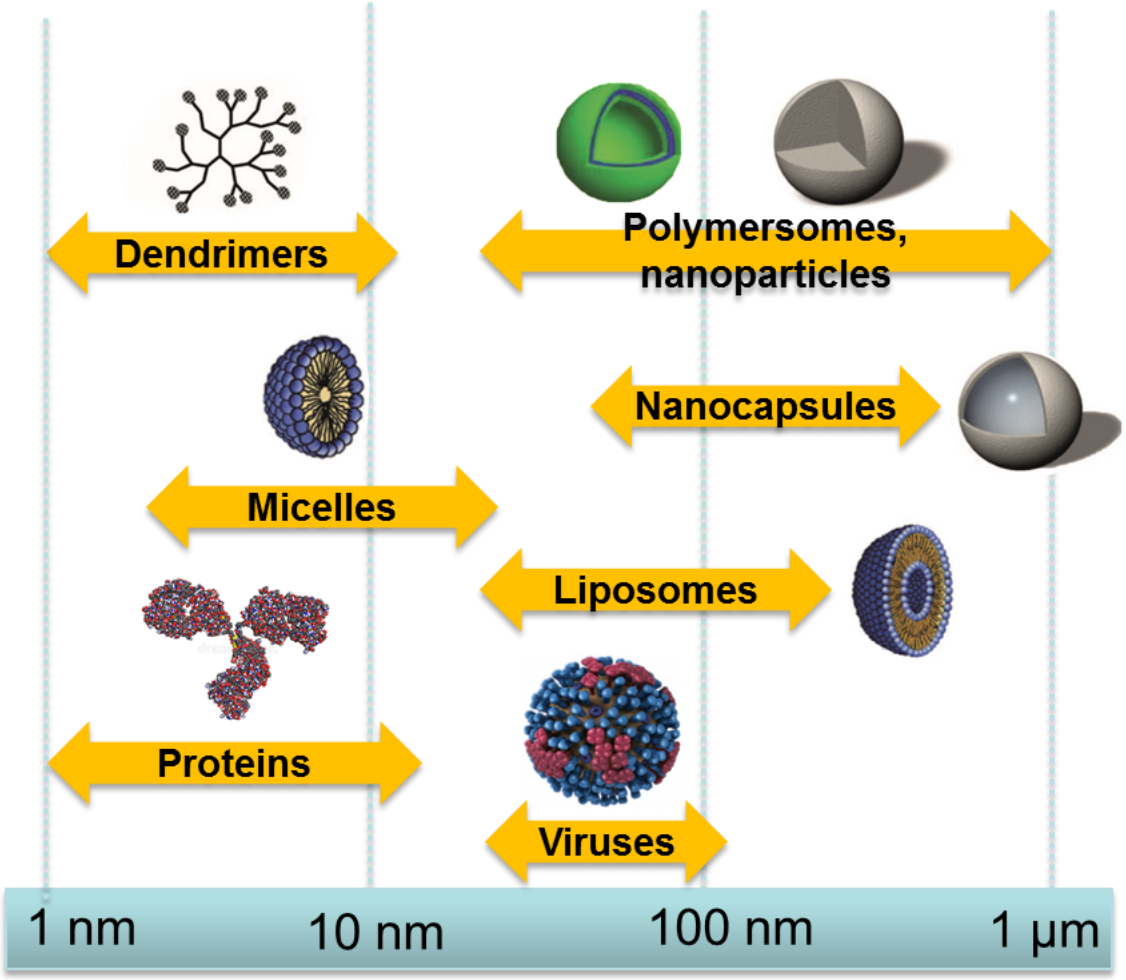
FFF 2.0: Field Flow Fractionation for the comprehensive Characterization of Proteins and Nanoparticles

Reaching the next level to characterize nanoparticles and proteins with Field-Flow Fractionation coupled to Multi-Angle Light Scattering

Dr. Christoph Johann, Wyatt Technology

Proteins conjugated with Nanoparticles …



- Drug Delivery
- Molecular Biology
- Industrial applications

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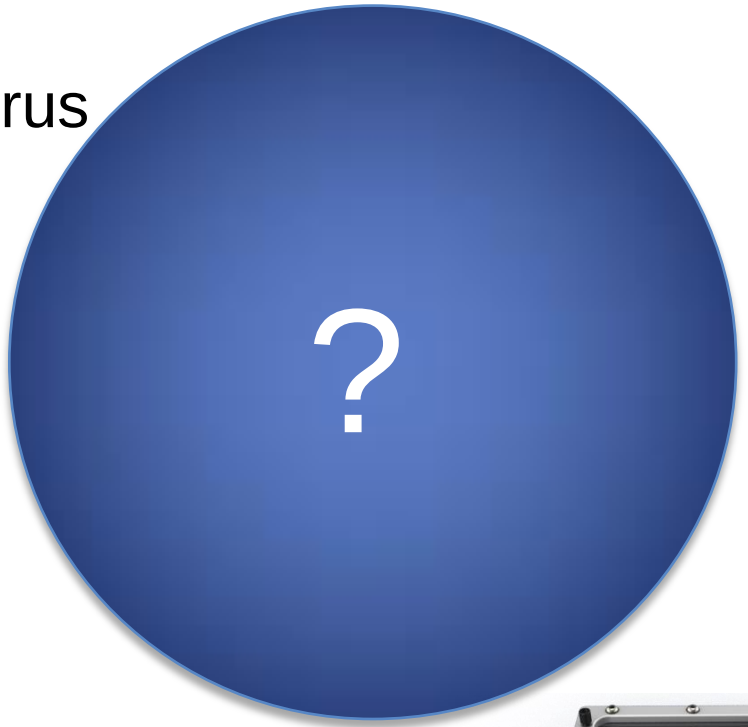
… need new analytical methods

Peptide Antibody Virus



HPLC-MS SEC
UHPLC-MS U-SEC

Field-Flow Fractionation

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Eclipse FFF: Why use Field-Flow Fractionation

- True particle size distribution, can produce fractions
- High resolution
- Wide separation range from small macromolecules (nm) to large particles (µm)
- Powerful for analysis complex samples
- Gentle, low shear and non-destructive
- On-line absolute characterization from MALS
- Determines charge and charge distribution
- Robust and easy to use

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Wyatt Eclipse – Complete System

- Seamlessly integrated and automated system
- Sophisticated data processing of shape, conformation, charge distribution
- Software supported method development

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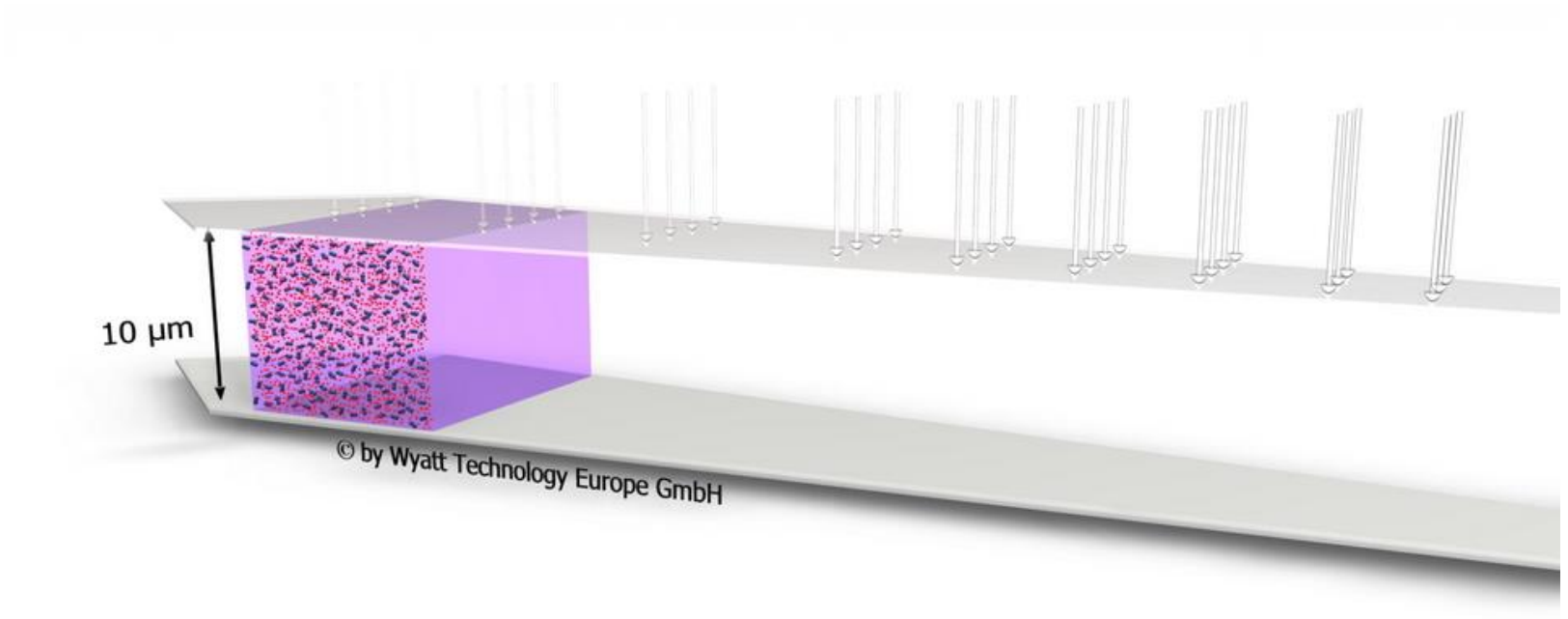
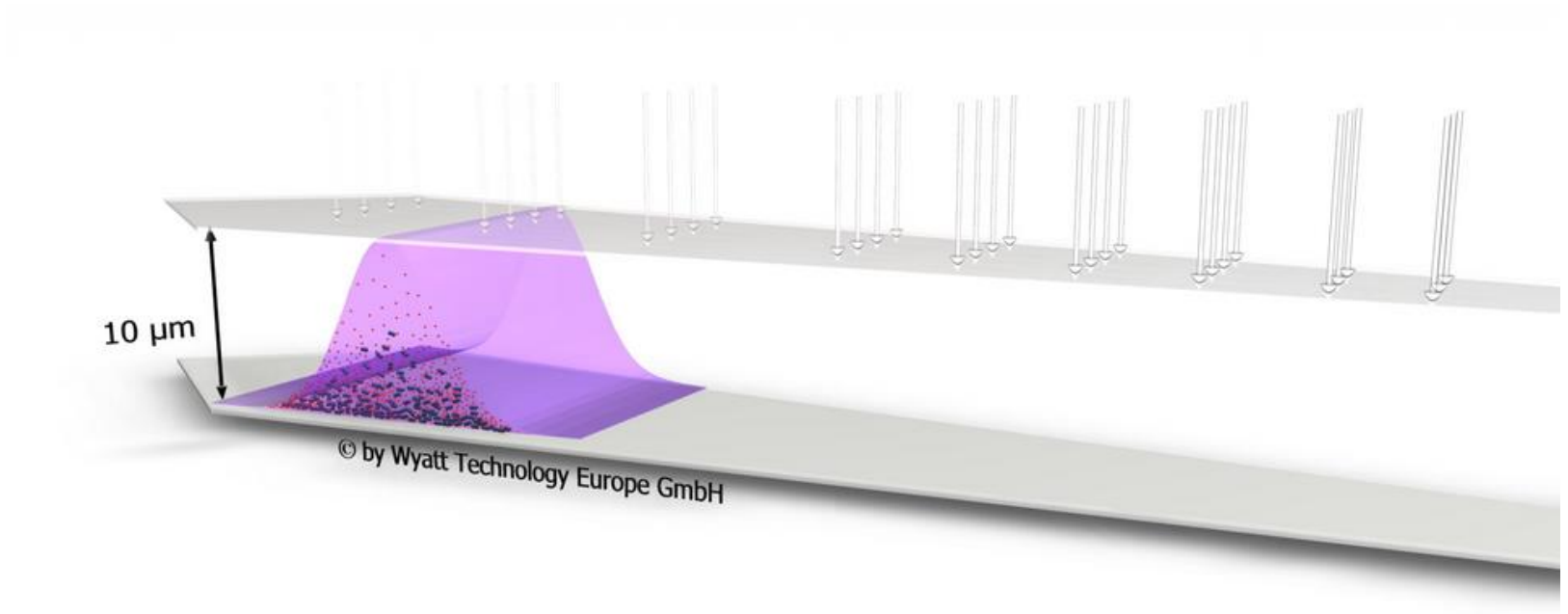
How Flow-FFF Separation Works



