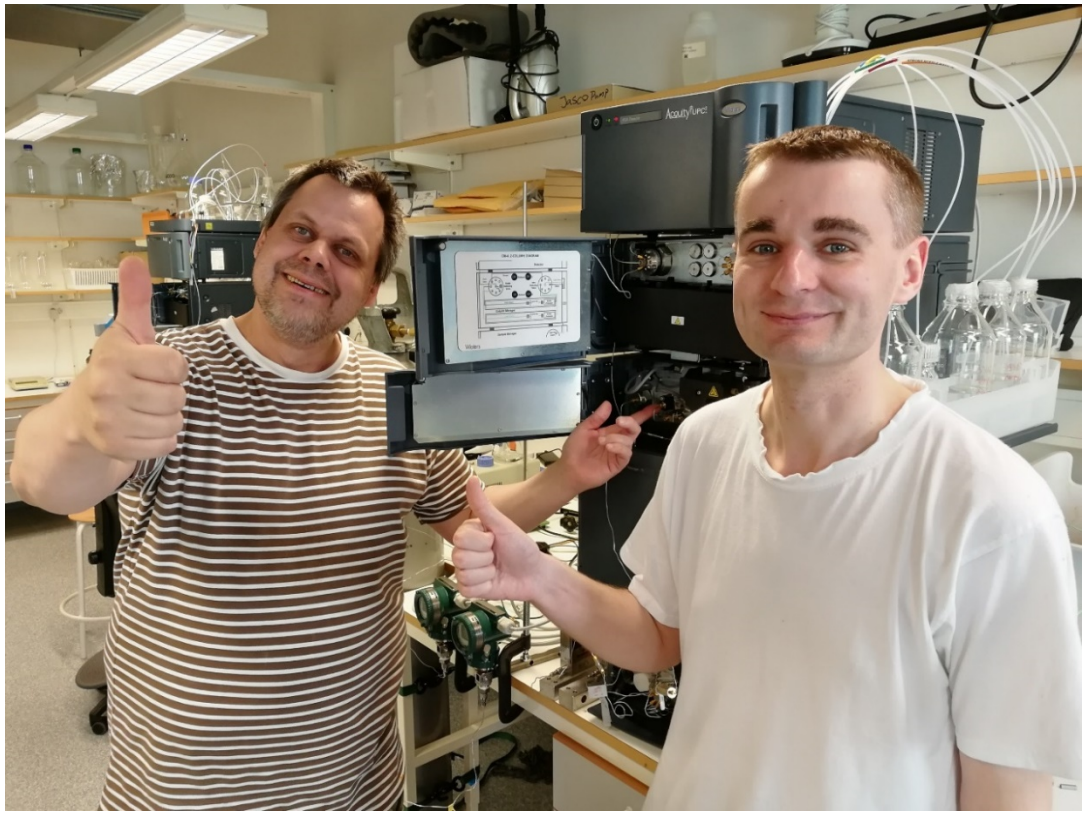
A Surface Biotechnology Center was founded in 2000 to continue the protein separation science at Uppsala University. The Center was sponsored by Amersham Biosciences and professor Karin Caldwell was the leader.

Karin Caldwell & Coworkers



Jörgen Samuelsson (Right) at the Downstream Proc. Unit

Our Site at Karlstad University



Jörgen Samuelsson (left) and Marek Leśko (right) in the background our SFC instrument Waters UPC2 and furthest away our Waters UHPLC instrument.

Emelie Glenne and "Wille" 6 months old.

Our site at BMC, Uppsala University

Dr. Martin Enmark, in the background our Agilent 1200 Instrument.

What Are We Doing ?

Combining Theory & Practice for Deeper understand how molecules interact with each other and with separation media/biosensor chips

- **Deeper Understanding**: Experimental data are processed with numerical tools identifying energy of interactions and number of sites without a priori model assumptions which leads to deeper understanding.
- **Process Optimization**: Models and algorithms are developed to predict optimal conditions for high throughput analytical/preparative methods.

Industrial Partners:

Astra-Zeneca Medical Chemistry, Astra-Zeneca Pharmaceutical Development, Waters Sverige AB, Attana AB, Agilent Sweden, Cambrex Karlskoga Corporation, Akzo Nobel Pulp and Performance Chemicals AB (today Noryon), Ridgeview AB, Attana AB

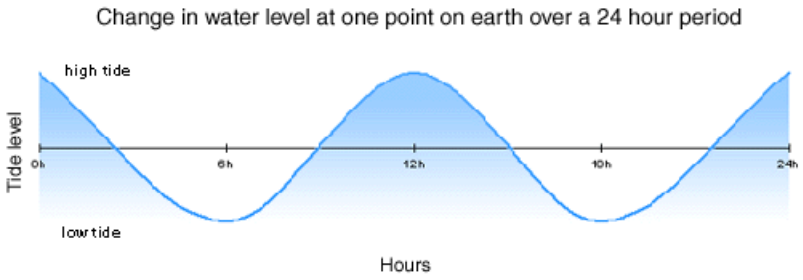
Academically Partners:

Prof. Marja-Liisa Riekkola & coworkers, Prof. Andrew Shalliker & coworkers, Prof. Krysztof Kaczmarski & coworker, Assc. Prof. Alberto Cavazzini, prof. Charlotta Turner & coworkers

