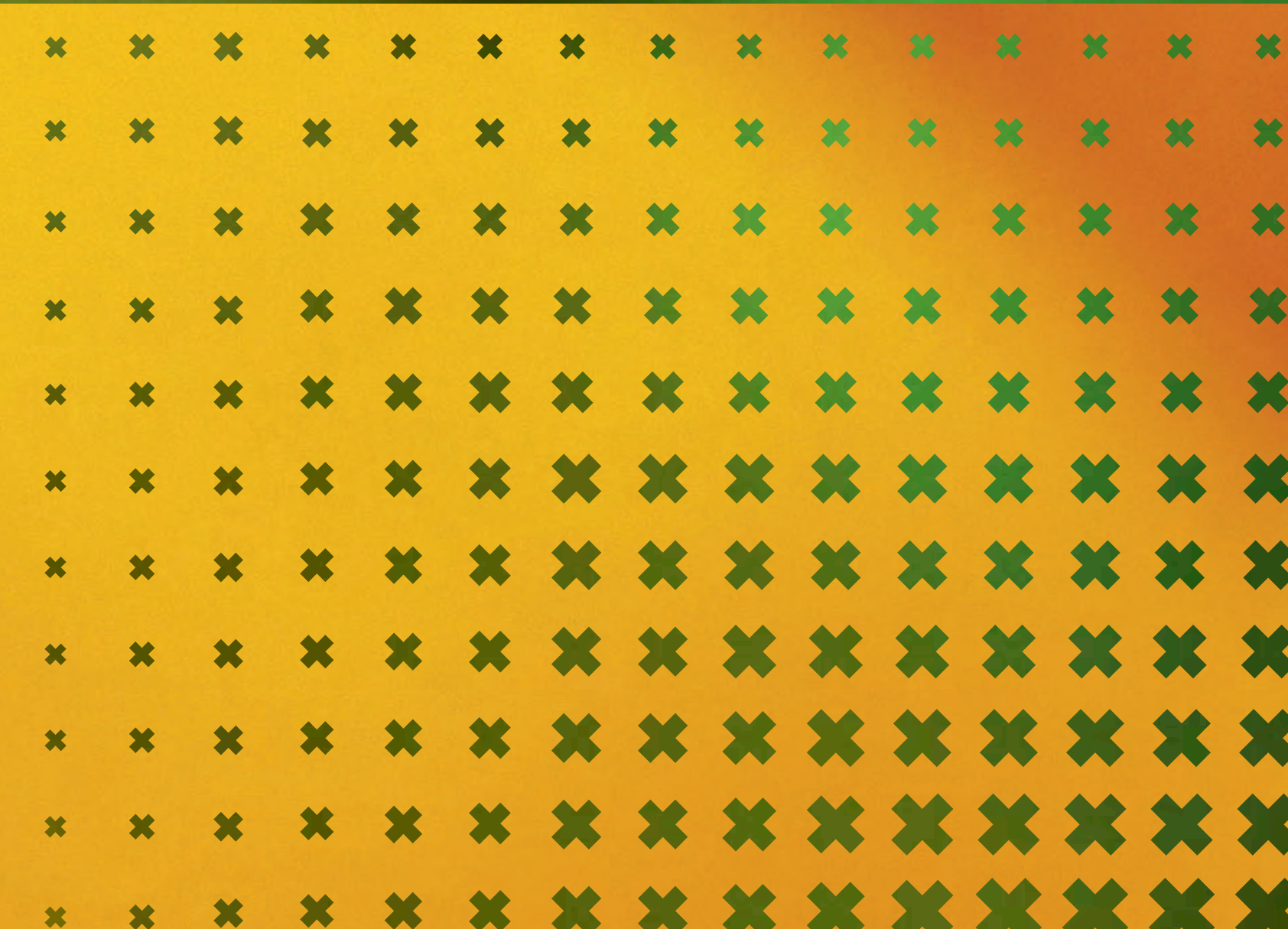




Valorisation at HIMS

*Chemistry
research
that matters*

Van 't Hoff Institute
for Molecular Sciences





Analytical Chemistry is forensic science at the molecular level. Analytical Scientists are involved in establishing which types of molecules are present, how many of them there are and, increasingly, what they are – or have been – doing. Thus, we are naturally involved with forensics, but also with chemistry, materials, art, food and medicine. In all these areas the analytical-chemistry group within HIMS collaborate with leading companies and institutions.

The Amsterdam universities are recognized as a unique national centre for Analytical Sciences in The Netherlands. We develop, improve and optimize analytical (separation) methods and technologies. We develop advanced software ('chemometrics') to turn large amounts of data into useful information. We work together with world-leading high-tech-instrument companies to make our findings accessible to other scientists.

One- and two-dimensional separation techniques

Gas and liquid chromatography

Mass spectrometry

Electro-migration techniques

Field-flow fractionation

Data analysis and chemometrics

Biomolecular systems Transition



Towards HYPERformance liquid chromatography

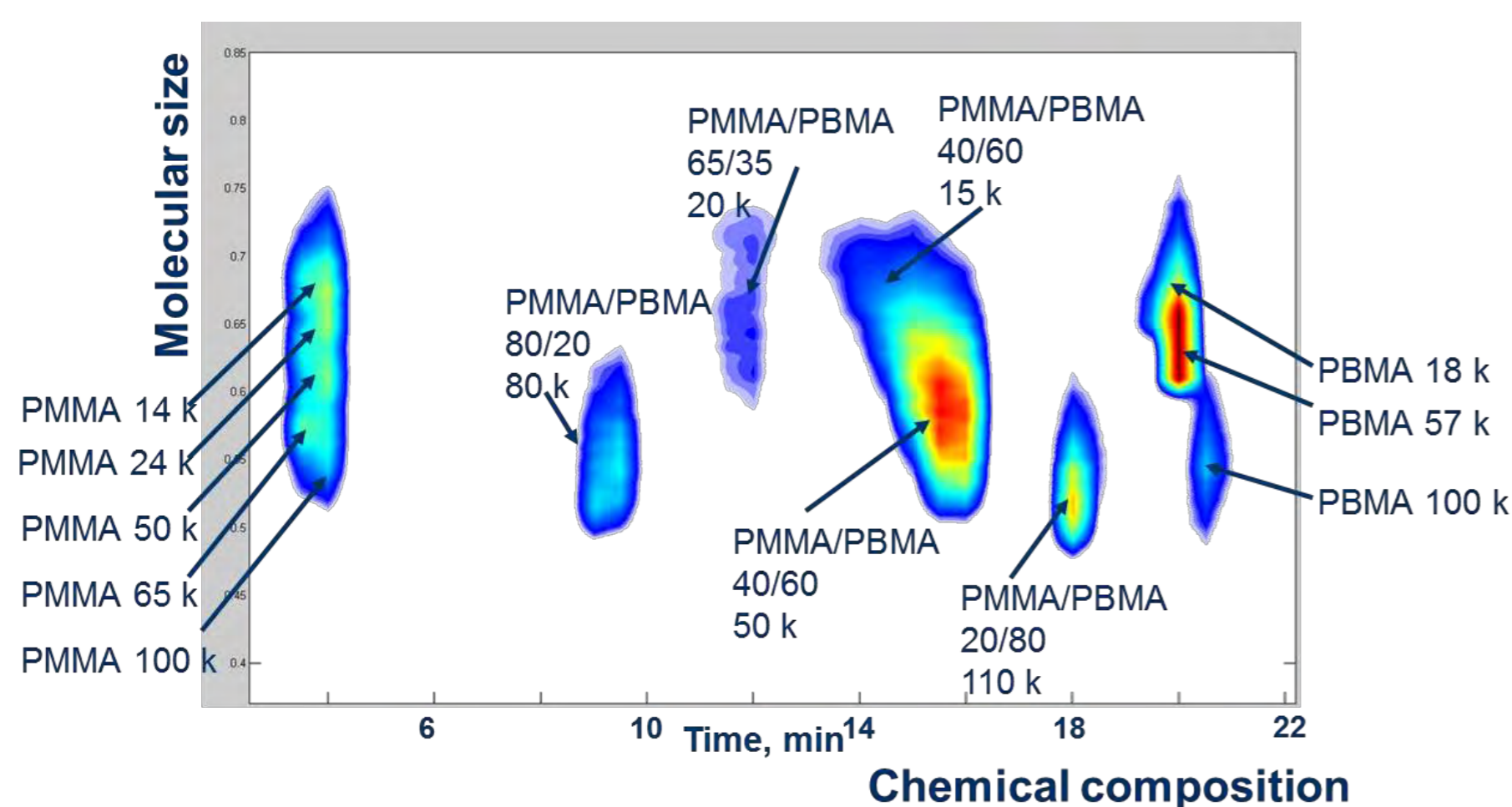
During the last decade liquid chromatography has developed from a high-performance or high-pressure level (commonly known as HPLC) to what is known as ultra-high-performance liquid chromatography (UHPLC). While UHPLC technology (smaller particles, smaller system volumes, higher pressures) definitely entails progress, it does not constitute a great leap forward. In contrast, progressing from conventional one-dimensional liquid chromatography to comprehensive two-dimensional liquid chromatography (LCxLC) and eventually “spatial” chromatography allows the separation of many more components in a much shorter time. Several projects in our group are aimed at taking LC to a next level, identified as HYPERformance liquid chromatography and conveniently abbreviated as HPLC.