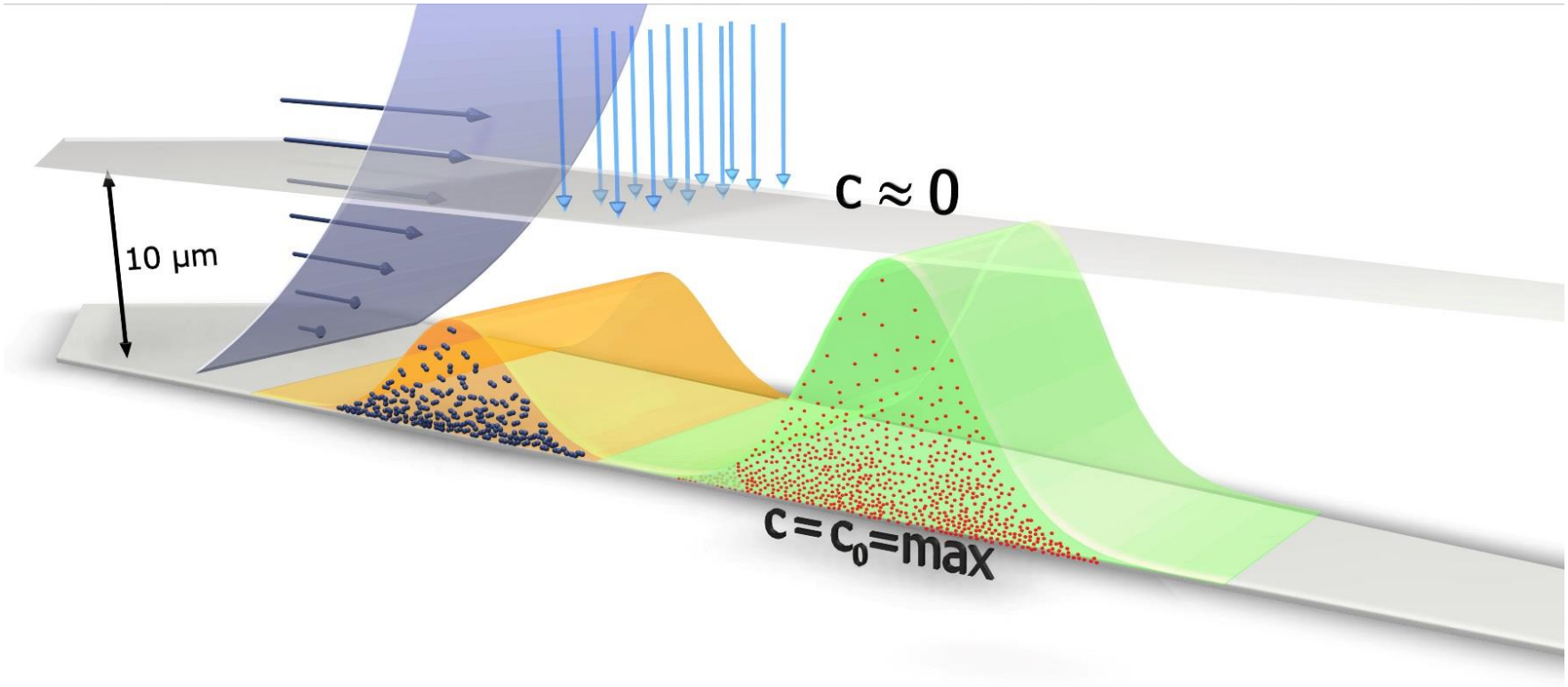
- Sample injected – by interaction with the cross-flow it is concentrated against the porous bottom wall

Flow-FFF with addition of Electrical Field



- The electrical field is added after focusing has taken place

FFF principle



- Separation based on diffusion against a cross-flow in a laminar flow stream

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Flow-FFF - Retention Equation

$$t_R = \frac{w^2}{6D_i} \ln\left(1 + \frac{F_x}{F_c}\right)$$

t_r retention time
w channel thickness
F_x cross-flow rate
F_c flow rate to the detector
D_i diffusion coefficient

[1] R. N. Qureshi, Wim Th. Kok, LCGC Europe Jan 2010

Retention depends on the flow rate ratio only, not the length or width of the channel (with given D_i and channel height)

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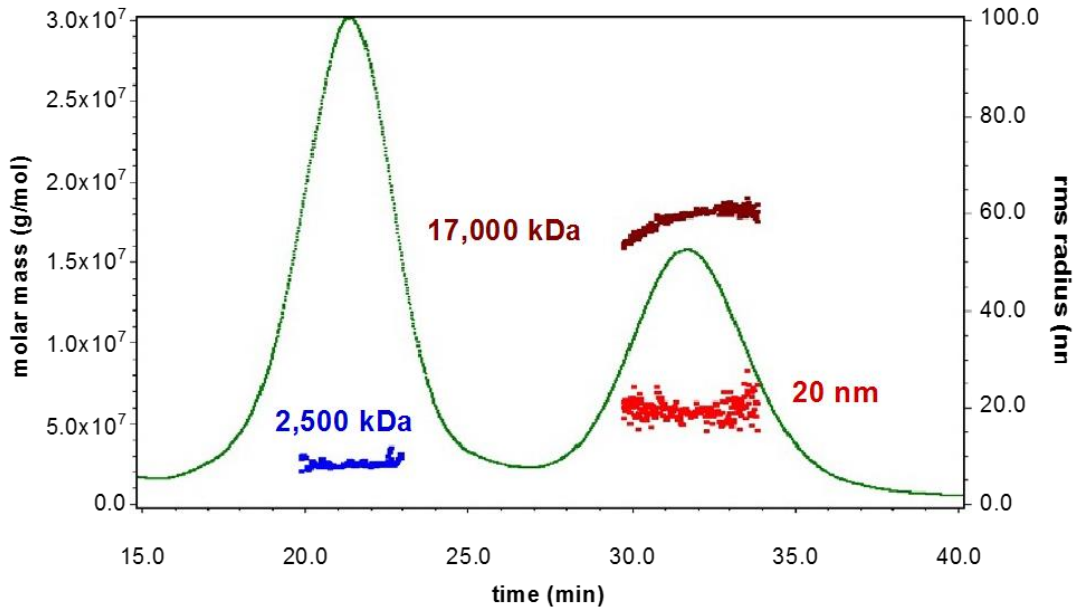
Human Polyoma VPL particles for gene delivery



- Analysis is critical for optimizing VLP reassembly

Citkowicz, A., et al. (2008). "Characterization of virus-like particle assembly for DNA delivery using asymmetrical flow field-flow fractionation and light scattering." Anal Biochem **376**(2): 163-172, **2008**