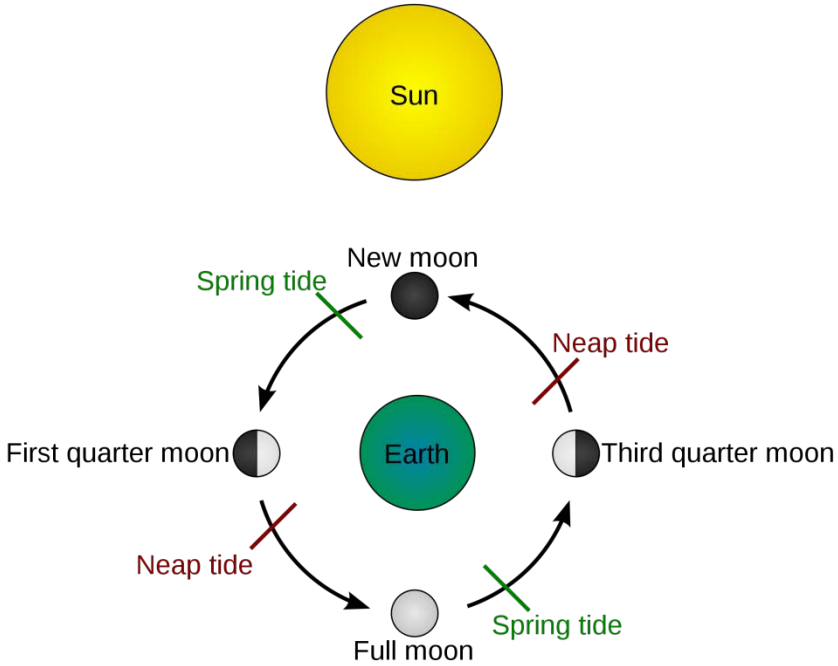
Empirical versus Mechanistic Model

Example – the Tide

Empirical model
based on observations



Mechanistic model
based on physical laws

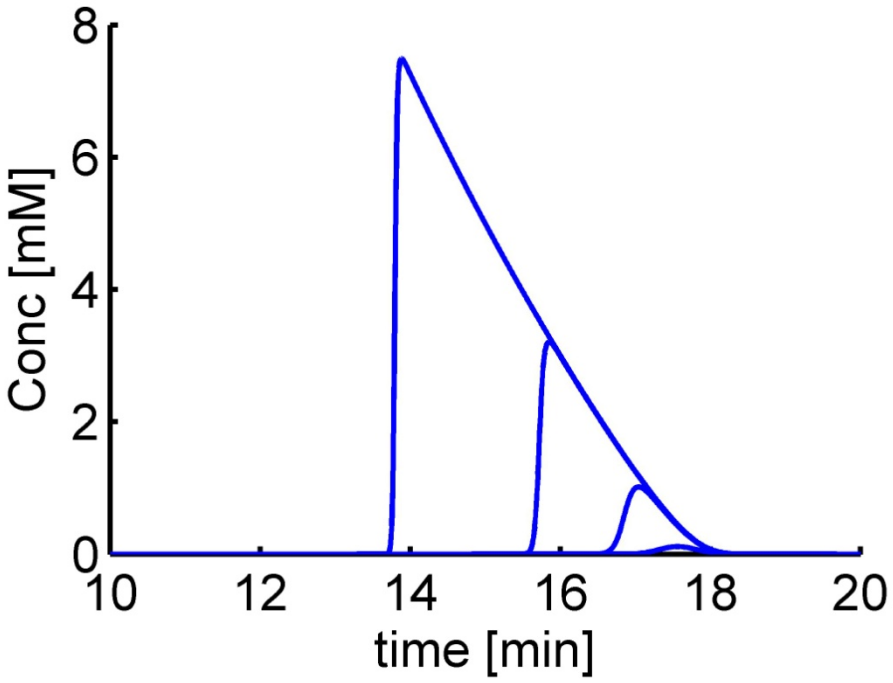


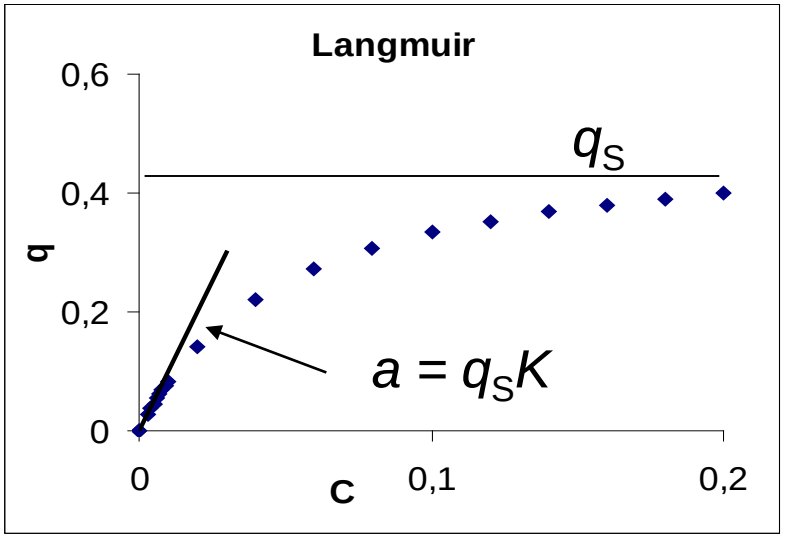
Our Current Research Projects

The Fundamental Separation Science Group at Karlstad University

1. Mechanistic modelling of Liquid Chromatography separations/Biosensor assays
2. Scaling up issues & transport Phenomena
3. Scientific approach to QbD/Quality Control
4. Peptide separations using Supercritical Fluid Chromatography
5. Therapeutic oligonucleotides – chromatographic Analysis & Purification

Preparative Peaks and Their Relation to Adsorption Isotherms





Large injected concentration:
Gaussian => Tailing peaks

Because: The column stationary phase has a limited surface, so a limited amount of analyte can be adsorbed.

$$q = \frac{Kq_sC}{1+KC}$$

q =	Adsorbed concentration
C =	Mobile phase concentration
K =	Equilibrium constant
q_s =	Monolayer capacity
a =	Initial slope

Chiral Separations with a Cellulase Protein

Stationary phase:

- Diol Silica - Cellobiohydrolase I’
- pI = 3.9 - Binding Site: 40Å Long Tunnel

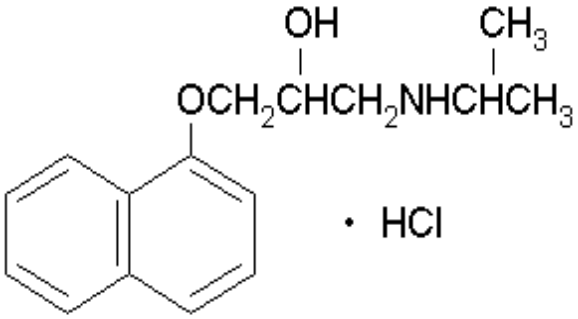
Mobile Phase:

- Acetate Buffers at pH 4.7 - 6.0

Sample Components:

- R- and S- β-Receptor Antagonists

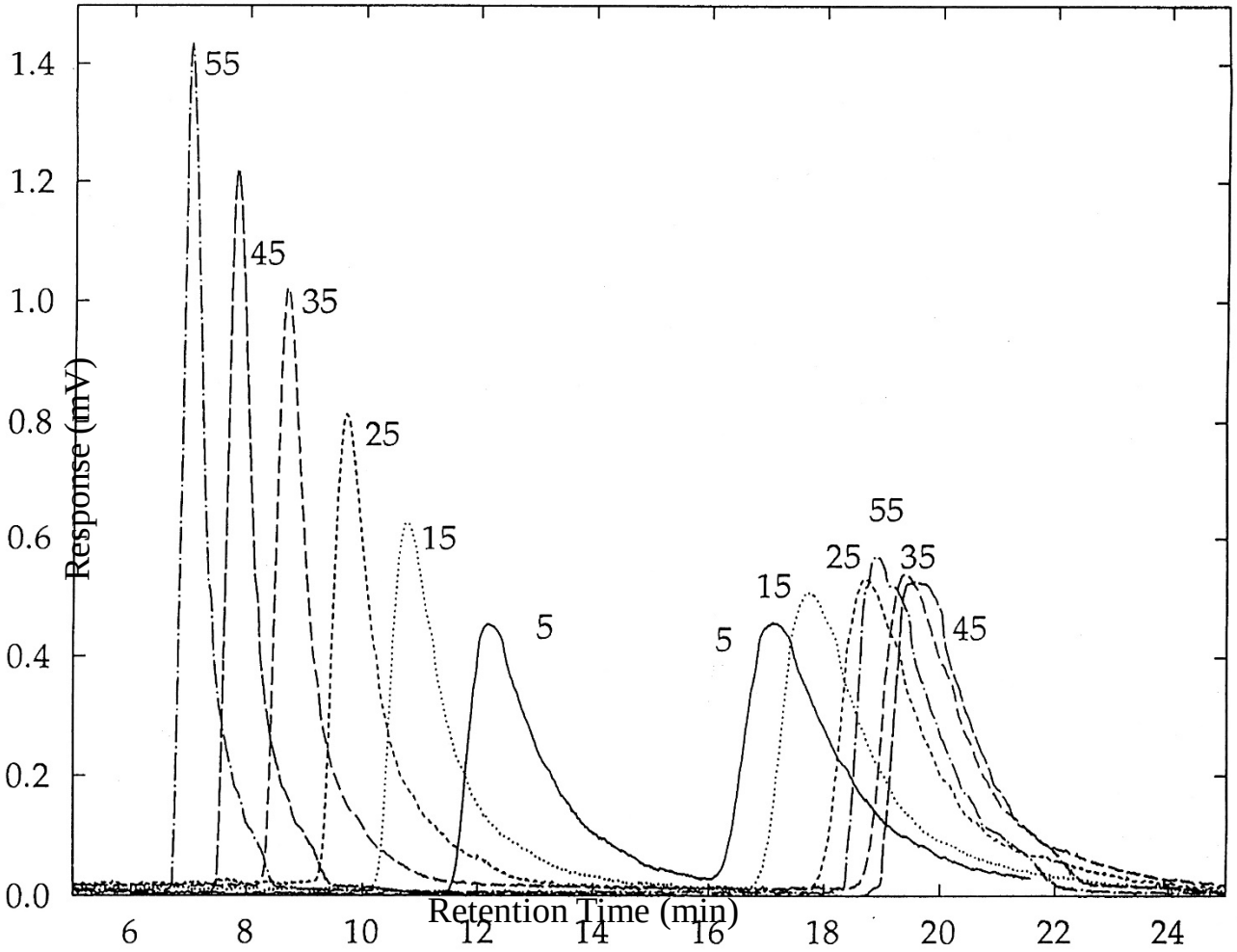
**New Name = Cel7A*



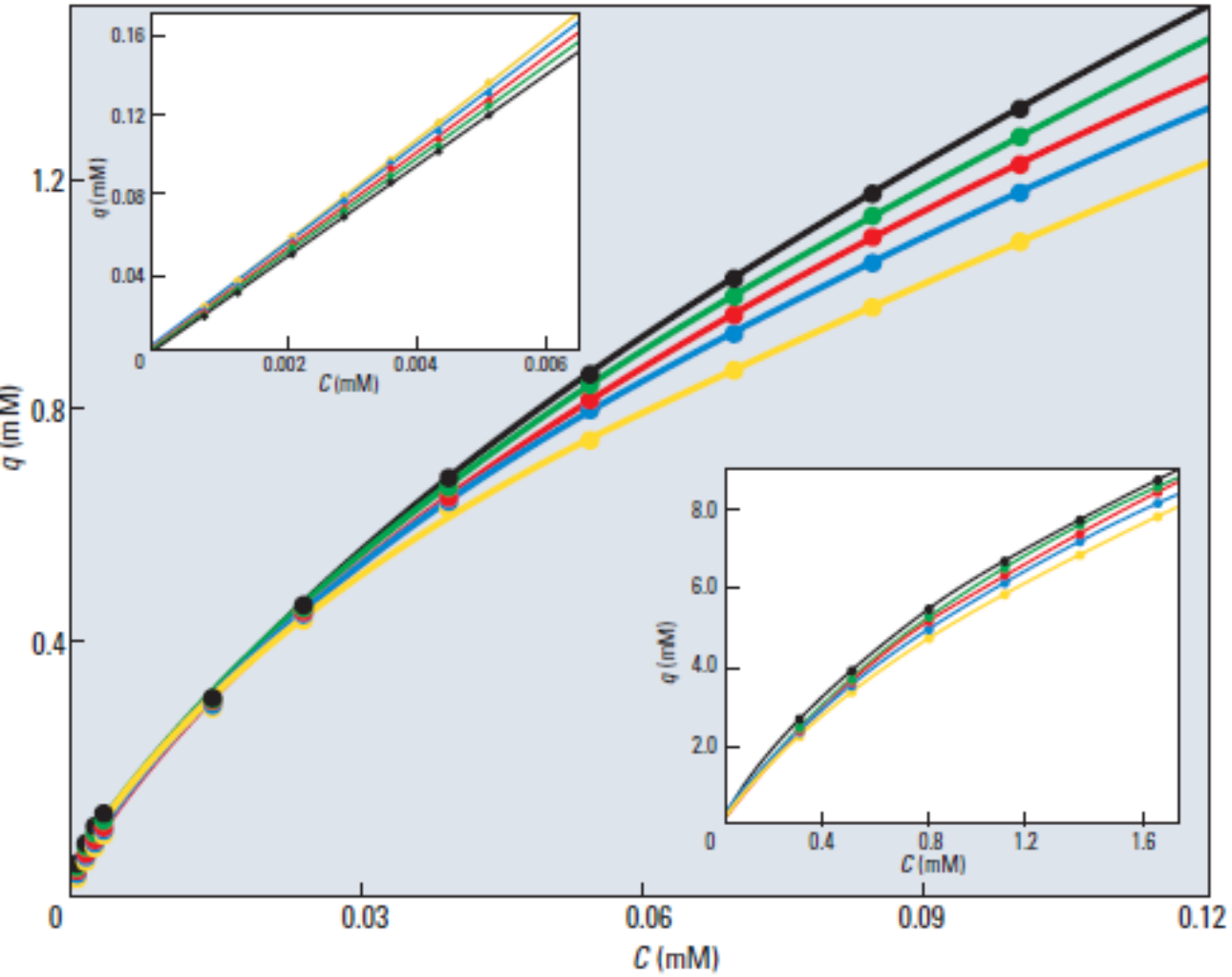
(R and S Propranolol)

Elution Profiles of R and S propranolol in different temperatures

S-Propranolol is most Retained Enantiomer; Eluent: Sodium Acetic Buffer at pH = 5.47



Adsorption isotherms of S propranolol *at different temperature*



Main Figure = medium concentration range

Inset upper-left corner = lowest range

Inset lower right corner: highest concentration range.

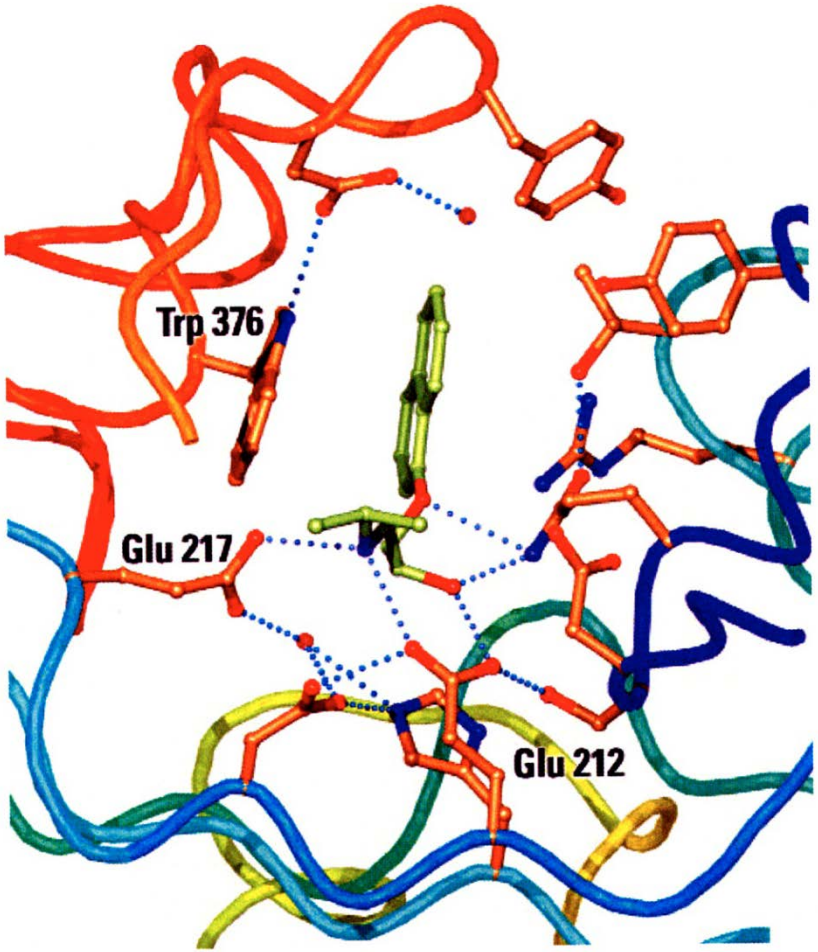
The data were calculated using the best bi-Langmuir isotherms. Stationary phase is immobilized Cel7A on silica; eluent is acetic acid buffered at pH 5.5.