Our objectives are to

- ✘ Increase the peak capacity (the number of peaks that can be separated) by at least an order of magnitude
- ✘ Increase the peak production rate (peak capacity per unit time) by at least an order of magnitude
- ✘ Enhance the linear range of detection by at least an order of magnitude

Ultra-performance LCxLC

Elena Uliyanchenko has decreased the required analysis time for LCxLC separation of copolymers according to composition (horizontal direction) and size (vertical direction) down from a typical 4 hours to some 20 minutes, making application of the technique in industry much more attractive.

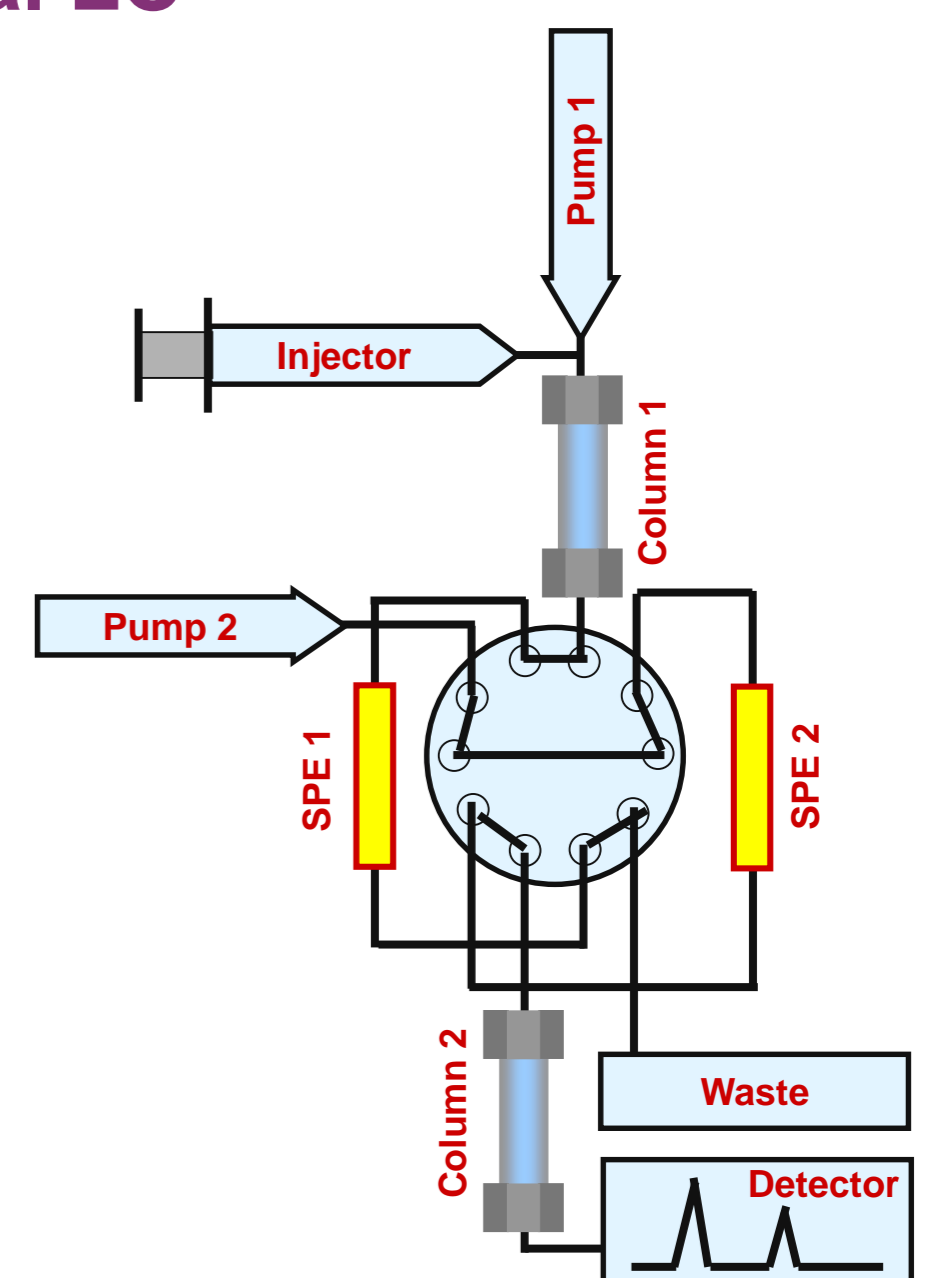
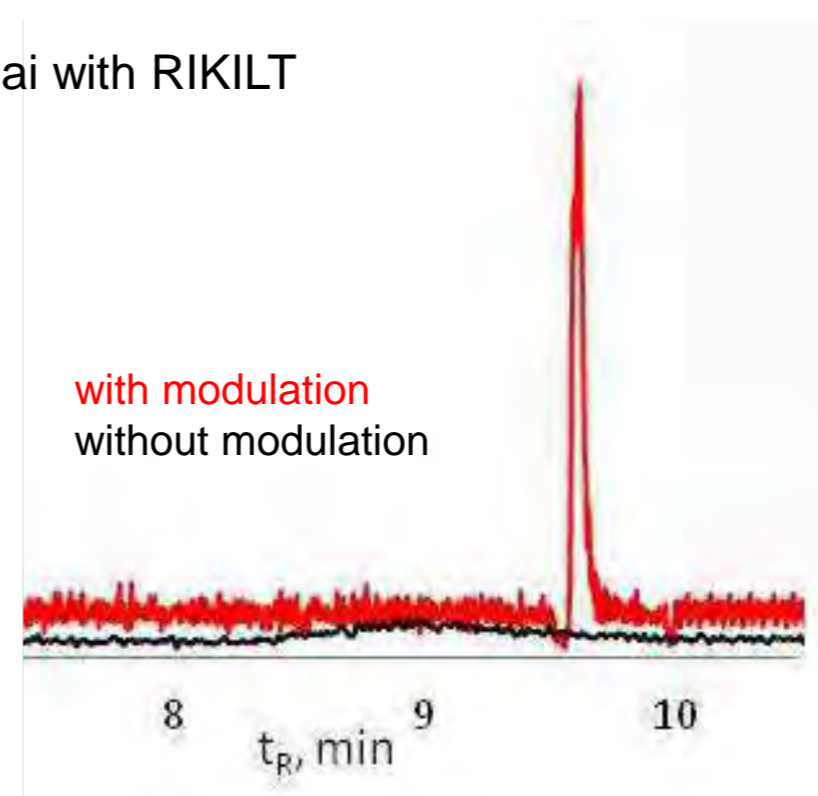