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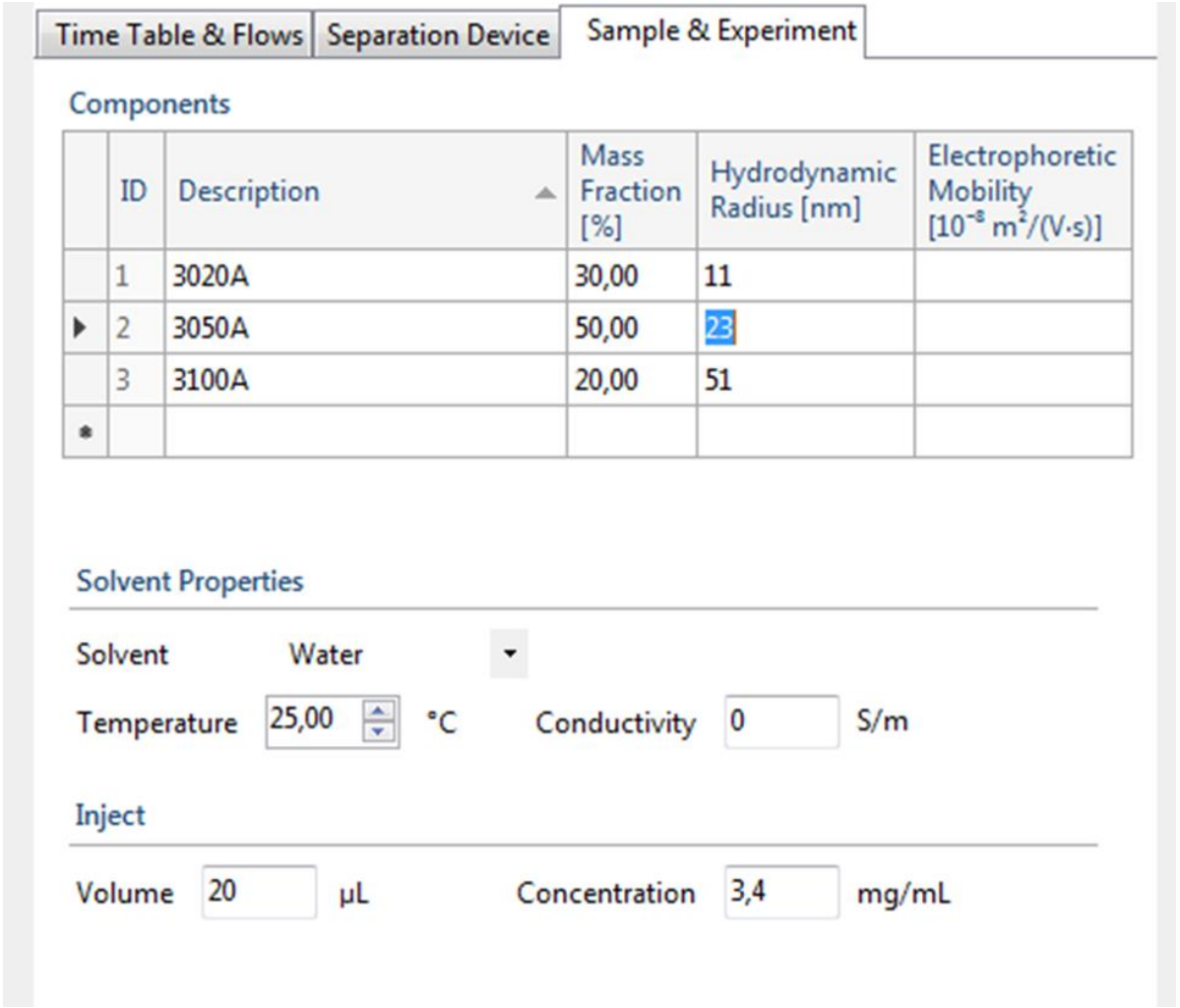
VISION – integrated software suite

Method development with SCOUT

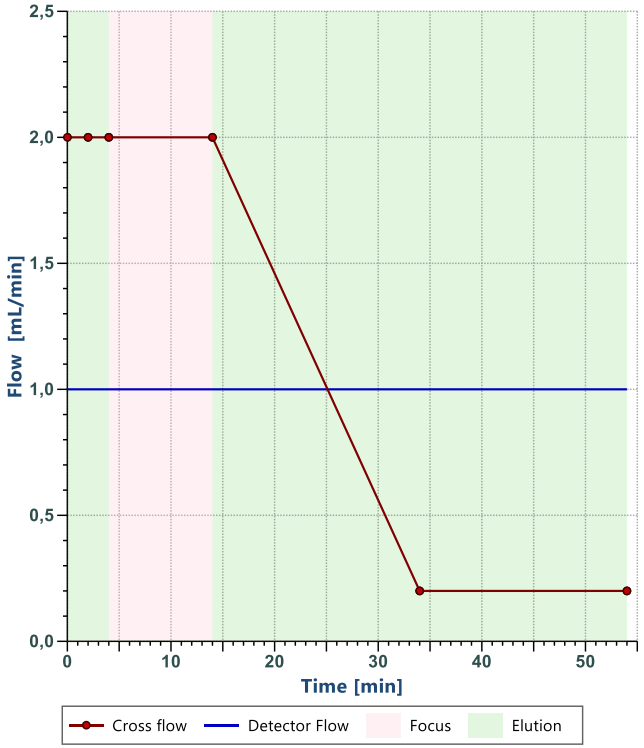
- Define a set of sizes which represent the sample

