POSTNOVA

Field-Flow Fractionation: Solving the Challenges where Size Exclusion Chromatography meets its Limitations

Trevor Havard, Gerhard Heinzmann, Florian Meier, Thorsten Klein

The Origin of FFF

1966 – Origin of Field-Flow Fractionation invented by Prof. Giddings

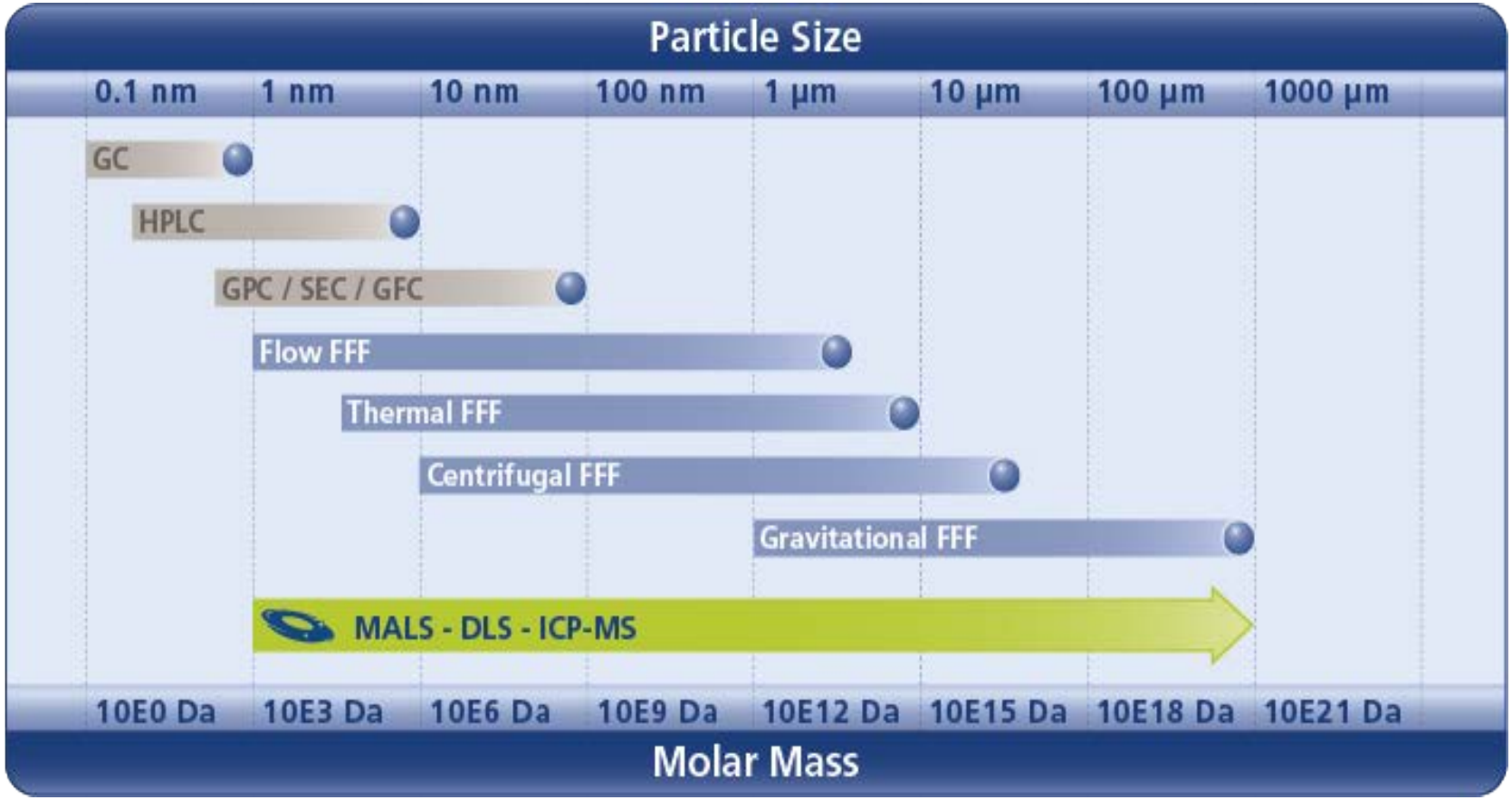
- Field-Flow Fractionation invented in 1966 by Prof. Calvin Giddings [1] at University of Utah, Salt Lake City, USA.
- He established the Field-Flow Fractionation Research Center at University of Utah in 1972.
- In 1986 he founded FFFractionation, worlds first company which started to commercialize the FFF technology.
- The obvious expectation was that FFF would replace SEC/GPC as it had so many theoretical advantages.
- With the development of Nanotechnology FFF finally comes of age and takes the stage

FFFresearch Center, Univ. Utah

[1] J.C. Giddings, „New separation concept based on a coupling of concentration and flow non-uniformities“, Sep. Sci., 1, 123-125 (1966)
[2] J.C. Giddings, „Dynamics of Chromatography“, (1965)
[3] J.C. Giddings, „Unified Separation Science“, (1992)