E. Uliyanchenko, P.J.C.H. Cools, Sj. van der Wal and P.J. Schoenmakers, *Anal.Chem.* **84** (18) (2012) 7802-7809

One-and-a-half-dimensional LC

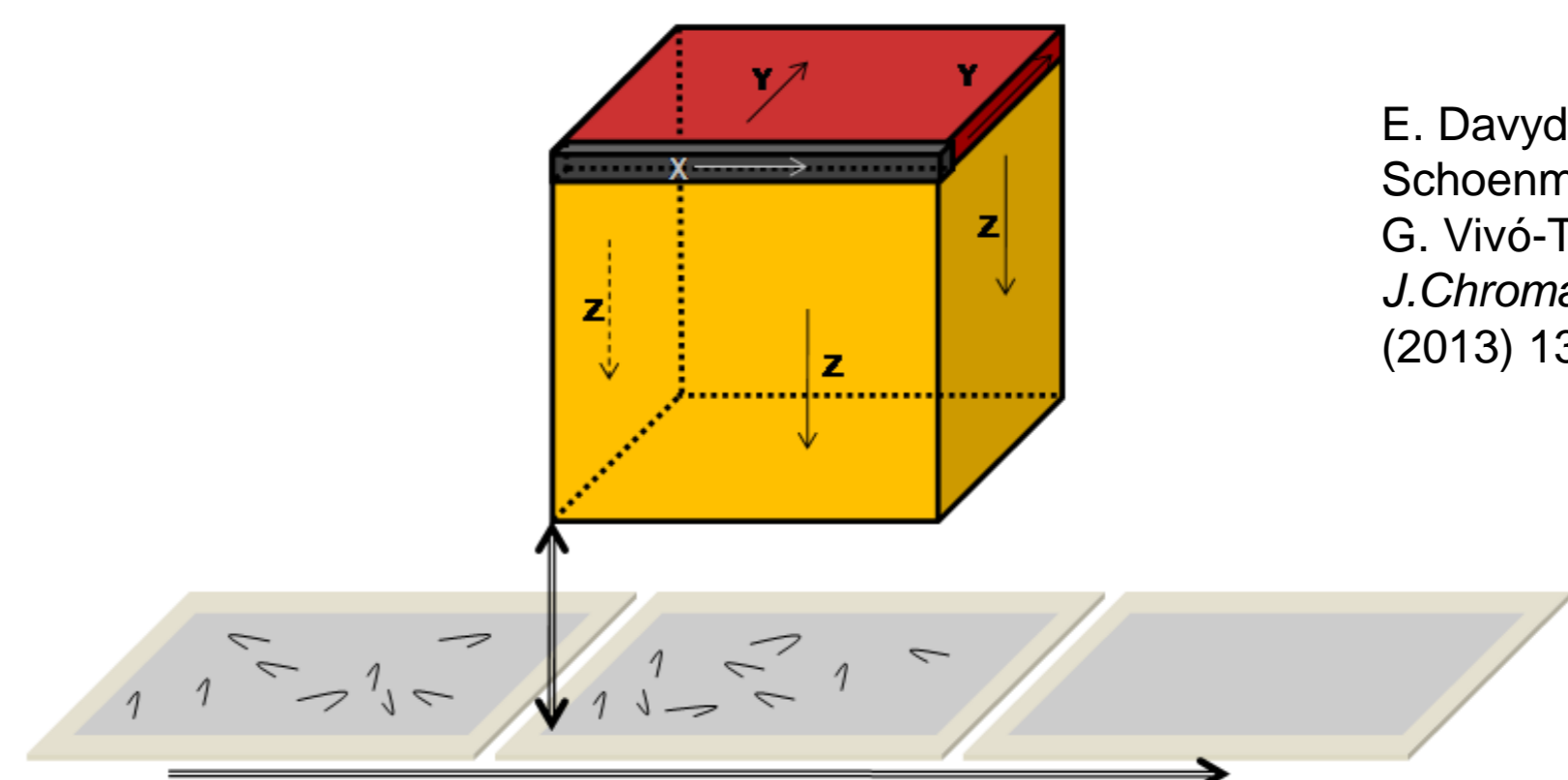
LCxLC modulator technology can be used to decrease the detection limits in LC-MS, as is illustrated in this example for the determination of testosterone in cow urine.

Anna Baglai with RIKILT

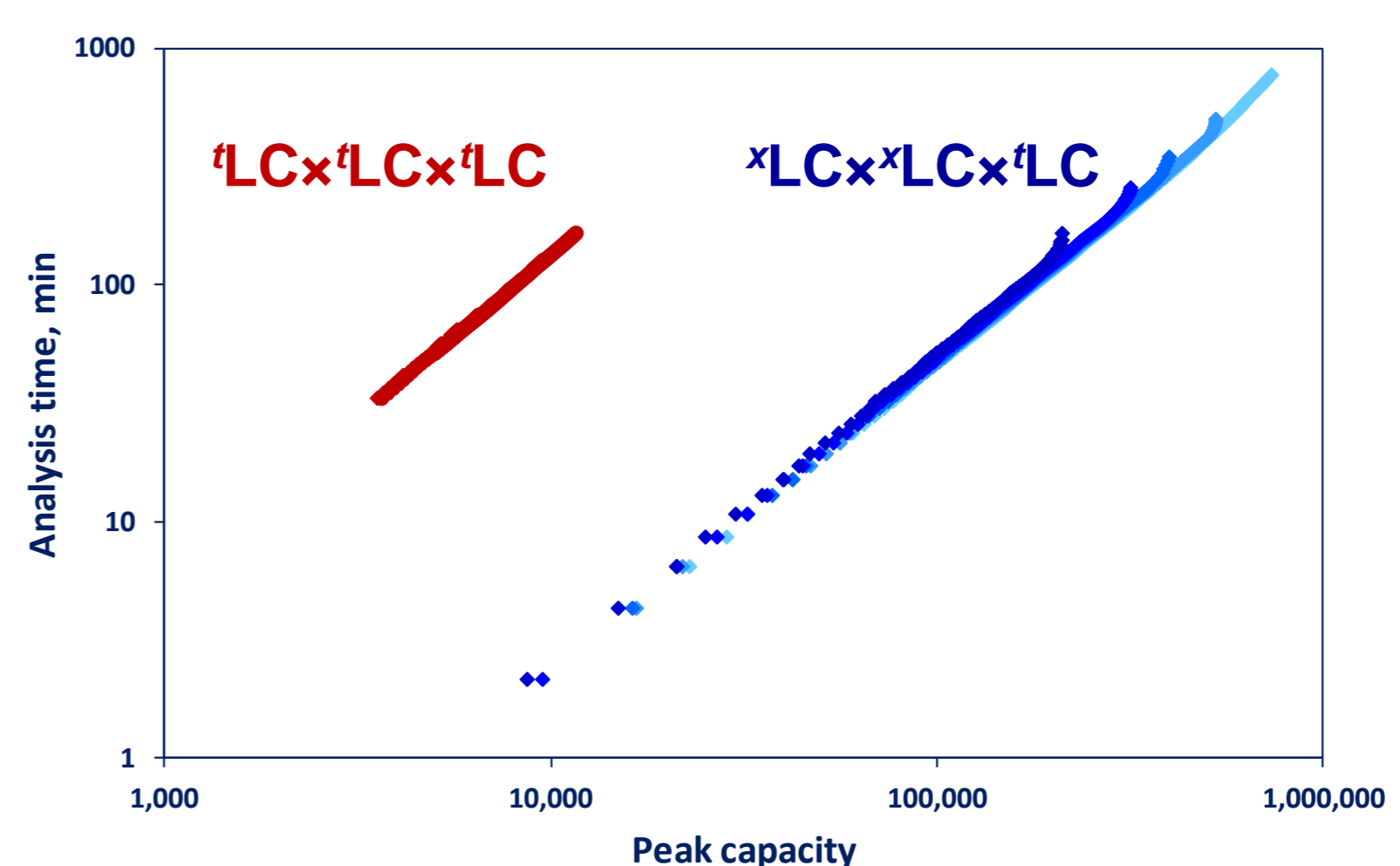


Three-dimensional “spatial” LC

The concept of spatial LCxLCxLC promises peak capacities up to one million within a reasonable time.



E. Davydova, P.J. Schoenmakers and G. Vivó-Truyols, *J.Chromatogr.A* 1271 (2013) 137-143.



With Sebastiaan Eeltink (VU Brussel)
Patent application numbers 20120171086, 20120164744, 20120162637

Valorization goals

- To develop and implement new or greatly improved separation methods
- To greatly enhance the applicability of advanced separation methods (such as LCxLC) in industry
- To solve very complex separation problems



Analytical Chemistry

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- E. Uliyanchenko, Sj. van der Wal and P.J. Schoenmakers, *Polym. Chem.* **3** (2012) 2313-2335.
- P.J. Schoenmakers and P.J. Aarnoutse, *Anal.Chem.* **86** (2014) 6172-6179.