Sample Definition

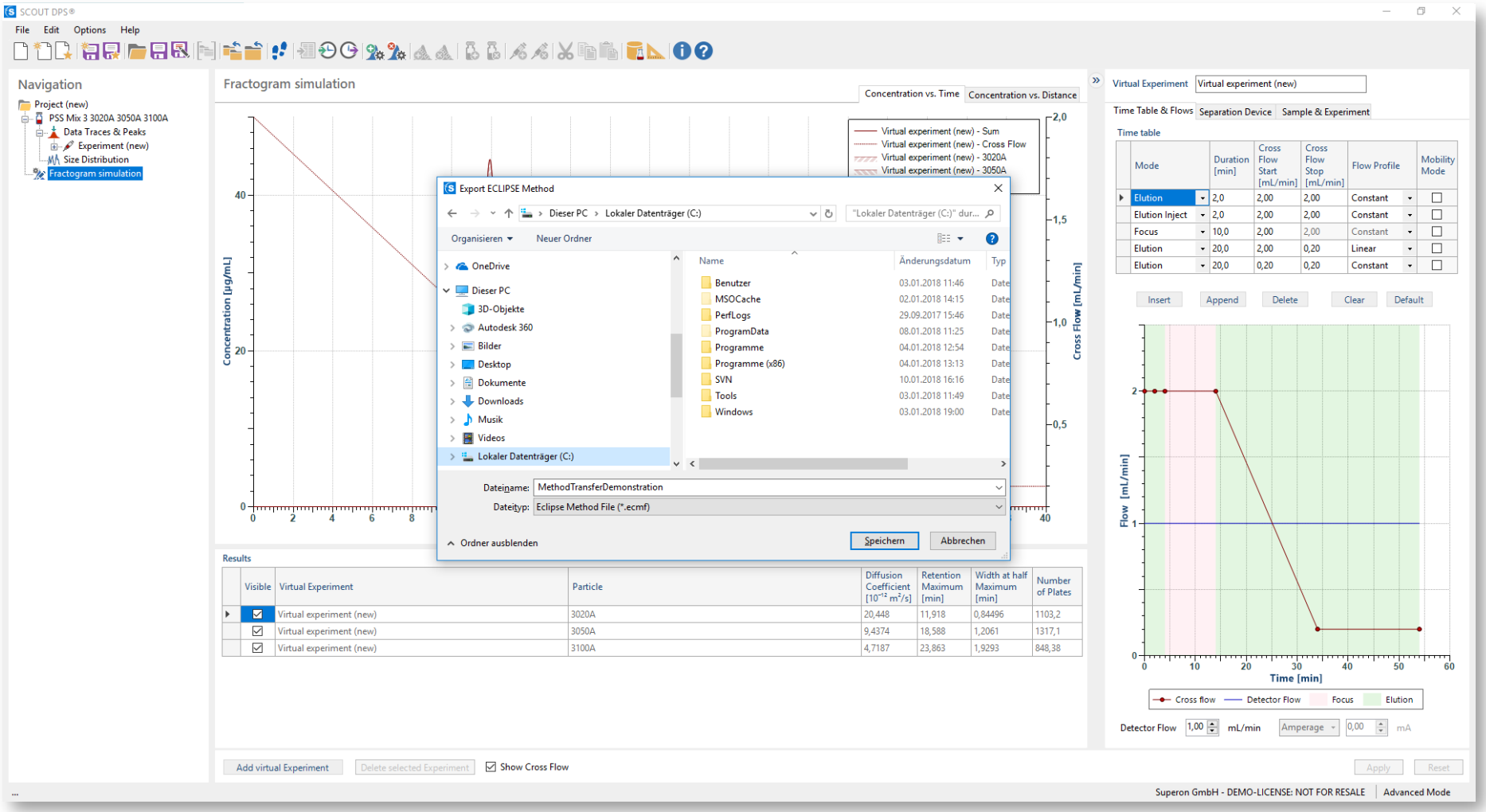


Method development with SCOUT

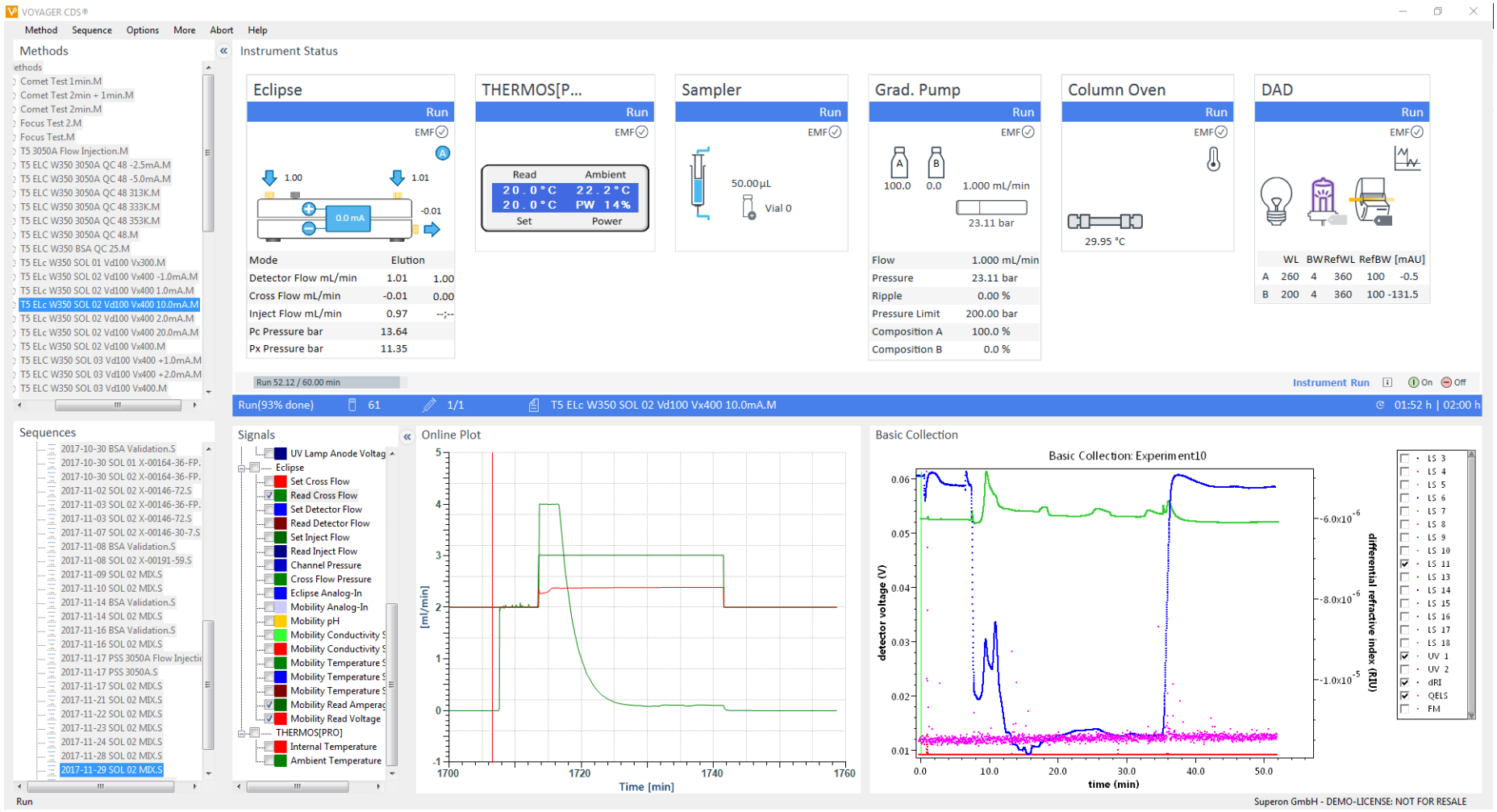
- Define a set of sizes which represent the sample
- Select a channel geometry
- Develop the flow program to show a satisfactory separation
- Export the method to VOYAGER and run it