Black = 278 K; Green, 288 K; Red, 298 K; Blue, 308 K; Yellow, 318 K.

T Fornstedt et al. In Journal of the American Chemical Society 119 (6), 1254-1264

R. Arnell et al in Analytical chemistry, Vol 78, pages 1682-168

Agreement with X-ray Crystallographic Studies



Proved Interactions[1]: Ion Binding between Positively Charged Amine of Propranolol and Residues Glu212 and Glu 217.

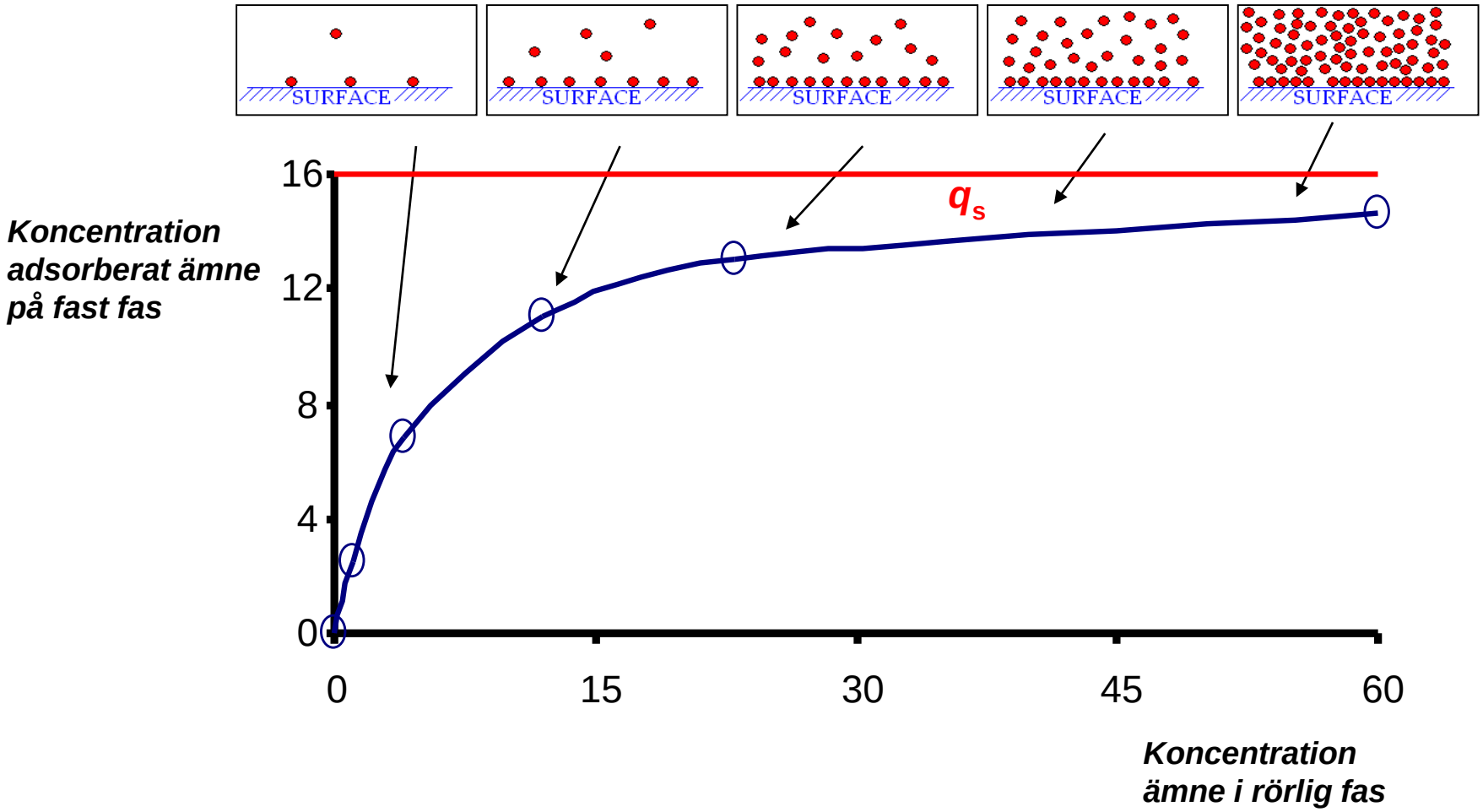
Probable Interaction: Hydrophobic Stacking with Trp 376

[1]Ståhlberg, J.; et al. J. Mol. Biol. 2001, 305, 79.

Picture used with permission From Jerry Ståhlberg

Adsorption isotherm at steady state

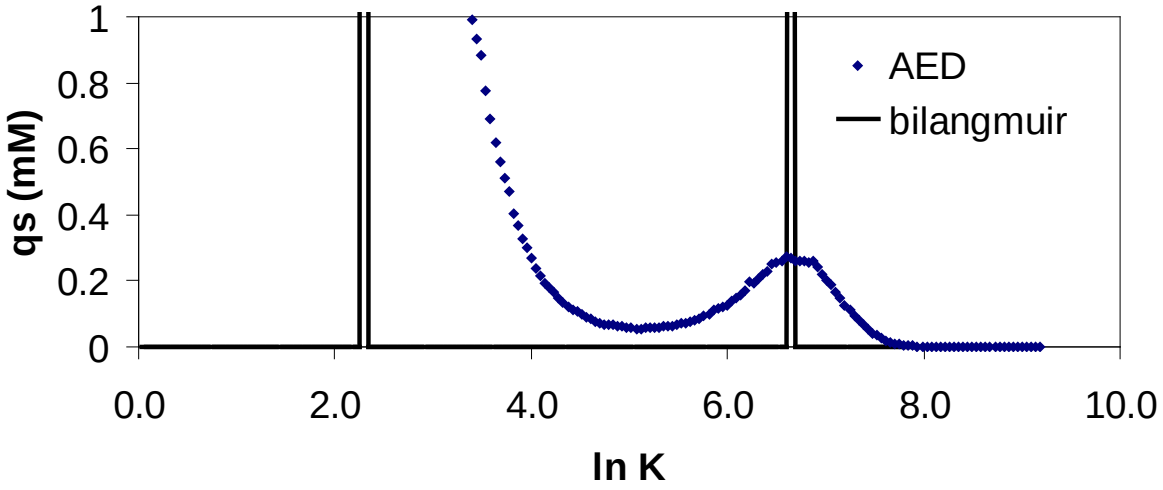
Visar sambandet mellan koncentrationen i rörliga fasen och adsorberad koncentration på den stillastående, fasta, fasens yta