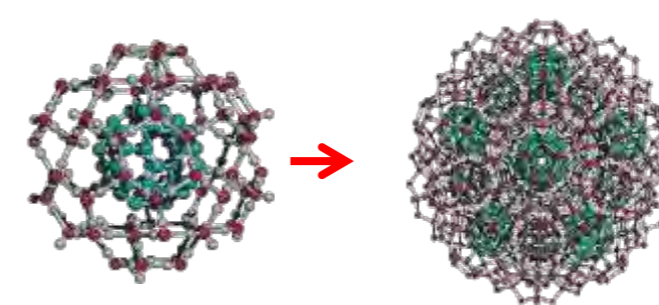
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Separation methods for macromolecules and particles

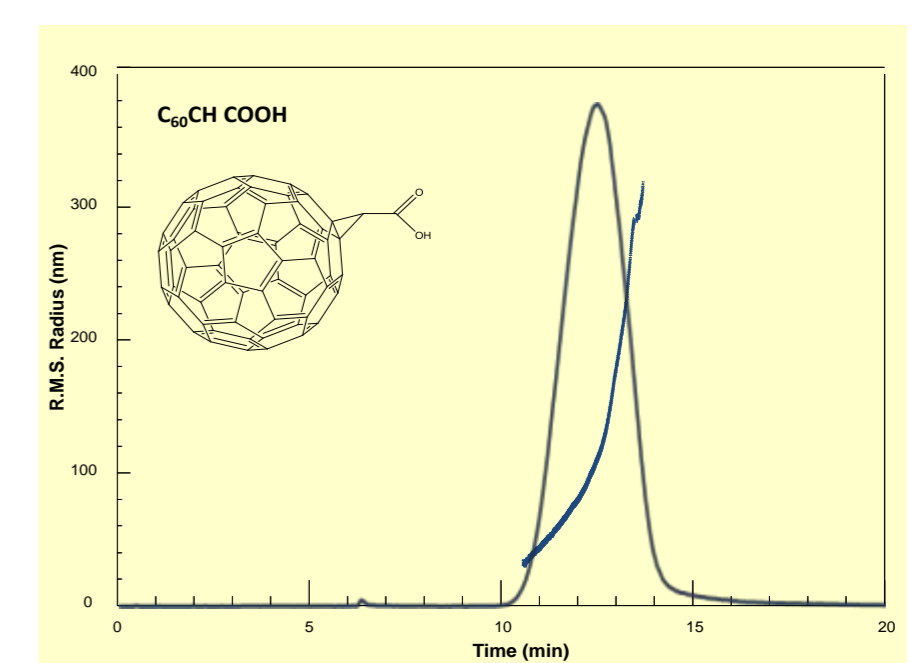
The conventional separation method for polymers/macromolecules is Size-Exclusion Chromatography (SEC). In SEC, the separation is according to molecular size. However, several other techniques have been developed in recent years that extend the size range of SEC, or that give other selectivities. Techniques studied within the Analytical Chemistry group of HIMS are:

- **Capillary Electrophoresis (CE).** This technique is suitable for water-soluble synthetic or natural macromolecules. CE can be used to study size distributions, functionality (end groups) or, e.g., the degree of substitution of modified polymers.
- **Asymmetrical Flow Field-Flow Fractionation (AF4).** Like SEC, AF4 separates according to size. However, its size range is much larger. AF4 can be applied to study supramolecular complexes, the aggregation of proteins, and to measure the size-distribution of particles.
- **Hydrodynamic Chromatography (HDC).** This technique can be especially suited for the analysis of solid particles, vesicles and liposomes. It has inherently a very high separation efficiency.

Aggregation of fullerenes

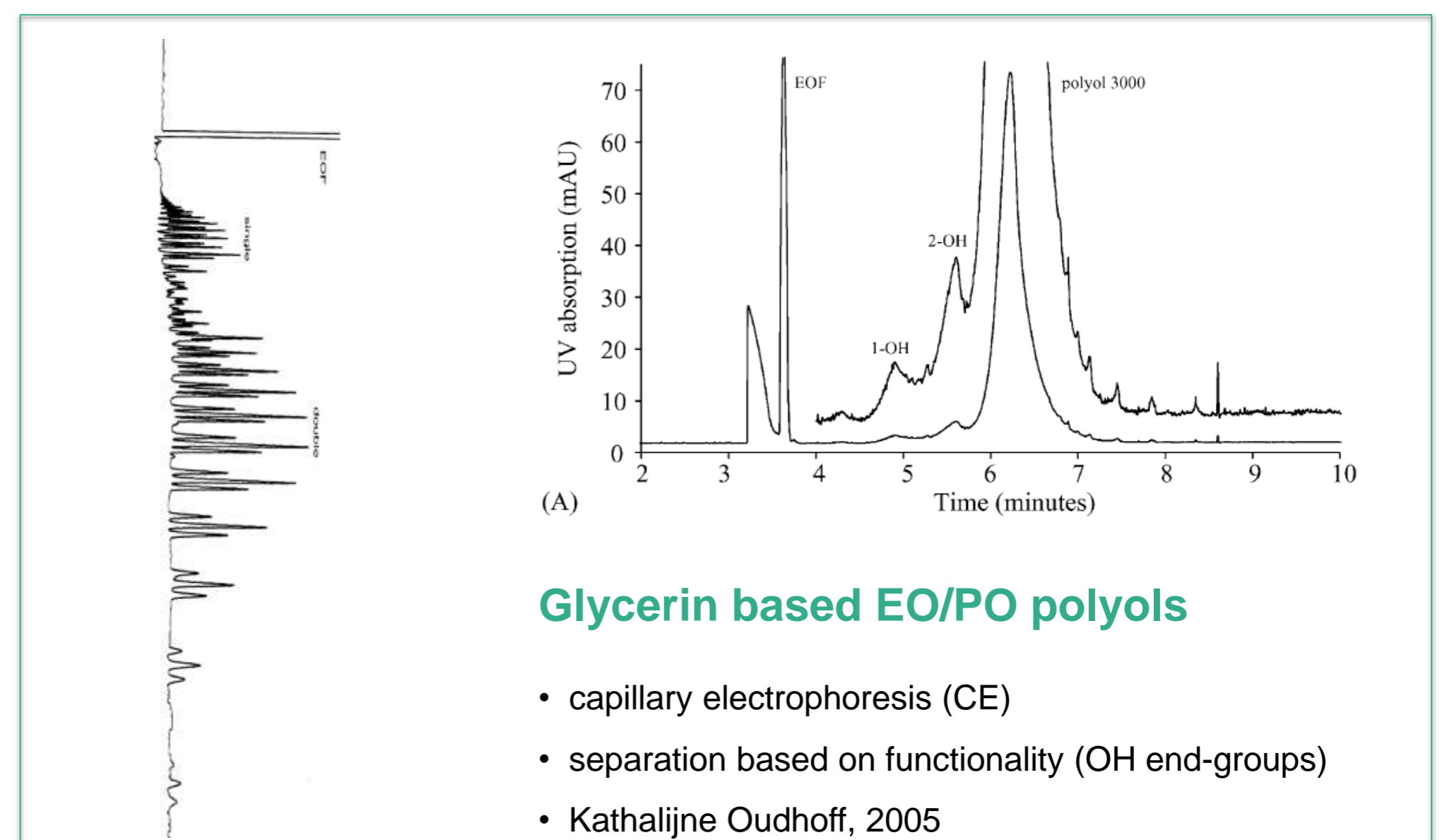
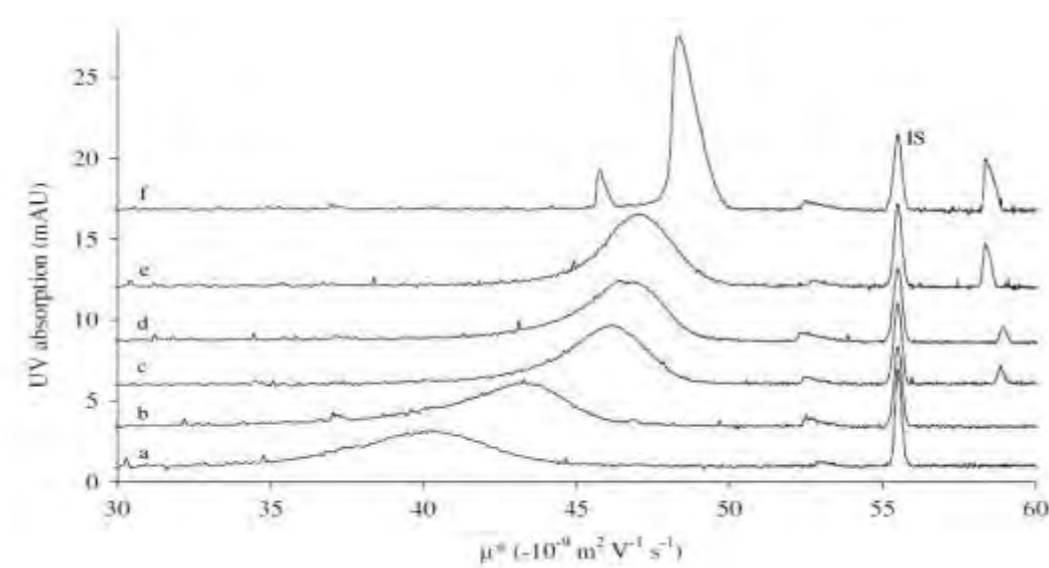


- field-flow fractionation (AF4)
- aggregation behaviour
- relation with environmental fate
- Alina Astefani, 2014