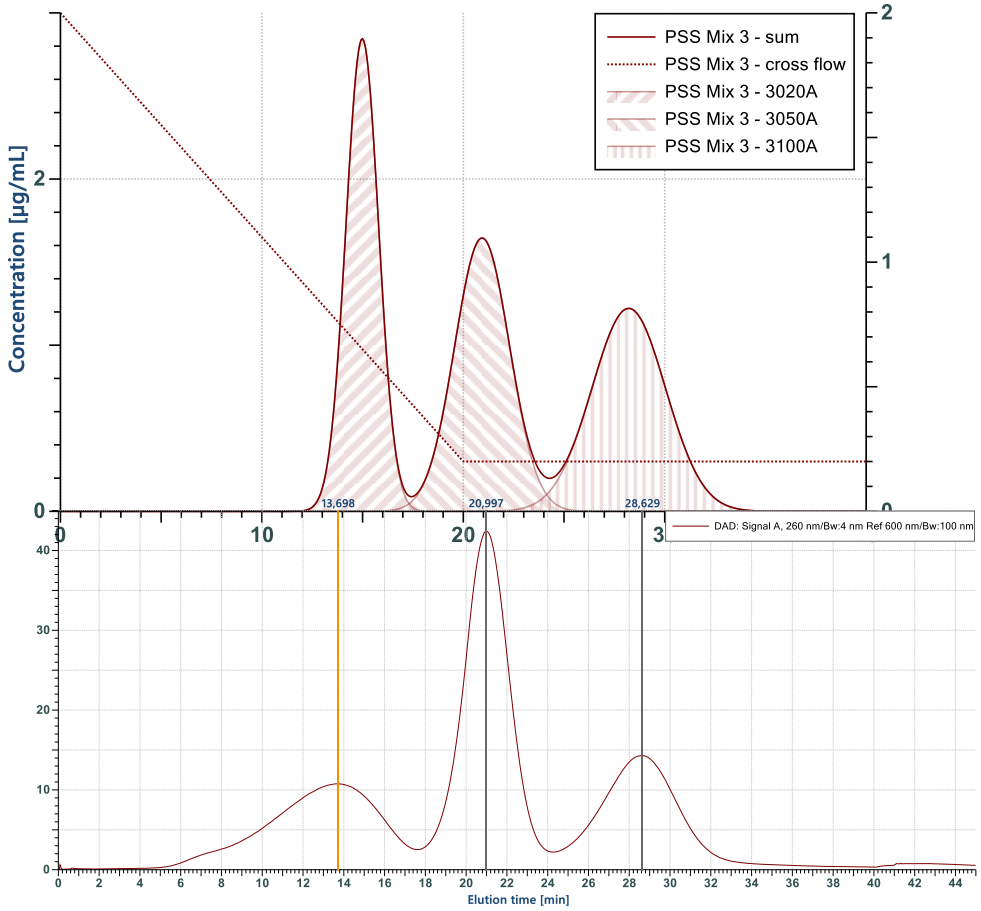
Method development in SCOUT II



Running a sample sequence in VOYAGER

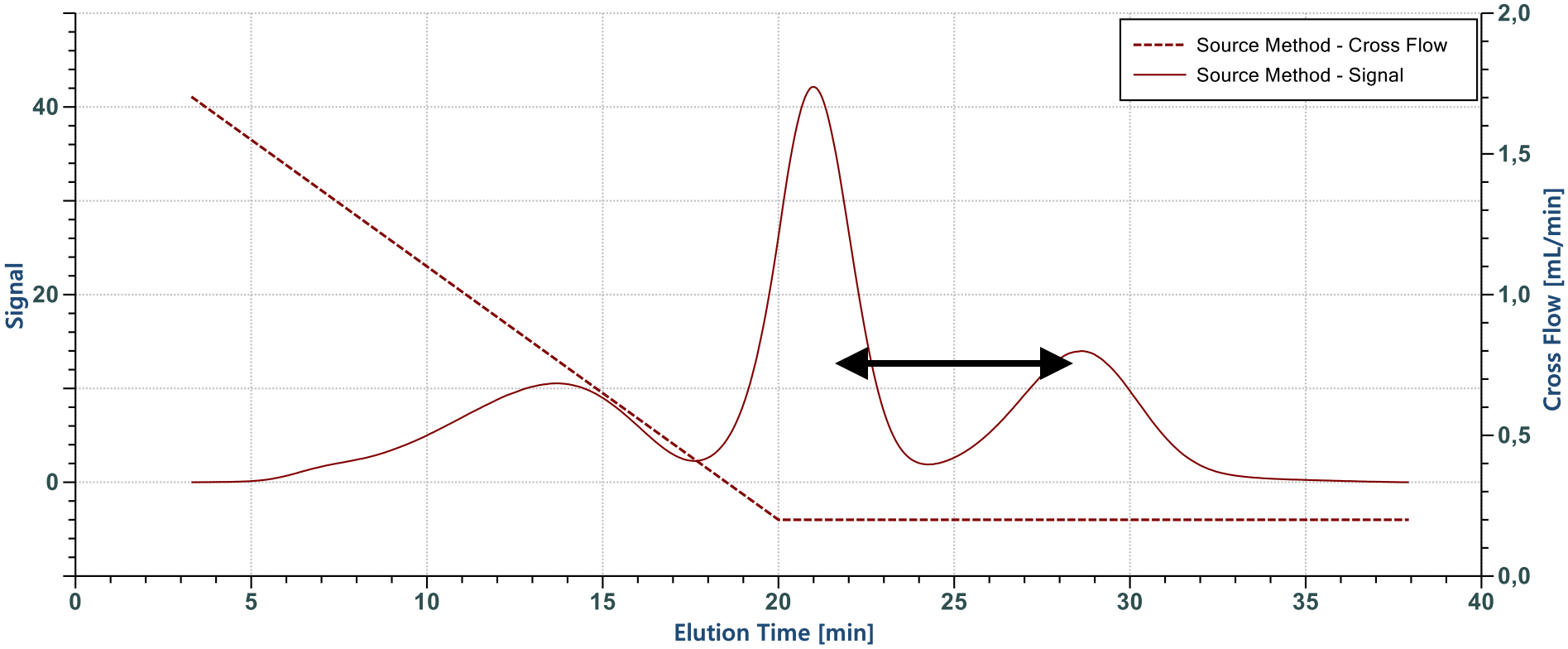


Comparison of simulation and experiment

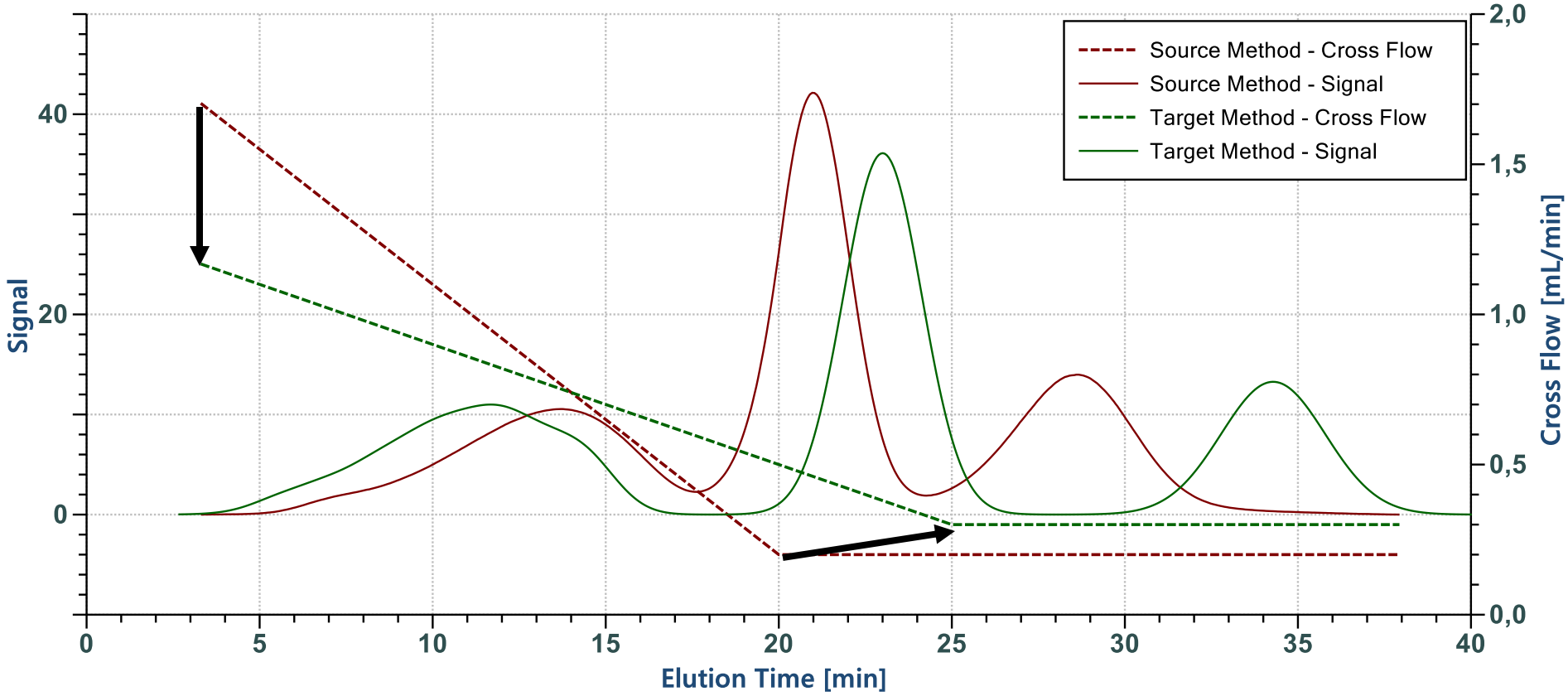


- Simulation for the three polystyrene latex samples
- Experiment with excellent agreement to the simulation