Adsorption Energy Distributions (AEDs)

Synthetic raw data for bi-Langmuir adsorption isotherm

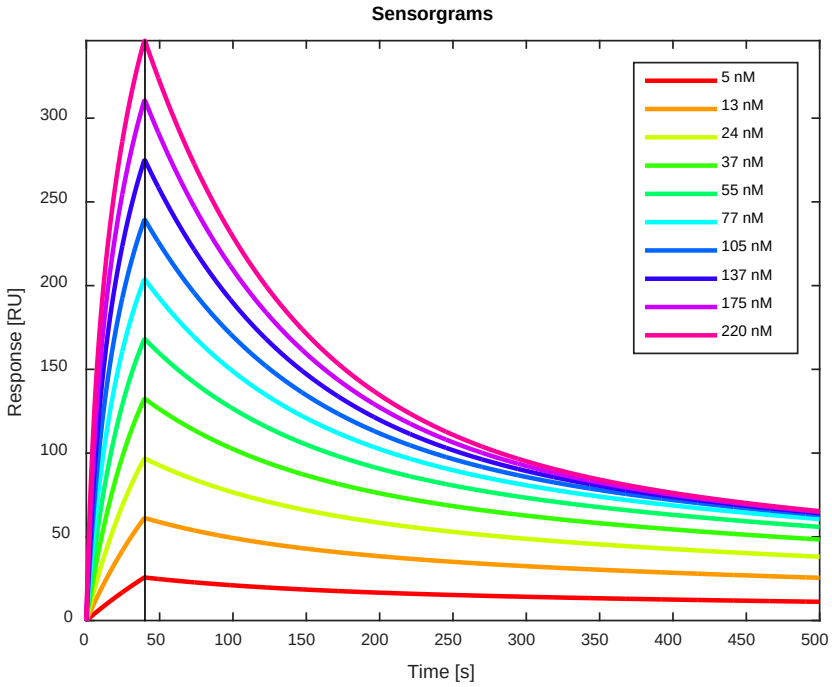
$q_{S,1}$ = 0.4 M , K_1 = 10 M^{-1}, $q_{S,2}$ = 0.0075 M , K_2 = 750 M^{-1}

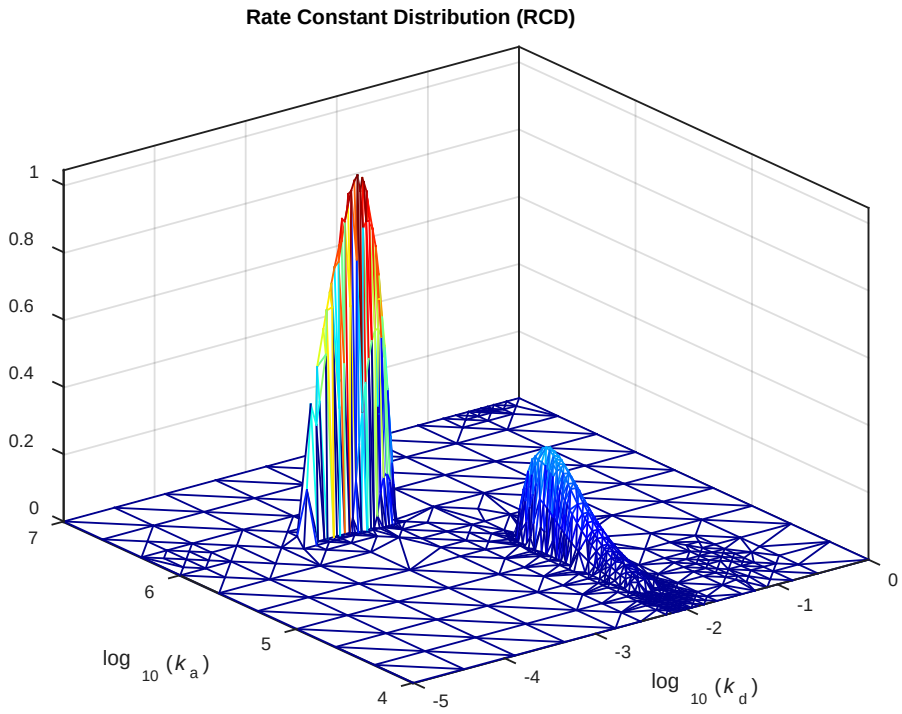


- Poor resolution at low-K due to low saturation of adsorption isotherm at high-C

- But K_2 predicted as 730 M^{-1} and $q_{s,2}$ as 0.0076 M.