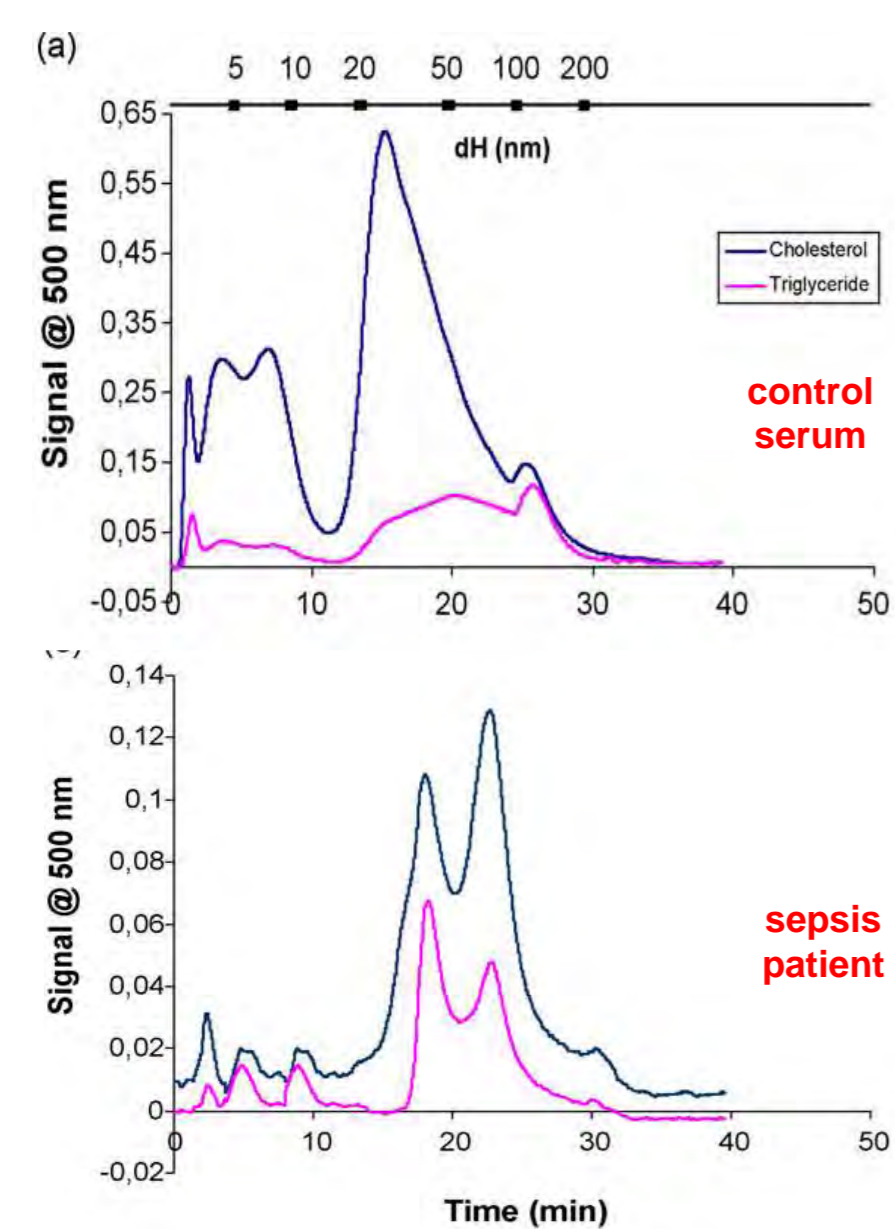
Carboxy Methyl Cellulose

- capillary electrophoresis
- determination of the degree of substitution



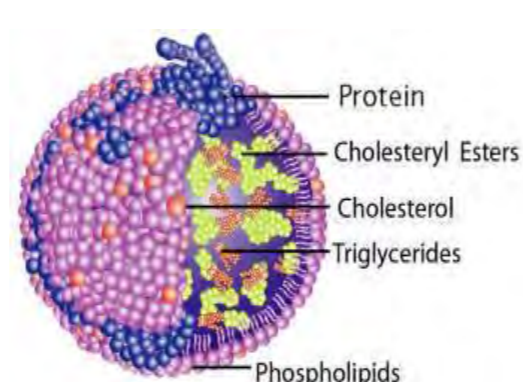
Glycerin based EO/PO polyols

- capillary electrophoresis (CE)
- separation based on functionality (OH end-groups)
- Kathalijne Oudhoff, 2005



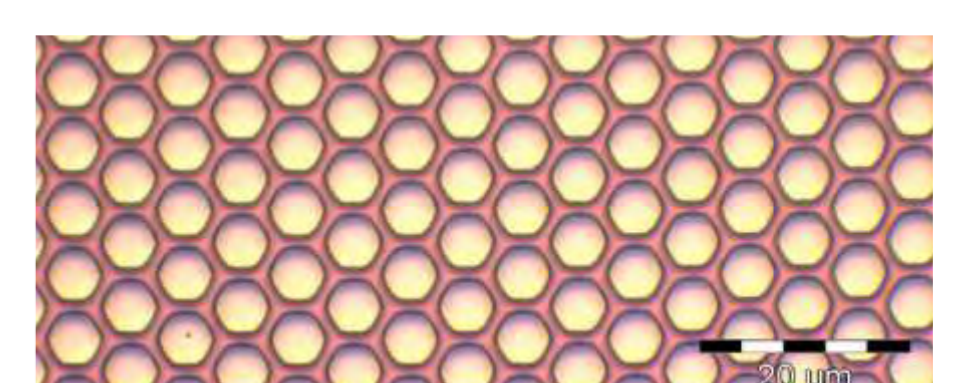
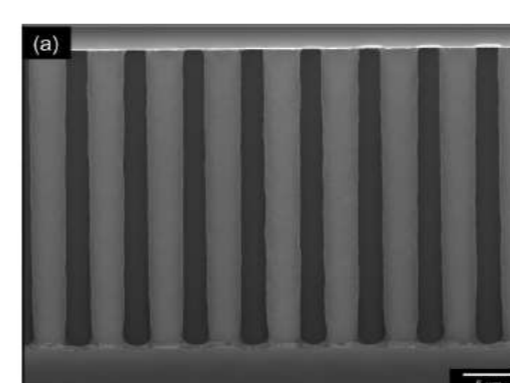
Lipoprotein characterization

- field-flow fractionation (AF4)
- separation based on size
- HDL – LDL – VLDL
- cholesterol and triglycerides
- Rashid Qureshi, 2010



Microfluidics

Various research projects have been / are carried out on the development of microfluidic separation devices. Pillar-structured channels have been fabricated and evaluated that give excellent separation efficiency for Hydrodynamic Chromatography. The microfluidic channels will be tested for the HDC separation of vesicles and liposomes.



Analytical Chemistry

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References

Professional education

In-house courses can be given on various levels (MBO/HBO/acad, NL/Eng), on:

- Basic statistics for analytical chemists
- HPLC: principles and optimization
- Capillary Electrophoresis



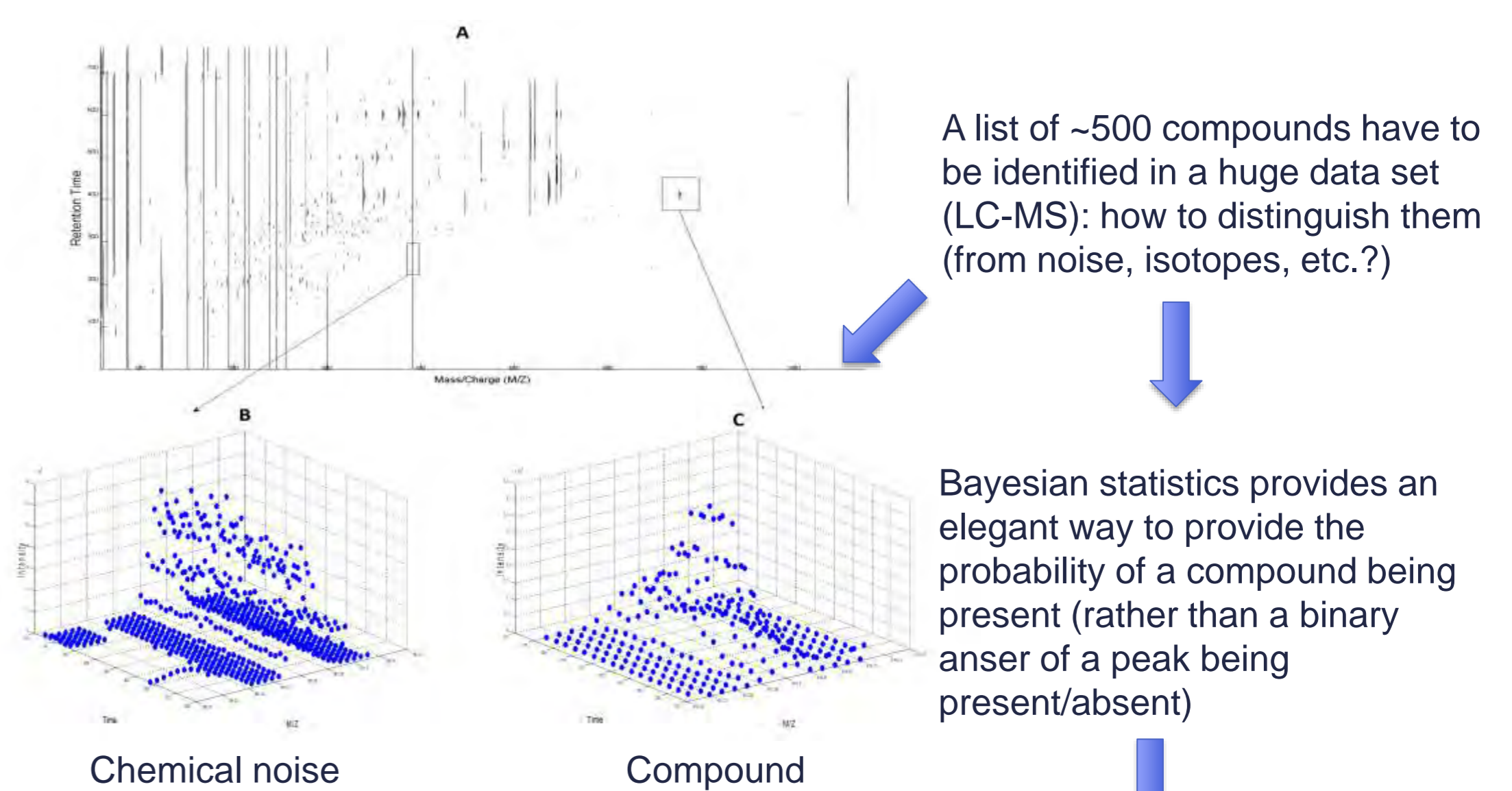
Bayesian statistics to deal with data analysis automation