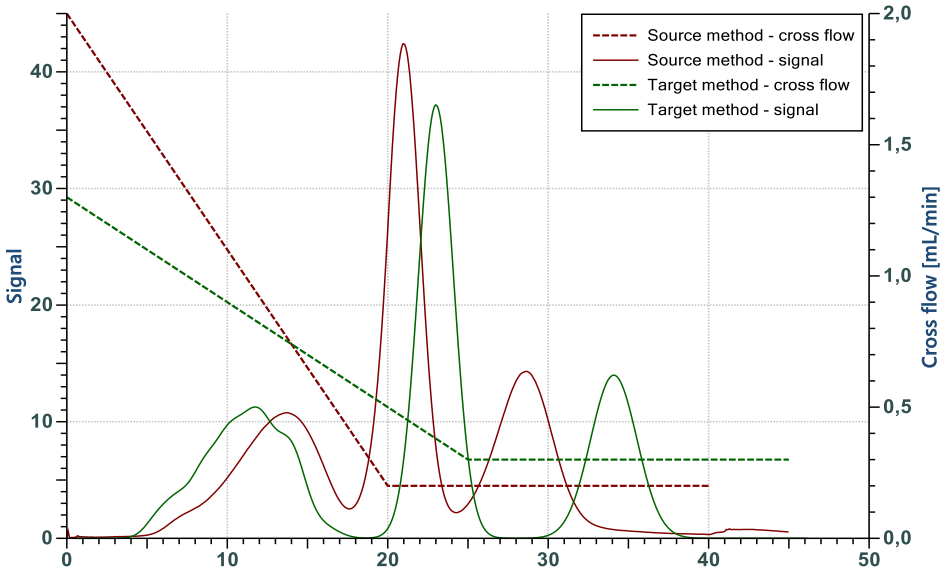
Method Optimization in SCOUT

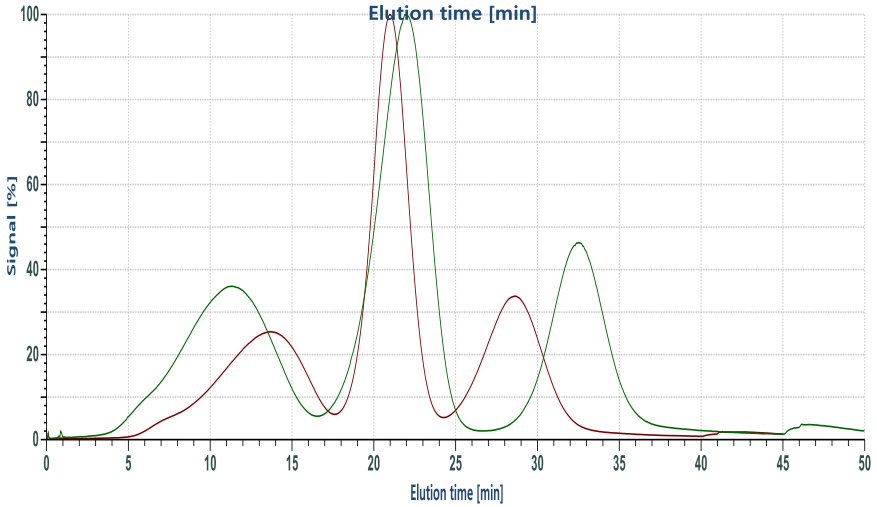


Method Optimization in SCOUT



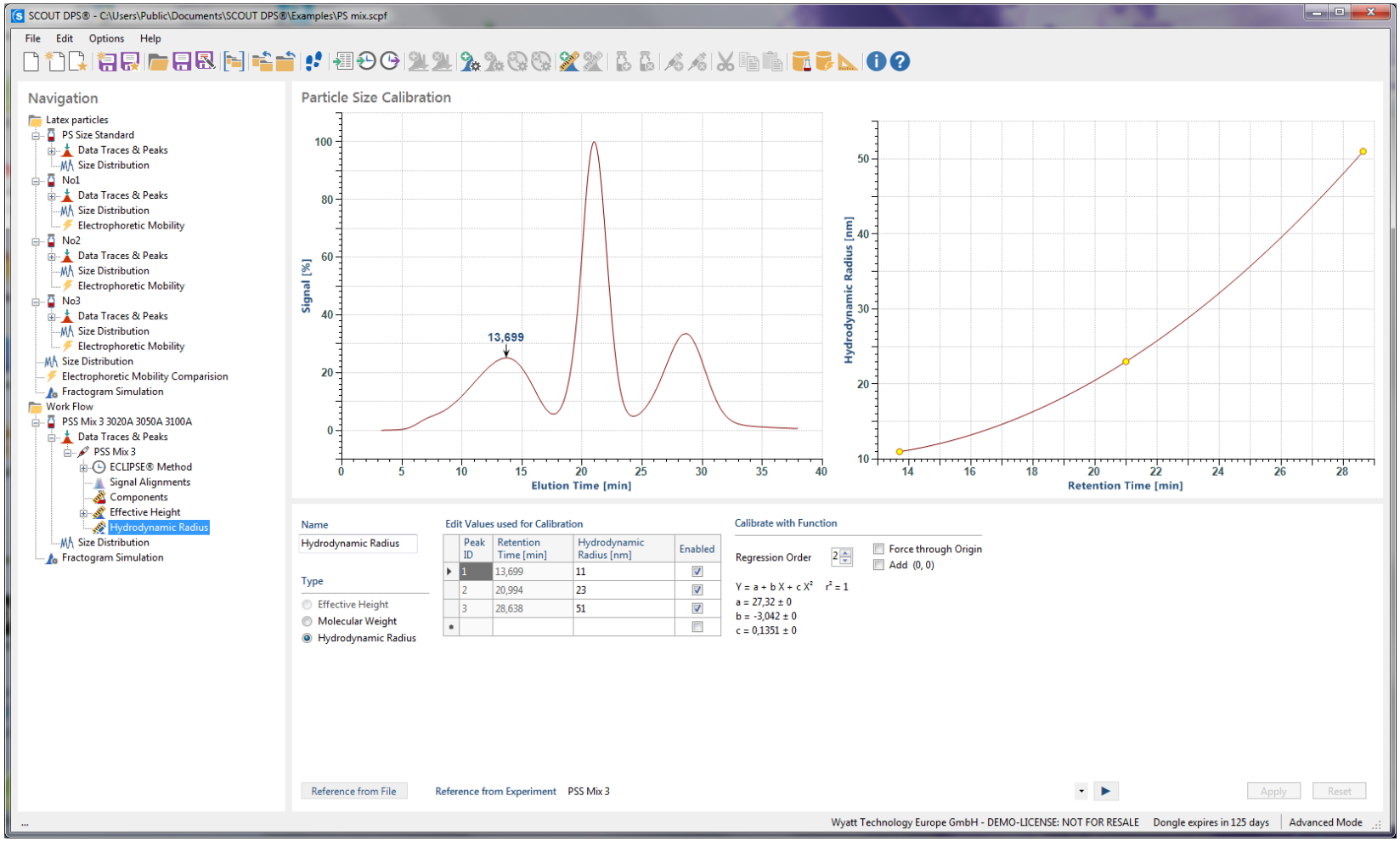
Comparison simulation and experiment



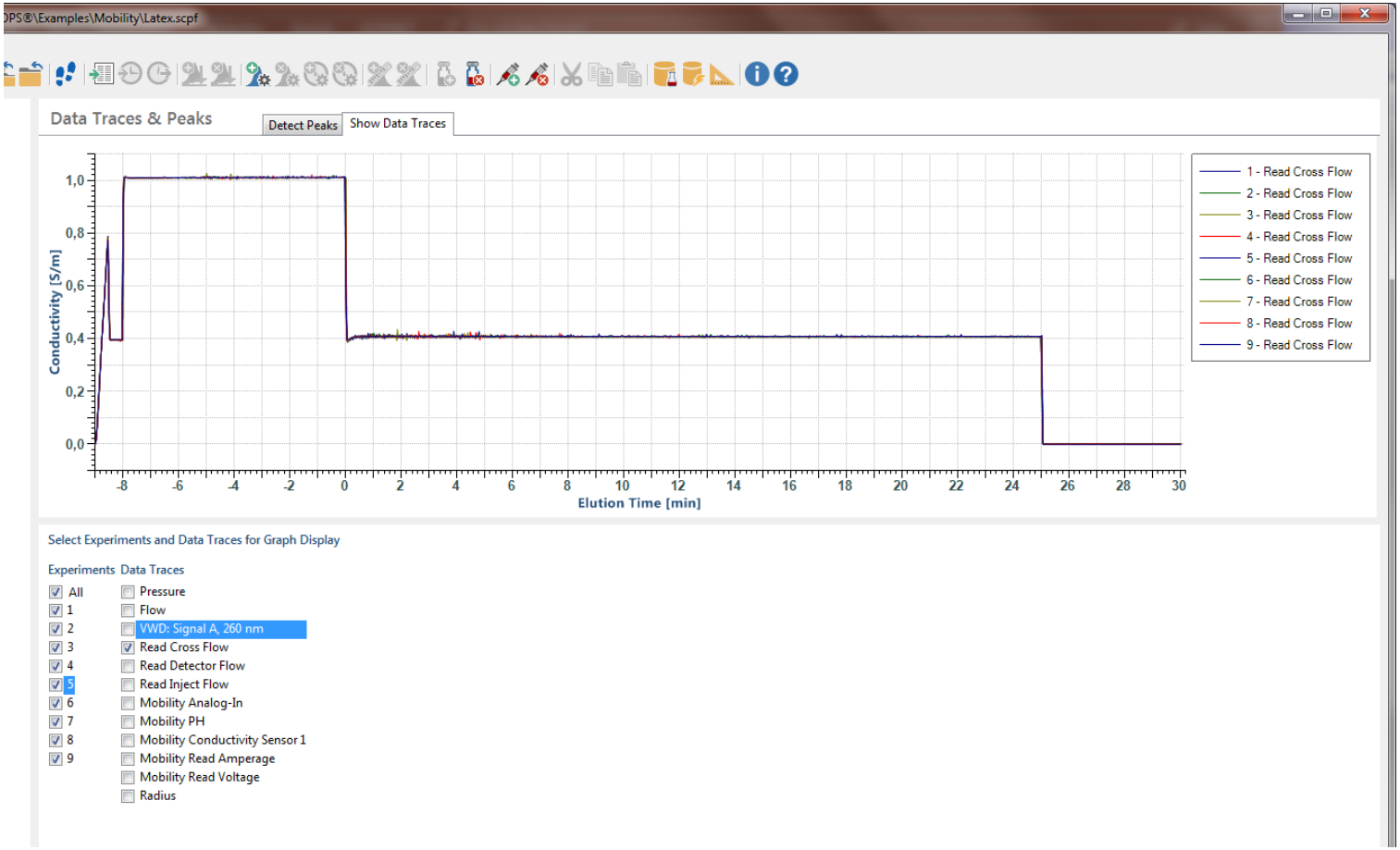


- Overlay of experiment and simulation
- Overlay of the initial and optimized experiment

Size distribution based on calibration



Post run view of system traces



Comprehensive set of results

- Hydrodynamic radius
- Electrophoretic mobility
- Zeta potential
- Conductivity
- pH values
- Temperature

VISION + MOBILITY

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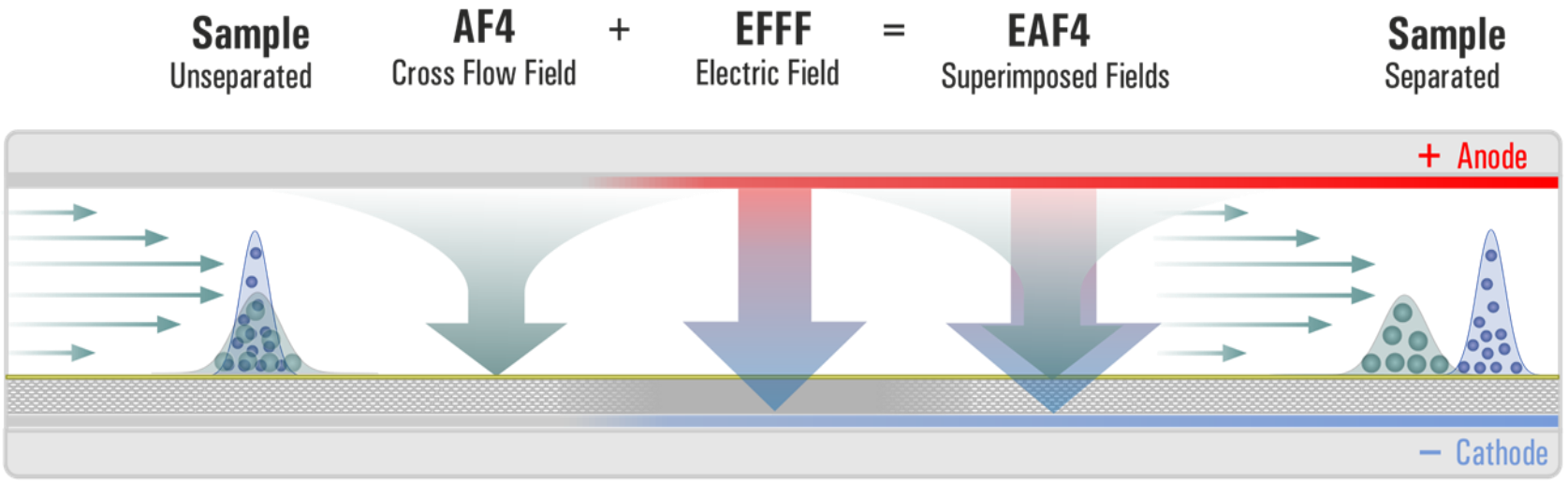
Principle of Electrical Flow-FFF



- EAF4 uses both force fields to generate a separation plus measurement of electrophoretic mobility