Rate Constant Distribution - approach for non-steady state Biosensor data

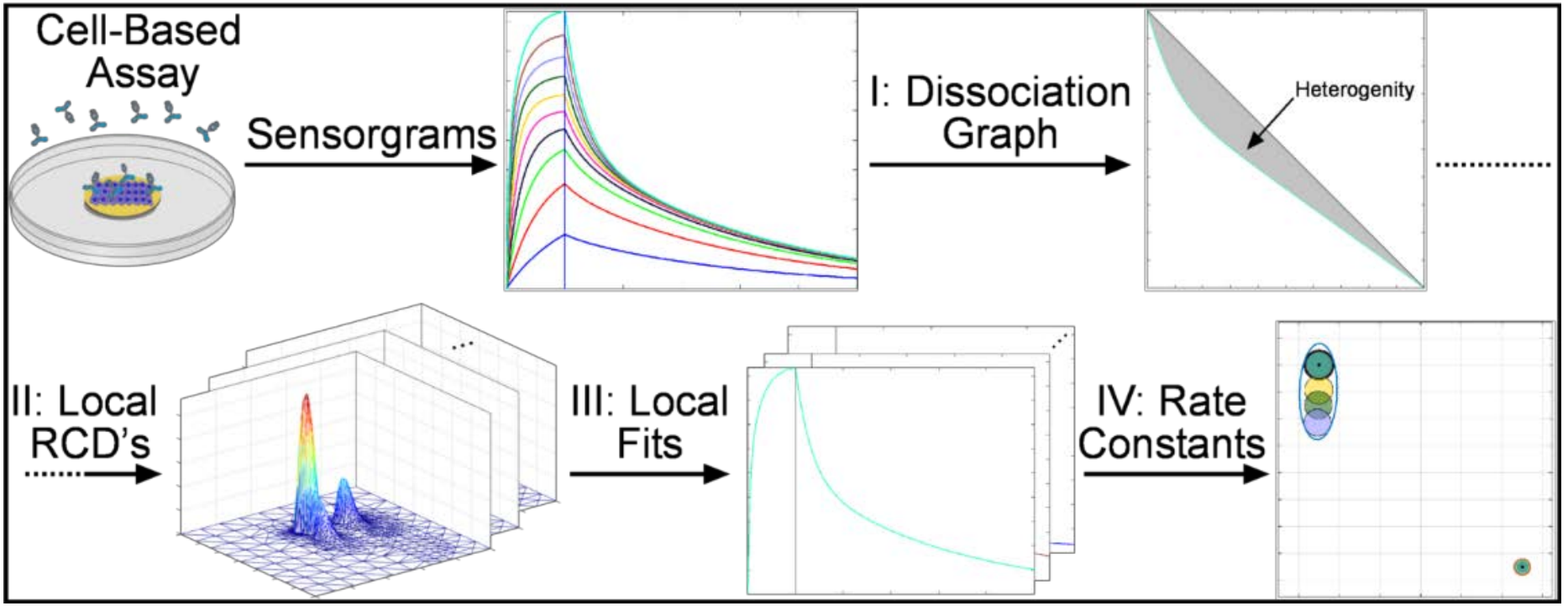




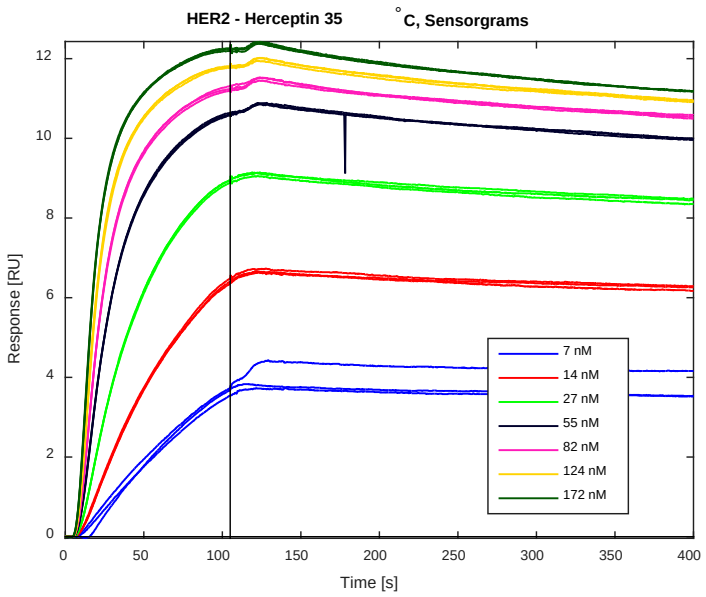
In the RCD the number of peaks indicate the number of different interactions in the system and the peak max position gives the median rate constants. Note that no a priori assumption is made here about the number of interactions!

Four-Step-Strategy- Summarized

I: Plot dissociation graph for top sensorgram concentrations
II: Calculate rate constant distributions for each sensorgram level
III: Estimate rate constants for each sensorgram level
IV: Cluster the rate constants for easer interpenetration



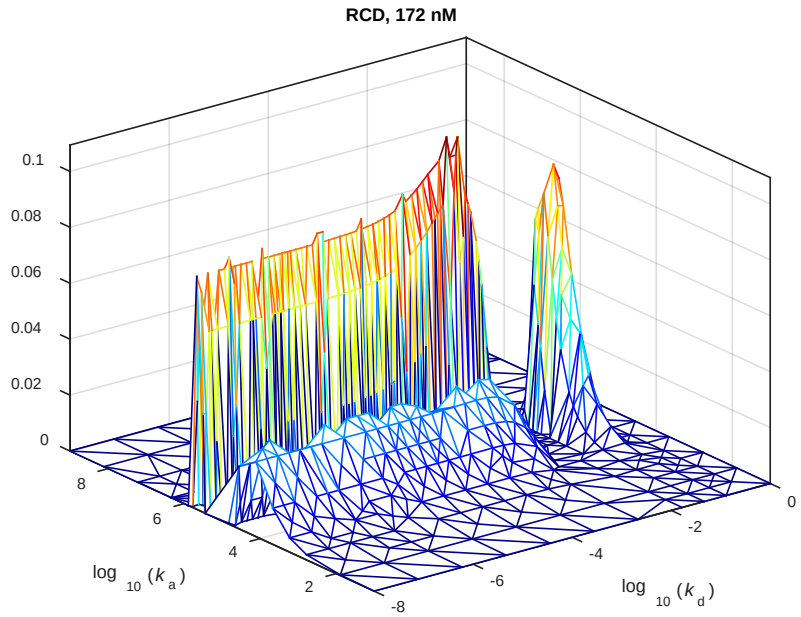
Example 3: Her2 – Herceptin Interactions

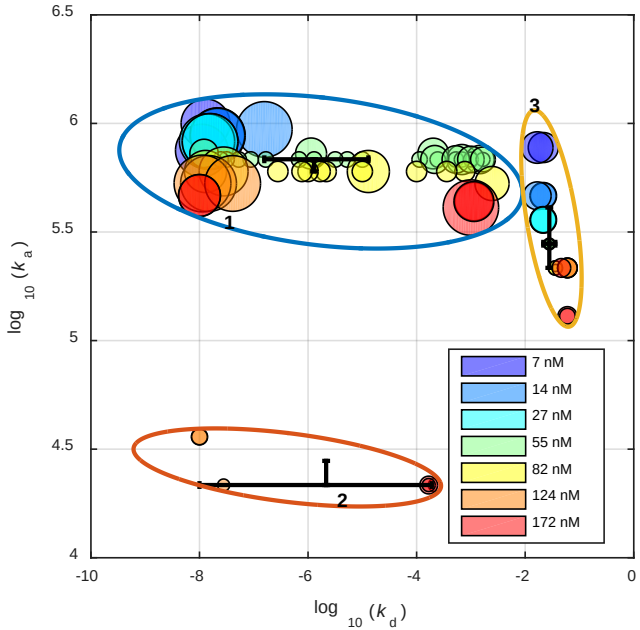


Ligand : Her2

Analyte : Herceptin

Notice the very slow disassociation!





Conclusion non steady state analysis

We have shown that the proposed new strategy can successfully handle very complicated Biosensor interactions and is a significant improvement compared to existing standard software to analyze Biosensor data.

In order to reliable estimates of rate constants one needs *both* high quality input sensorgram data and improved numerical data processing strategies.

In order to use the numerical tools for more advanced application, such as for diagnostic purposes/Quality Control, we plan to further develop and refine them.

The Quality by Design Project

General Aim:
To add firm separation theory to the analytical “Quality by Design” for easier and more convenient changes after approval of Drug.