We are witnessing a tremendous explosion of the data sizes produced by analytical instrumentation. For example, a single injection of a sample in a GCxGC-MS instrument produces around 15 GB/hour of data. Often, the processing of this data into information becomes the major bottleneck of the analysis. In our group we are investigating methods to automate this process.

The classical approach to data analysis automation:

Data	Algorithm	Information
	$\sum \theta \sin \epsilon$	There is a chromatographic peak at $t_r=12$ min. These 10 peaks in these 10 chromatograms belong to the same compound. ... etc.
In a sense, the machines are "taking responsibility" on the decision...		... and only the final result is shown.

Just an example: application to toxicology screening with NFI (Dutch forensic institute):



Our new approach approach to data analysis automation:

Data	Algorithm	Information
	$\sum \theta \sin \epsilon$	20% chance: Peaks 1-9 belong to the same compound. Peak 10 is different. 15% chance: Peaks 1-5,7-9 belong to the same compound. Peaks 6 and 10 are different. ... etc.
In a sense, the machines are "taking responsibility" on the decision...		not a collection of all (ranked) possibilities ... and only the final result is shown.

In this way, the user (and not the algorithm) takes the decision: the algorithm just helps the end user in taking this decision by delivering a collection of (ranked) possible answers.

Posterior odds	Likelihood ratio	Prior odds
$\frac{p(H_0 D)}{p(H_1 D)}$	$\frac{p(D H_0)}{p(D H_1)}$	$\frac{p(H_0)}{p(H_1)}$
The peak is not present	The peak is present	
Marginalize		
$p(D H_1)$		
This contains LC and MS information (and includes isotopic information)		

- The presence/absence of chemical noise
- The possible retention times of the peak (with shifts)
- The possible positions of the MS signal (detector uncertainty)
- The possible adducts.
- The number of isotopes considered.
- ...

The method is able to scan high resolution LC-MS and looking for more than 500 compounds.

Takes into account:

- Retention times
- m/z values
- Relative isotope ratios.

Sensitivity and specificity are better than commercial software packages (e.g. Mass hunter from Waters). It scans 0.5 Gb of data in ~ 6 min.

Valorization goals

Developing a global software package for automated data treatment from chromatography using Bayesian statistics.



Analytical chemistry
(chemometrics cluster)

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References

M. Lopatka, G. Vivó-Truyols, M. Sjerps, Anal. Chim. Acta, 817 (2014) 9-16.
M. Woldegebriel, G. Vivó-Truyols, J. Chromatogr. A, submitted.



Mass Spectrometry Powered Biomolecular Systems Analysis

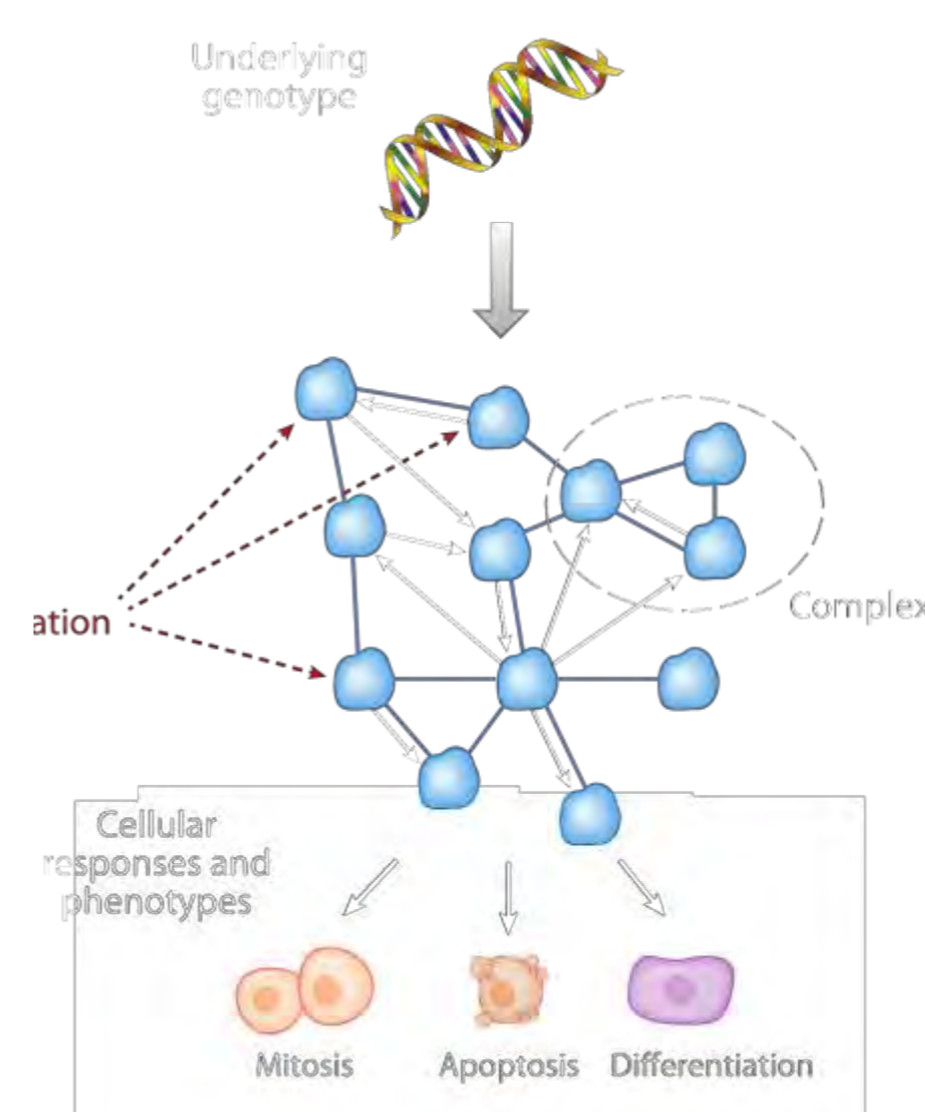
This new HIMS group opens new areas of activity for the Institute by pursuing fundamental and applied research into the **analysis of biomedical systems** for health and disease as well as **forensic science** to aid crime investigation. A third area of activity concerns *Science for Art**, that combines interdisciplinary research uniting art history, art conservation and science. * See Katrien Keune for more information

Our research uses mass spectrometers (MS) to characterise and quantify proteins in biological systems. Proteins are particularly interesting because they are critical for most biological processes and functions. The MS-based tools and allied technologies we develop enable the analysis of exquisite amounts of thousands of proteins that make up the molecular networks in cells, tissues and organs.

Knowledge about the interplay and the molecular state of proteins is important to understand how cells respond to their environment and influence the emergent properties of living systems. This holistic approach to study biological systems from a molecular perspective requires multidisciplinary teams where chemistry and bioinformatics are key elements.