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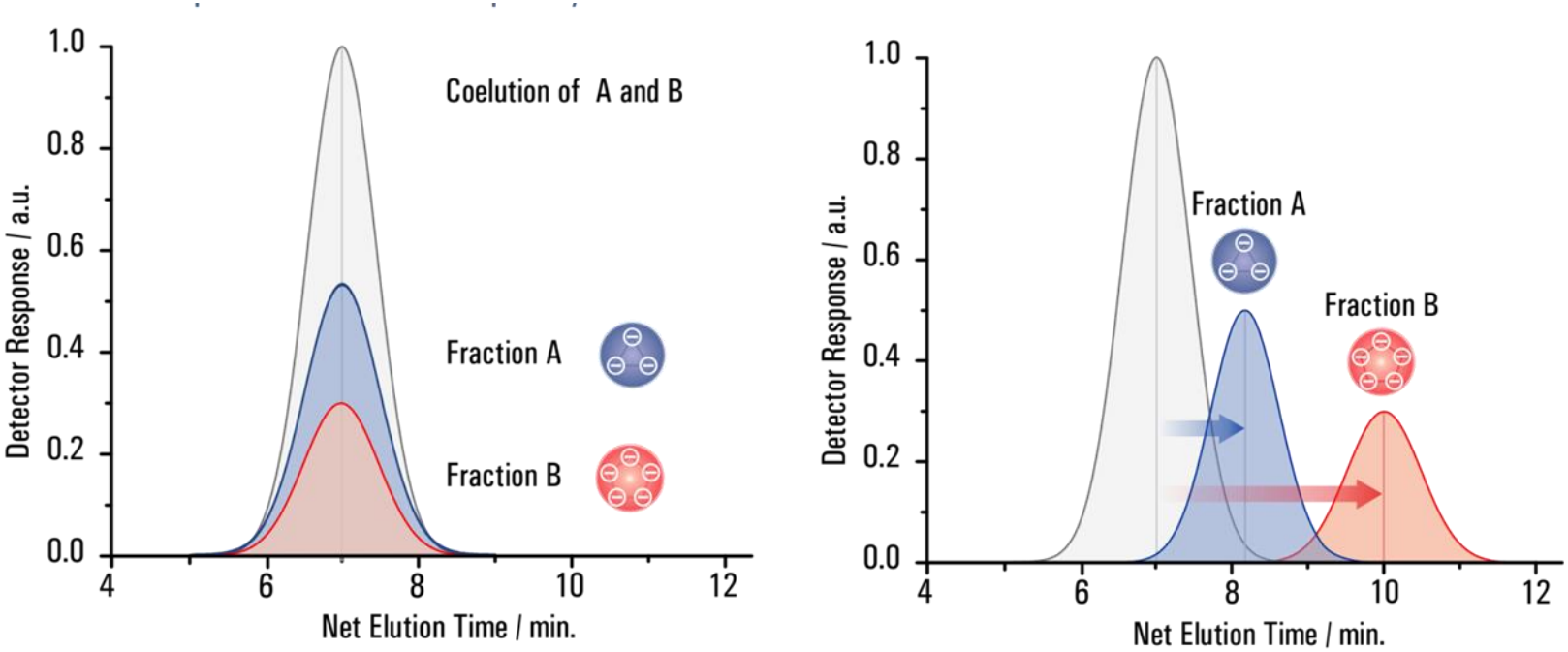
Improving Separation I



- Species of same size but different charge can be separated

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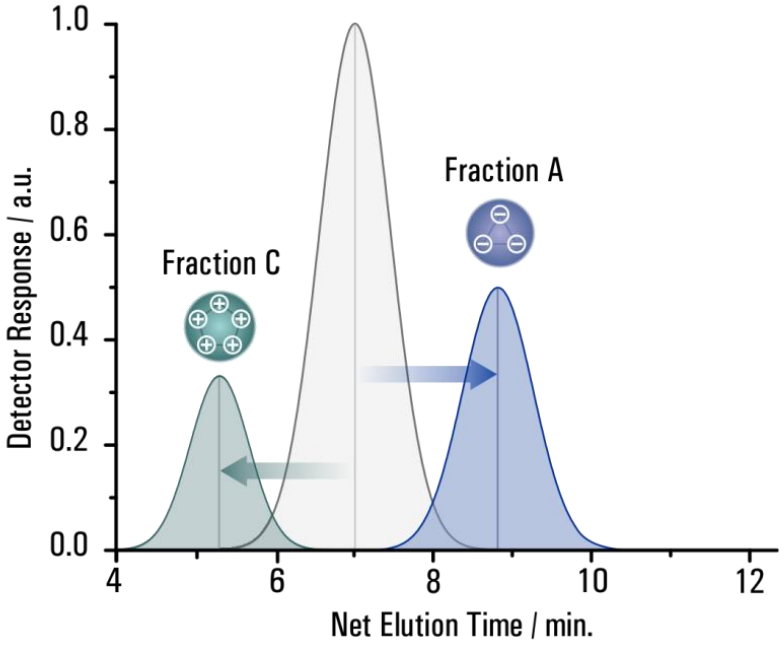
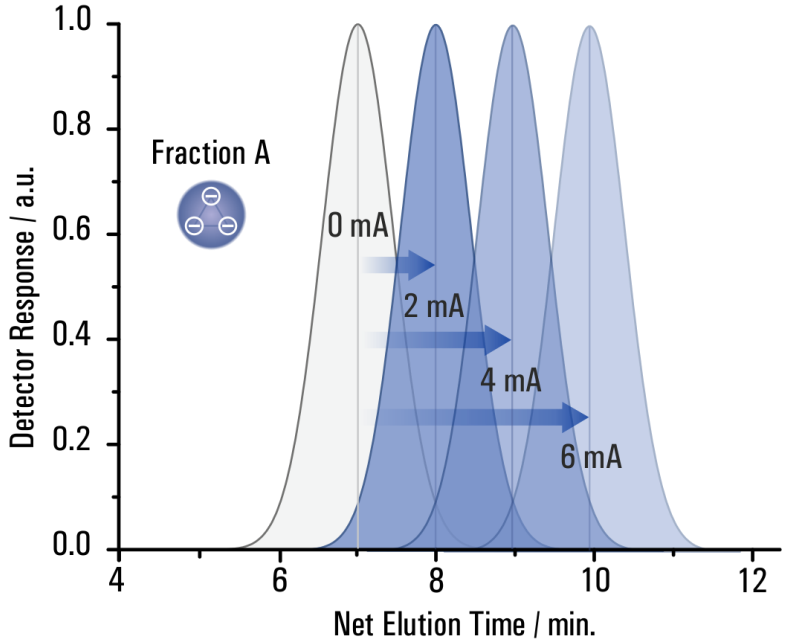
Improving Separation II





- Different charge will move to shorter and longer retention time
- With a series of increasing electrical field, the shift will increase proportionally

Theory I

$$t_R = \frac{w^2}{6D} \cdot \ln(1 + \frac{f \cdot F_c}{F_{out}})$$

- Retention in A4F depends on the ratio of flow rates
- Consider mobility and electrical field

$$\mu = \frac{v_{EP}}{E} \qquad E = \frac{U}{d} = \frac{I \cdot R}{d} \qquad E = \frac{I}{A_{el} \cdot k}$$

- μ can be determined, if the drift velocity v_{EP}, electrical current I and the conductivity k are known, therefore conductivity has to be measured
- Under the influence of a cross-flow and the electrical field E the drift velocity has two components

$$v = v_c + v_{EP} \qquad v_c = \frac{F_c}{A_{el}}$$

Theory II

$$v_{EP} = \left(e^{\frac{t_{Ri} \cdot \ln\left(1+\frac{f \cdot F_c}{F_{out}}\right)}{t_R}} - \left(1 + \frac{f \cdot F_c}{F_{out}}\right) \right) \cdot \frac{F_{out}}{A_{el} \cdot f}$$

- At constant cross-flow the drift velocity is given by a simple equation
- For cross-flow gradients, a discretization algorithm has to be used
- Knowing size from MALS, DLS or FFF retention time, the zeta potential can be calculated

analytical chemistry

Article

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Instrument and Method to Determine the Electrophoretic Mobility of Nanoparticles and Proteins by Combining Electrical and Flow Field-Flow Fractionation

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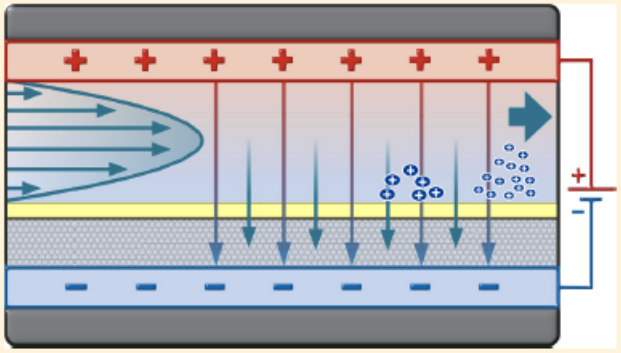
Vol. 87, 8, 4292-8, 2015

Supporting Information

ABSTRACT: A new FFF method is presented which combines asymmetrical flow-FFF (AF4) and electrical FFF (ElFFF) in one channel to electrical asymmetrical flow-FFF (EAF4) to overcome the restrictions of pure ElFFF. It allows for measuring electrophoretic mobility (μ) as a function of size. The method provides an absolute value and does not require calibration. Results of μ for two particle standards are in good agreement with values determined by phase analysis light scattering (PALS). There is no requirement for low ionic strength carriers with EAF4. This overcomes one of the main limitations of ElFFF, making it feasible to measure proteins under physiological conditions. EAF4 has the capability to determine μ for individual populations which are resolved into separate peaks. This is demonstrated for a mixture of three polystyrene latex particles with different sizes as well as for the monomer and dimer of BSA and an antibody. The experimental setup consists of an AF4 channel with added electrodes; one is placed beneath the frit at the bottom wall and the other covers the inside of the upper channel plate. This design minimizes contamination from the electrolysis reactions by keeping the particles distant from the electrodes. In addition the applied voltage range is low (1.5−5 V), which reduces the quantity of gaseous electrolysis products below a threshold that interferes with the laminar flow profile or detector signals. Besides measuring μ, the method can be useful to improve the separation between sample components compared to pure flow-FFF. For two proteins (BSA and a monoclonal antibody), enhanced resolution of the monomer and dimer is achieved by applying an electric field.