Specific Aim:
To investigate method transfer from HPLC to UHPLC and the use of Quality by Design to aid the transfer in the pharmaceutical industry

Partners:

Prof. Krzysztof Kaczmarski (Poland)
Prof. Alberto Cavazzini (Italy)

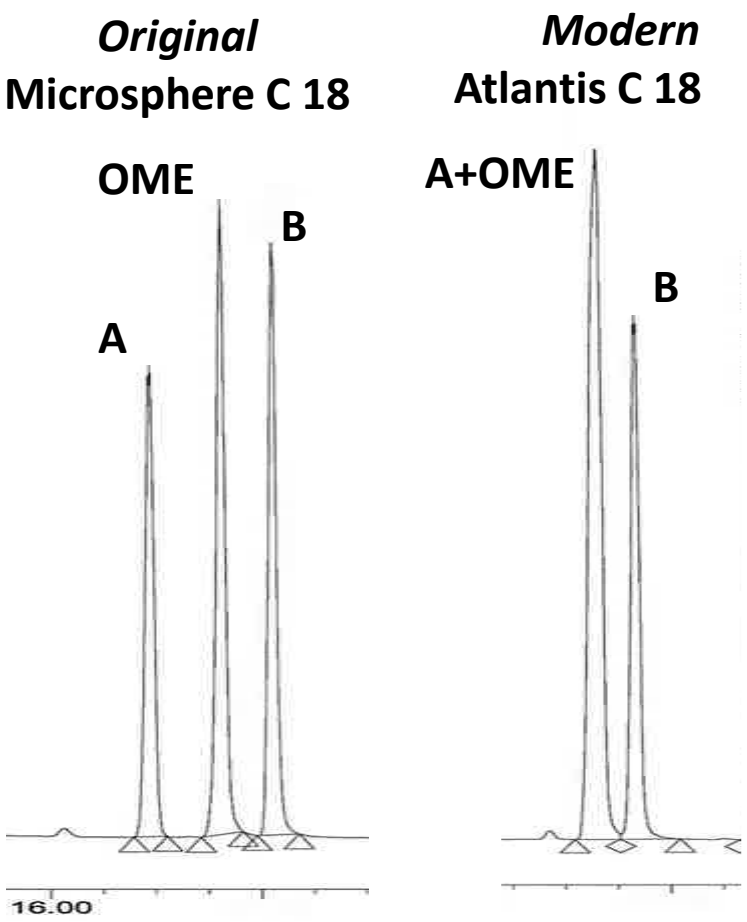
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

AstraZeneca

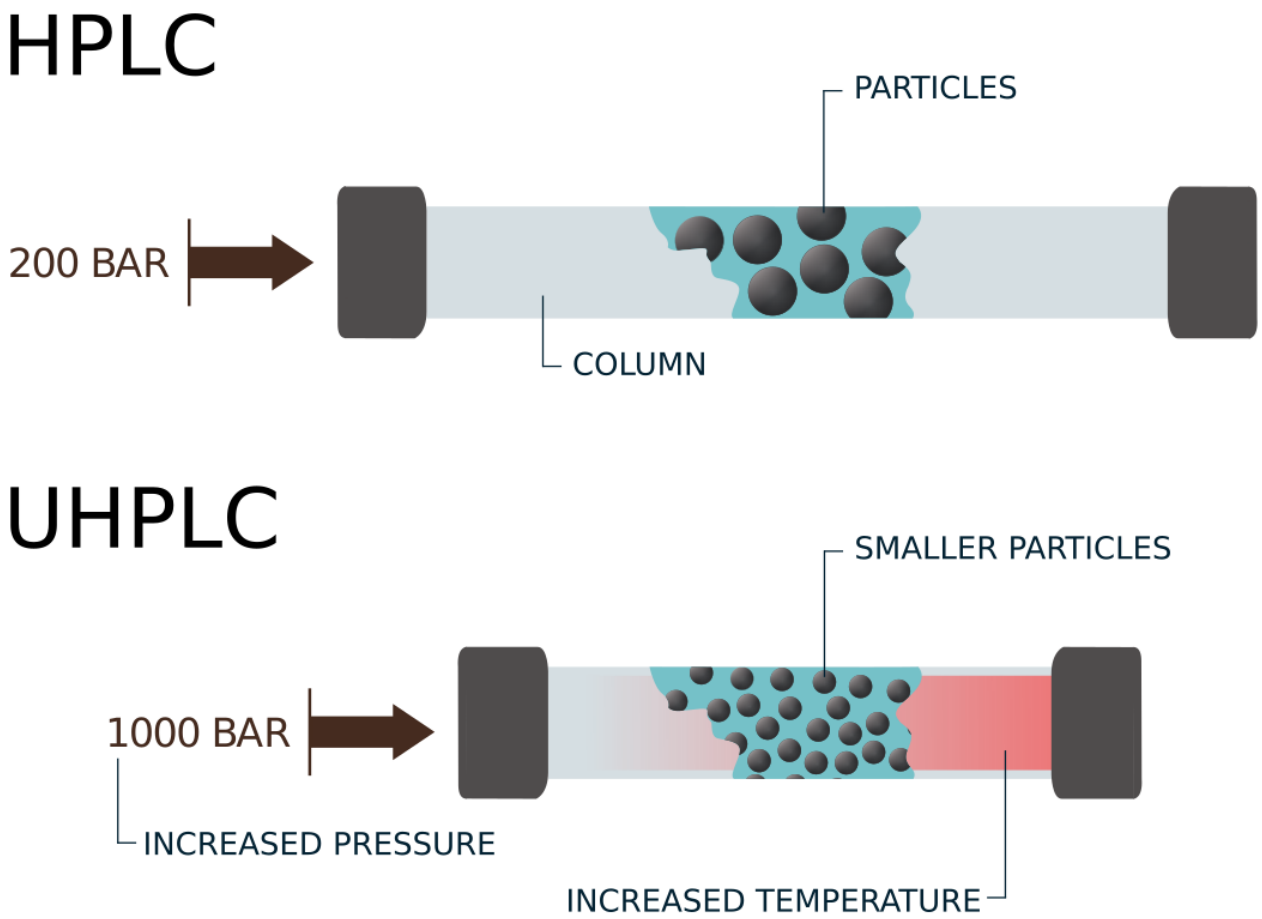
CAMBREX
Innovation. Experience. Performance.

A QC Method for Losec (*OME*) – Modern Columns didn't Work

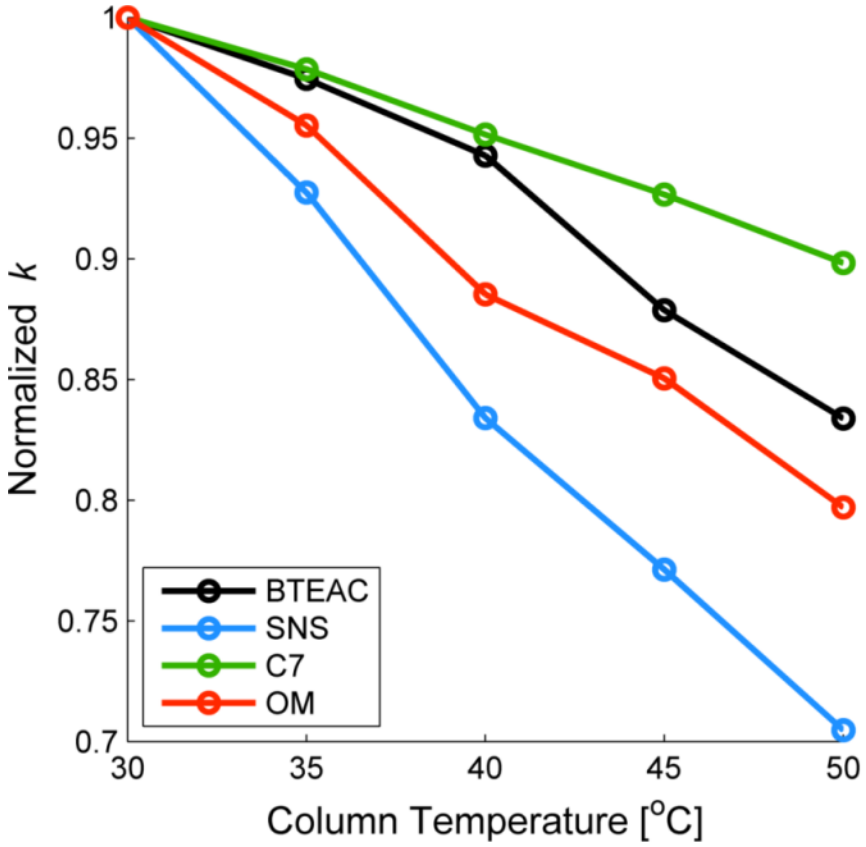
- Developed with one of the few pH-stable columns at the time
- Recently, manufacturing of the column stopped
- Changing to any of the new columns did not work