To achieve our goals we develop

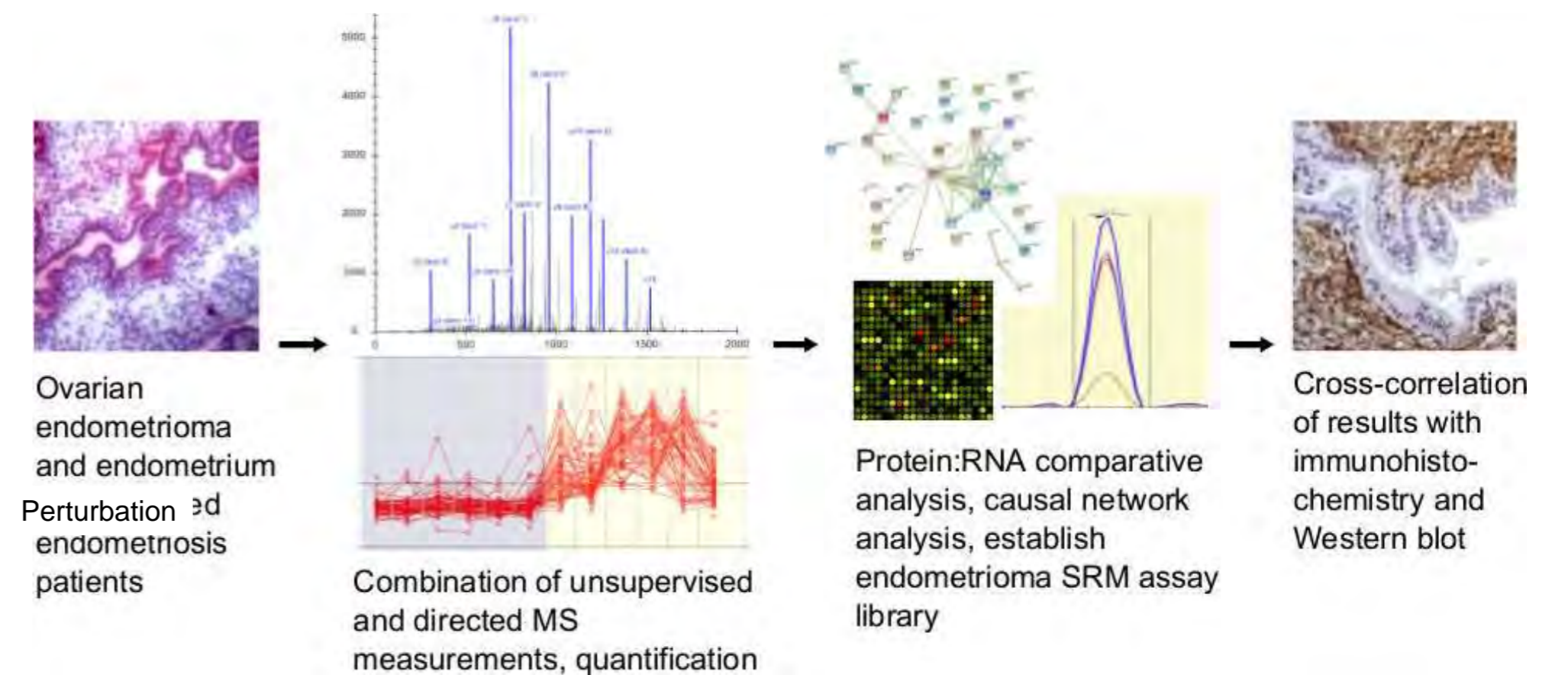
- Chemical procedures to aid preparation and separation methods in line with MS
- Enabling technologies to quantitate proteins in molecular networks in subcellular localities and in clinical tissues and their cellular substructures
- Enabling technologies for post-translation modification interrogation
- Bioinformatics that enables high-speed and high-accuracy proteome analysis



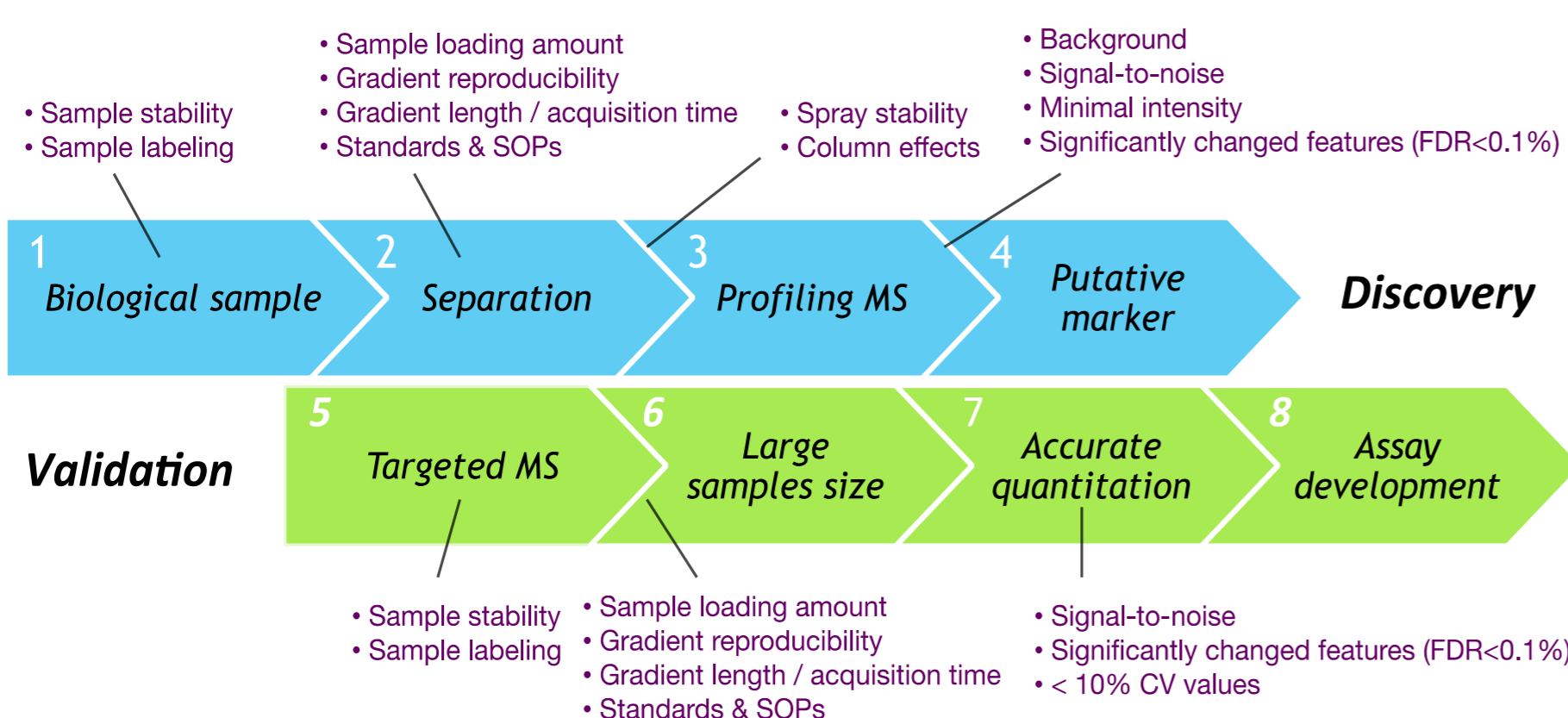
Technological advances have outstripped conceptual advances in biology. The days of one gene – one protein – one function are passed.

Networks of interacting molecules the key difference molecular between genotype and phenotype, where each node in a protein system represents a molecule of interest.

The analytical pathway from discovery to validation



New molecular information on potential therapeutic targets or tools for non-invasive diagnosis for endometriosis are important for patient care and treatment. From Vehmas et al. *Ovarian Endometriosis Signatures Established through Discovery and Directed Mass Spectrometry Analysis*, J Proteome Res. 2014



During the steps from discovery to validation each step must be optimised for each new project, and each sample. Methods must be sensitive, scalable, and enable translational research from bench to clinic and patient material to model systems.

Valorization goals

- To strategically underpin research in the Dutch biosciences community and to highlight the Netherlands as an important partner in research commercialisation.
- Developments of several analytical technologies for immediate spin off or through venture uptake.



Biomolecular Systems Analytics

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References

- Kannaste et al., J Proteome Res. 13 (2014) 1957-68.
- Westermarck Jet al., Mol Cell Proteomics 12 (2013) 1752-63.
- Santos et al., Rapid Commun Mass Spectrom. 26 (2012) 254-62.