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Validation and Comparison to PALS

Using industrial model samples

Comparison of EAF4 and PALS on polyacrylic latex samples

- This study was done in collaboration with The Dow Chemical Corporation, Dr. Wei Gao
- Aim of the study was to compare the electrophoretic mobility determination on two model acrylic latex particles between EAF4 (Wyatt Eclipse Mobility) and PALS (Wyatt Möbius)
- The samples were measured in parallel with both methods and identical sample preparation in the same laboratory to ensure that the results can be unambiguously compared
- Two samples were selected which were known to have different charge

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Design of the study

- For the EAF4 measurements, the two stock solutions of the latex samples were diluted 1:100; 1:1000, and 1:10000. Each of these suspensions was measured under identical conditions with the same volume injected
- PALS measurements were done with samples diluted 1:100 and 1:1000, one set of experiments on suspensions without further treatment, a second set after a dialysis had been performed
- In all cases 5 mM phosphate buffer at pH 7.4 was used to prepare the suspensions and it was the carrier solution for EAF4

Results Table

Sample ID	Dilution	Hydrodynamic Radius [nm]	EAF4 Electroph. Mob. [10^{-8} m²/(V·s)]	PALS Electroph. Mob. [10^{-8} m²/(V·s)]	PALS / No Dialysis Electroph. Mob. [10^{-8} m²/(V·s)]
LP1731	1:10k	56.0 ± 0.0	-5.50 ± 0.14	-4.83 ± 0.05	-4.77 ± 0.20
	1:1k	56.8 ± 0.5	**-5.15 ± 0.07**	**-5.20 ± 0.10**	-4.88 ± 0.07
	1:0.1k	57.0 ± 0.4	-5.06 ± 0.16		
LP1735	1:10k	43.0 ± 0.1	-3.51 ± 0.03	-3.77 ± 0.16	-4.01 ± 0.23
	1:1k	40.5 ± 0.2	**-3.67 ± 0.10**	**-3.76 ± 0.04**	-4.04 ± 0.15
	1:0.1k	40.4 ± 0.1	-3.86 ± 0.03		

- Hydrodynamic radius values are calculated from FFF retention time and they correlated well with DLS (data not shown)

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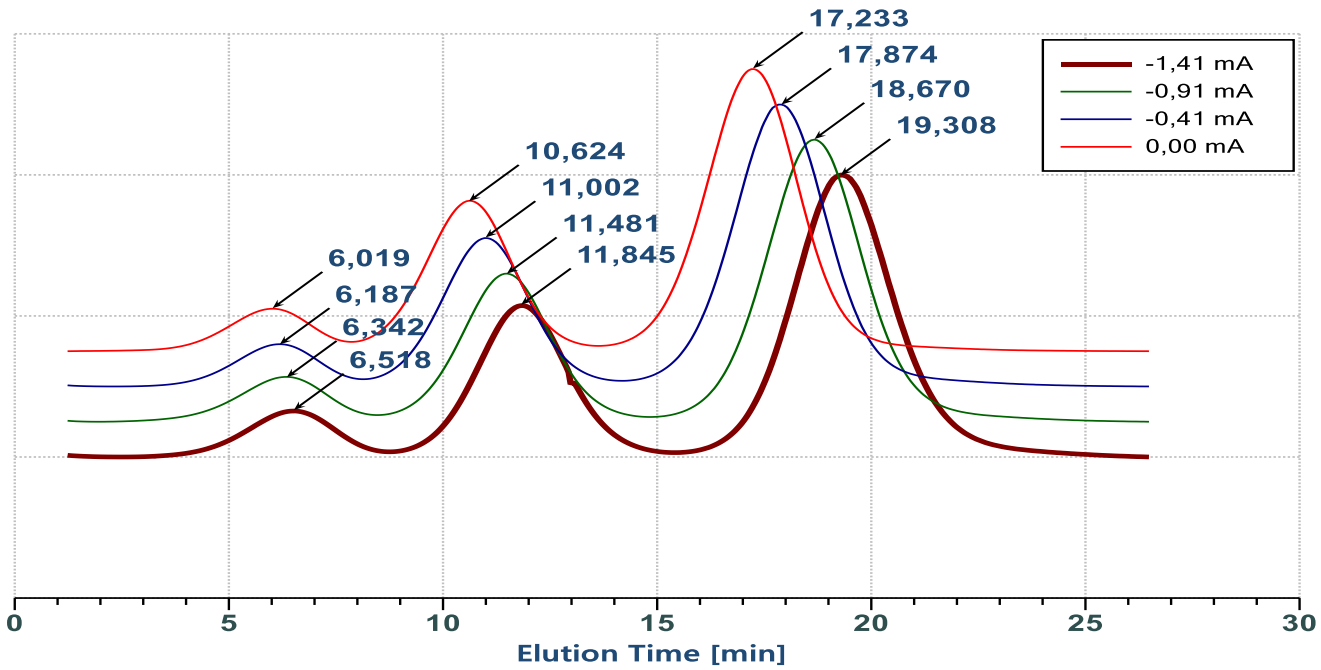
Discussion of the Comparative Measurements

- PALS results on dialyzed samples at 1:100 dilution were measured with identical mobility values compared to EAF4.
- The EAF4 results for both samples did depend slightly (by about 10%) on the dilution, with opposing trends for both samples.
- PALS results depended significantly on dialysis.

Mix of 3 Duke Latex 23, 46, 102 nm



Hydrodynamic Radius [nm]	Diffusion Coefficient [10^{-12} m²/s]	Electrophoretic Mobility [10^{-8} m²/(V·s)]	Net Charge	Zeta Potential [mV]
21,603 ± 0	10,5 ± 0	-4,2603 ± 0,14	-127,7 ± 4,11	-86,68 ± 2,79
42,669 ± 0	5,315 ± 0	-5,8125 ± 0,17	-405,6 ± 11,6	-117,6 ± 3,37
82,379 ± 0	2,753 ± 0	-5,385 ± 0,13	-925,9 ± 22,9	-107,3 ± 2,65

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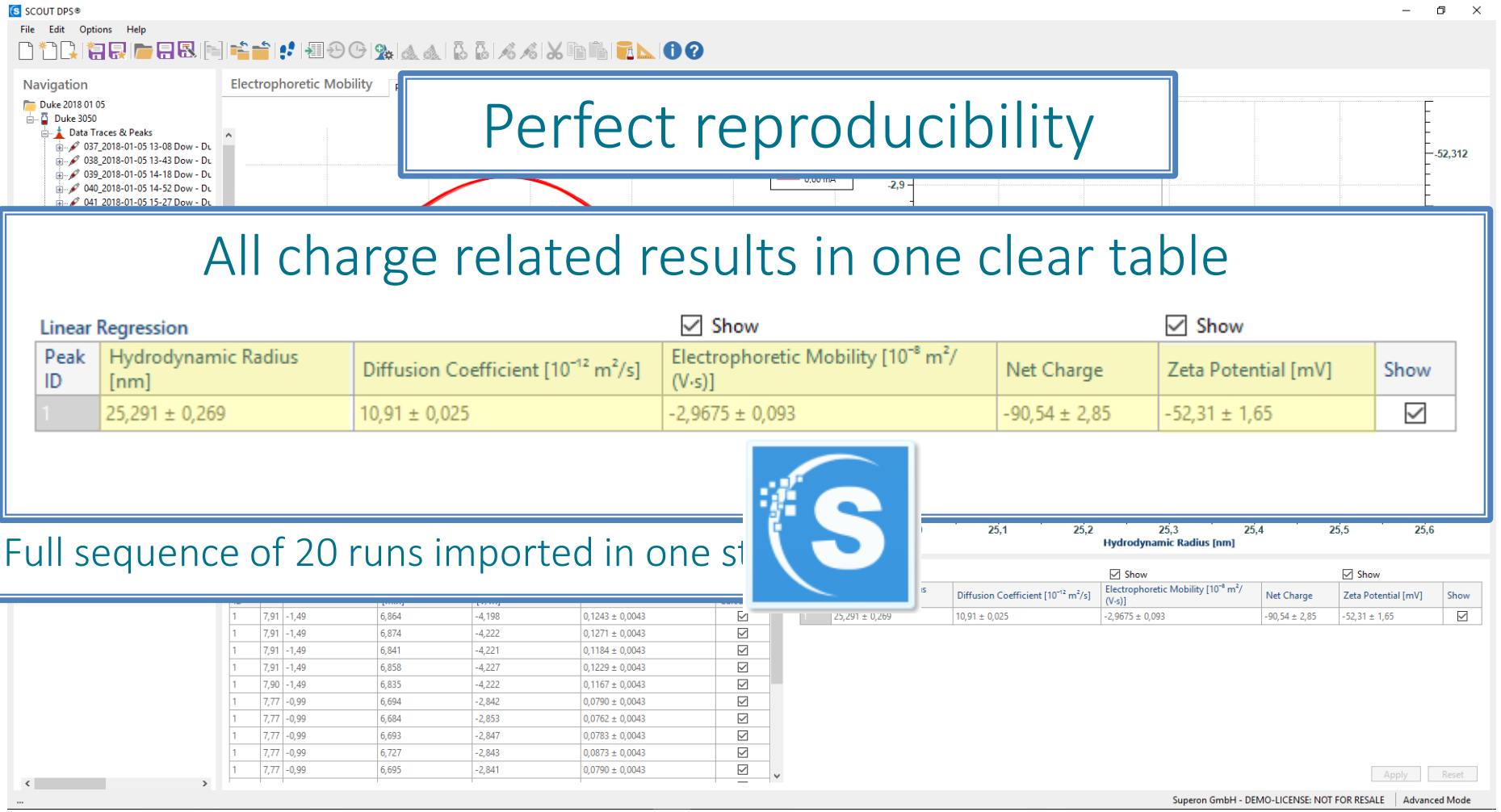
One-Click-Analysis – From template to instant results



Summary

- The Wyatt Eclipse Flow-FFF system is a powerful tool to characterize complex nanomaterials
- It is based on efficient separation coupled to online molar mass and size measurement with a Wyatt MALS detector
- Eclipse Mobility allows to determine charge and charge distribution in complex samples
- The system is fully integrated with the new software VISION and provides a seamless workflow from method development to final result
- We have seen application examples for polymer latex particles

Thank you for your attention

Questions?

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