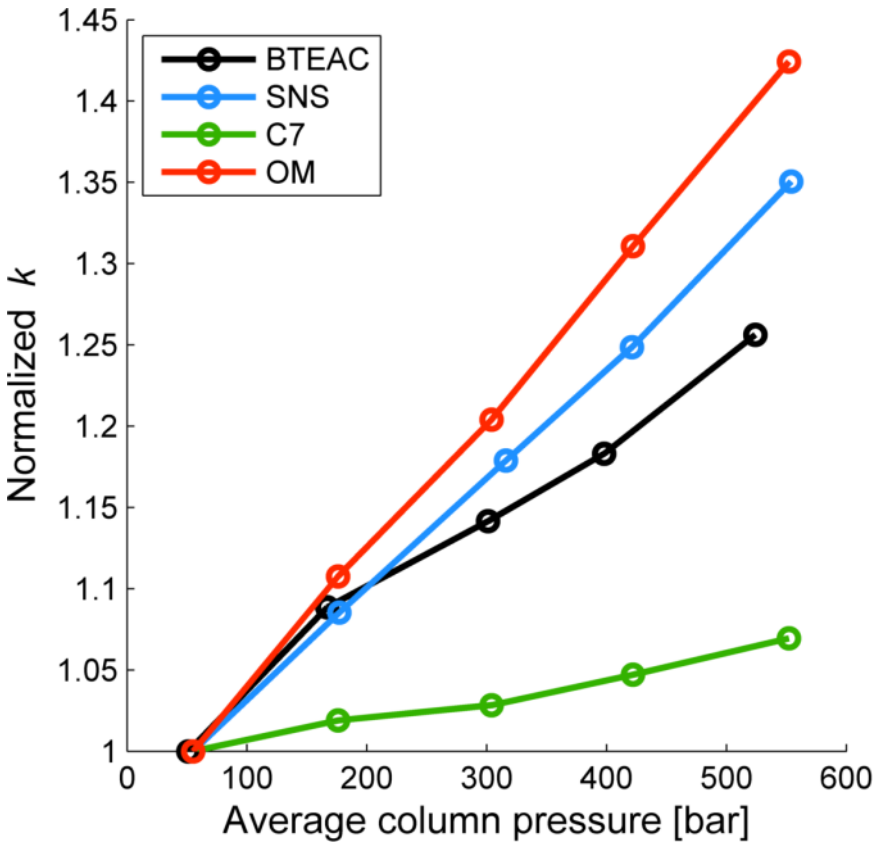


HPLC to UHPLC – Technical challenges

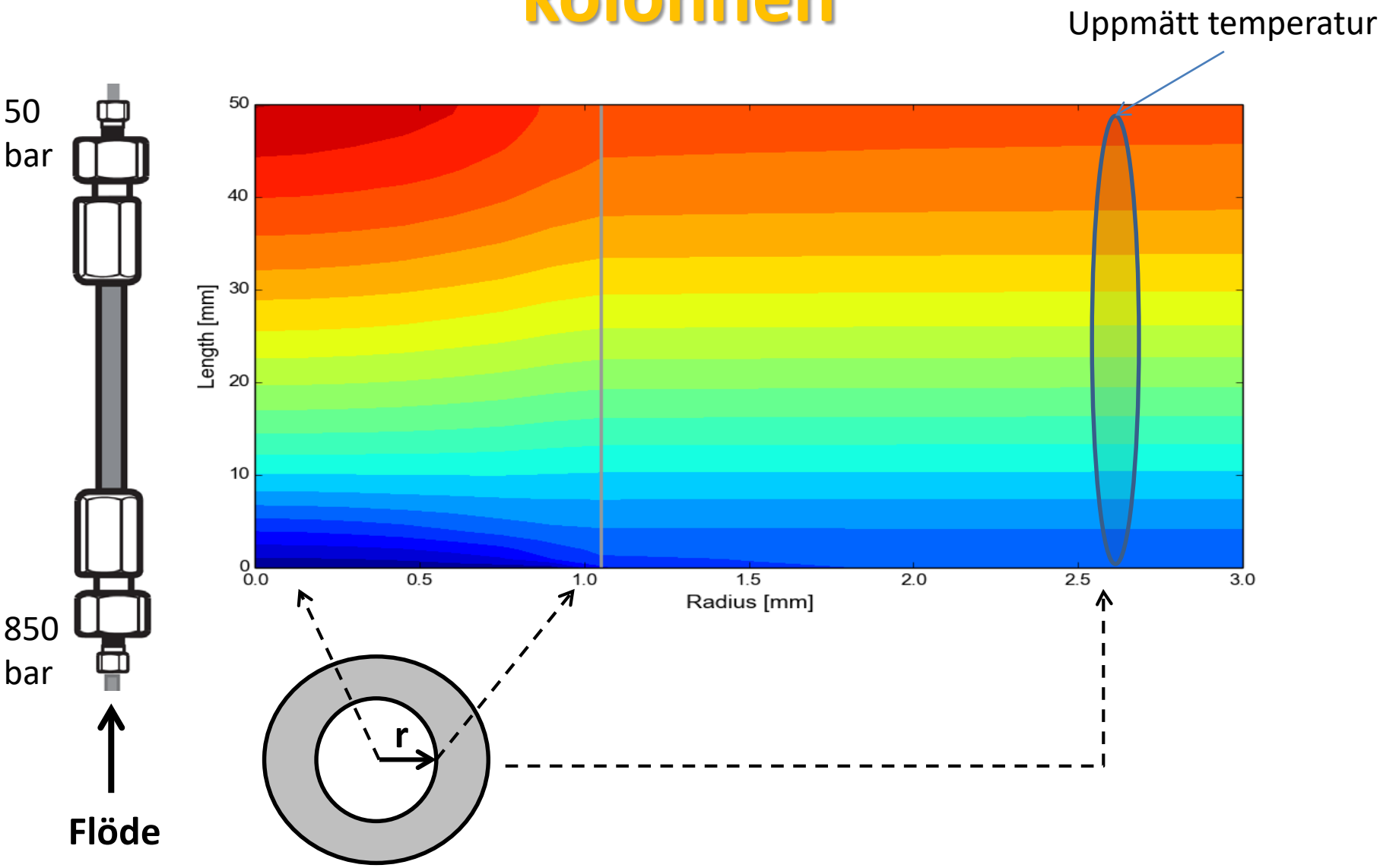


Impact of Temperature and Pressure on retention times





Radiell och axiell temperaturprofil i kolonnen



Acknowledgements

BIO-QC: Quality Control and Purification for New Biological Drugs with three academic institutions and four companies participating, among others AstraZeneca R&D Gothenburg, Sweden and Nouryon/Kromasil