Developing new chromatographic technology and methods for sustainable chemistry and proteomics

The versatility and power of chromatography

Chromatography is a versatile separation technique. Unlike other separation techniques, chromatography can separate based on various chemical and physical properties of the sample components, such as size, shape, charge, hydrophobicity, hydrogen bonding ability, π - π interactions, affinity for a particular ligand. With the appropriate selection of separation mechanisms it is now possible to separate 33 compounds per minute [1], making chromatography not only versatile but powerful.

The need for new technology and methods

Despite this the separation power of chromatography has not been exploited in sustainable chemistry or proteomics. This is partly due to limitations in chromatographic technology. Our research aims to address this problem by developing methods and technology specifically for these application areas.

We are looking for interested industry partners in sustainable chemistry and protein analysis who face separation/characterisation problems to join us in tailoring methods and technology for their specific applications

Current research directions

Chromatography for sustainable chemistry:

Two-dimensional chromatography for lignocellulosic biomass catalysis

Lignocellulosic biomass catalysis produces a vast variety of different compounds: sugars, aromatics, aliphatic compounds, phenols and other low molecular weight compounds which serve as important reagents in chemical synthesis. This makes for a complicated separation.

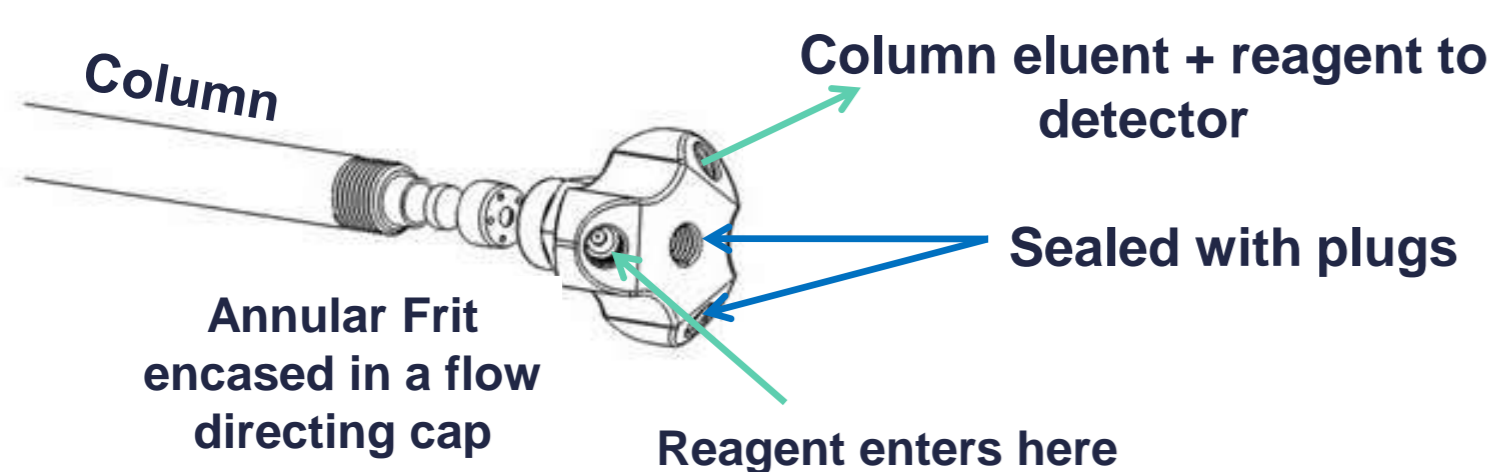
To date, typical separation tools are predominantly one-dimensional. We are investigating the use of the increased power of two-dimensional chromatography to this application.

Not all components adequately separated in 1D. Combining two selectivities can increase separation power

In collaboration with: Dr. N. Raveendran Shiju (HIMS)

Online reactions + separation = efficiency:

Reaction flow chromatography [3,4]



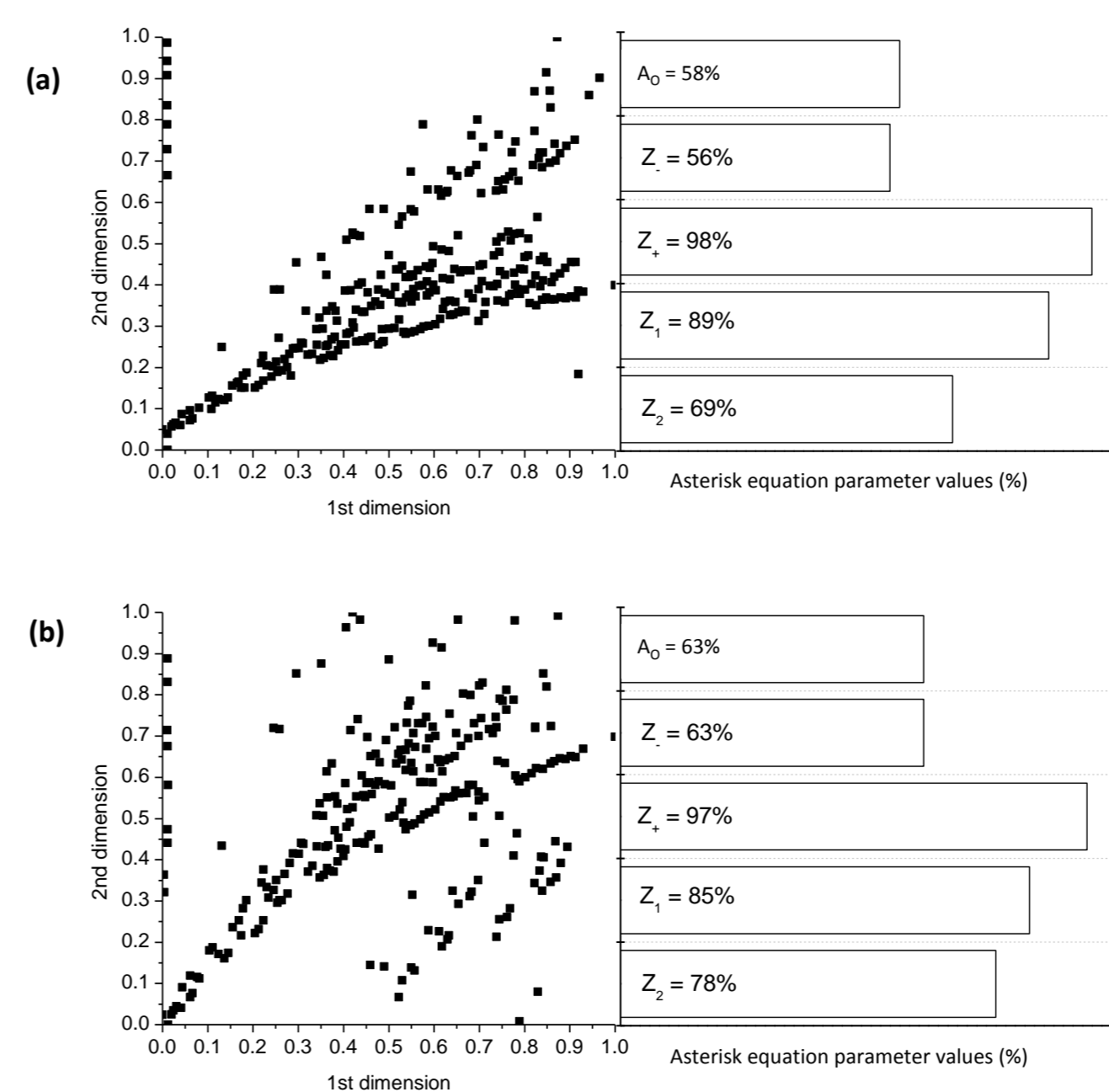
Reaction flow has been used for online assays and chemiluminescence

Figure 2: Diagram of reaction flow chromatography

In collaboration with: **Thermo Scientific** and **University of Western Sydney** (Bringing knowledge to life)

Making method development clearer:

The asterisk equations [2]



For maximum separation power in two-dimensional chromatography, it is necessary to couple two selectivities which have vastly different separation mechanisms.

The asterisk equations report metrics which assesses the optimality separations mechanism when combined. The equations are easily used in Microsoft Excel.

Work has begun on extending the equations to create a metric accounting for all aspects of separation quality

In collaboration with: **DEAKIN UNIVERSITY AUSTRALIA**

New chromatographic technology for protein separations

Efficient protein separations require stationary phases with high permeability, large pore size, high loadability yet chemically and mechanically stable. Core-shell stationary phases, while very efficient, have short lifetimes, reduced permeability and reduced loading capacity. We aim to create a new stationary phase which addresses the current limitations of existing technology

In collaboration with: Prof Gadi Rothenburg and Prof Garry Corthals (HIMS)

Valorization goals

Aside from the asterisk equations the remaining projects are in the process of being launched, subject to funding.



Corthals research group

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