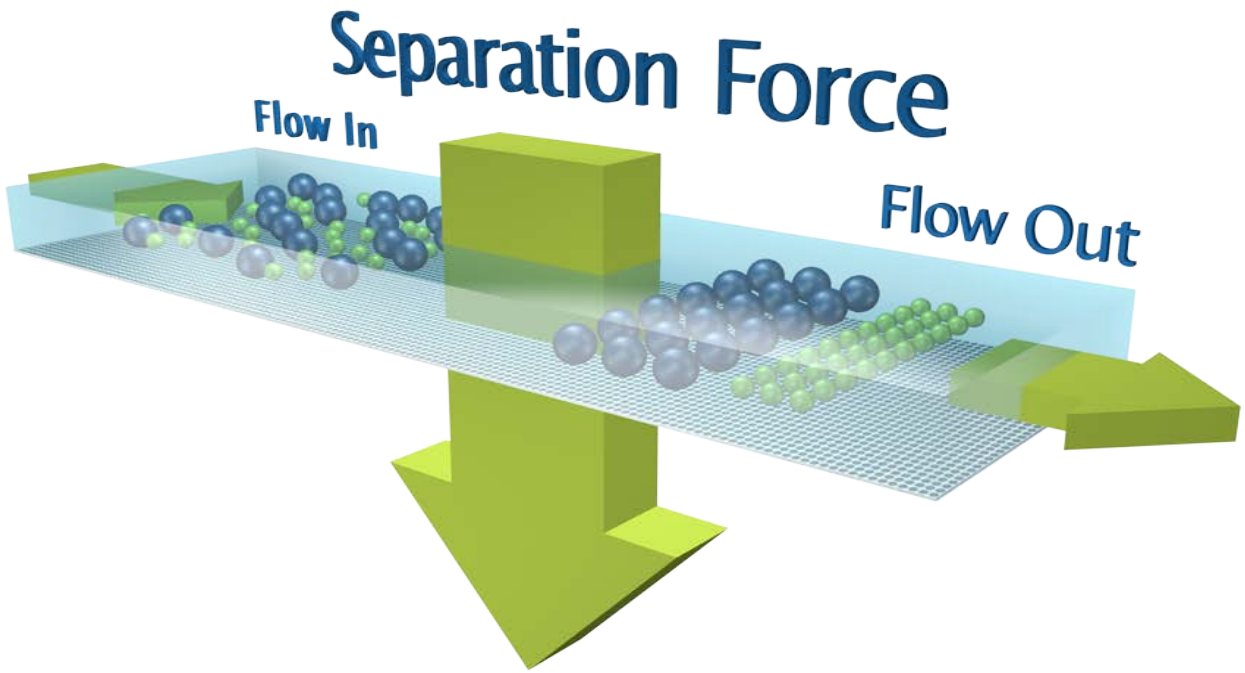
FFF Separation Range

Comparison Chromatography vs. Field-Flow Fractionation



FFF Principle

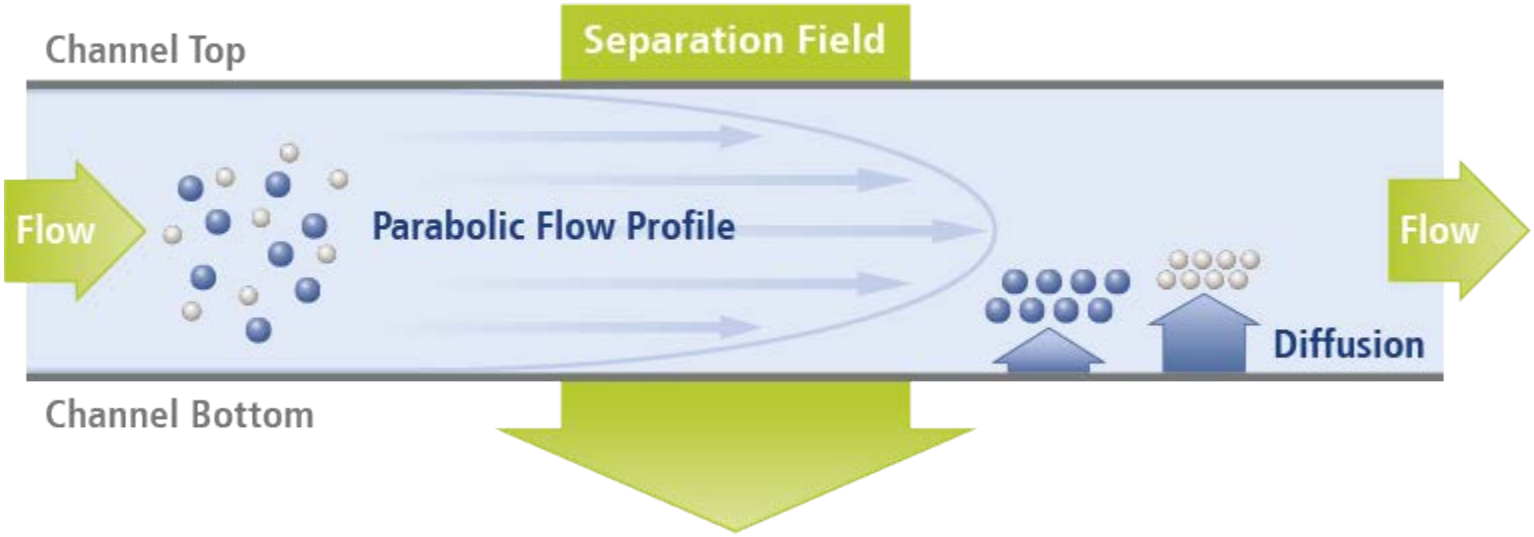
Separation Mechanism



- Separation in a narrow ribbon-like channel
- Laminar flow inside the channel
- External field perpendicular to the solvent flow

FFF Principle

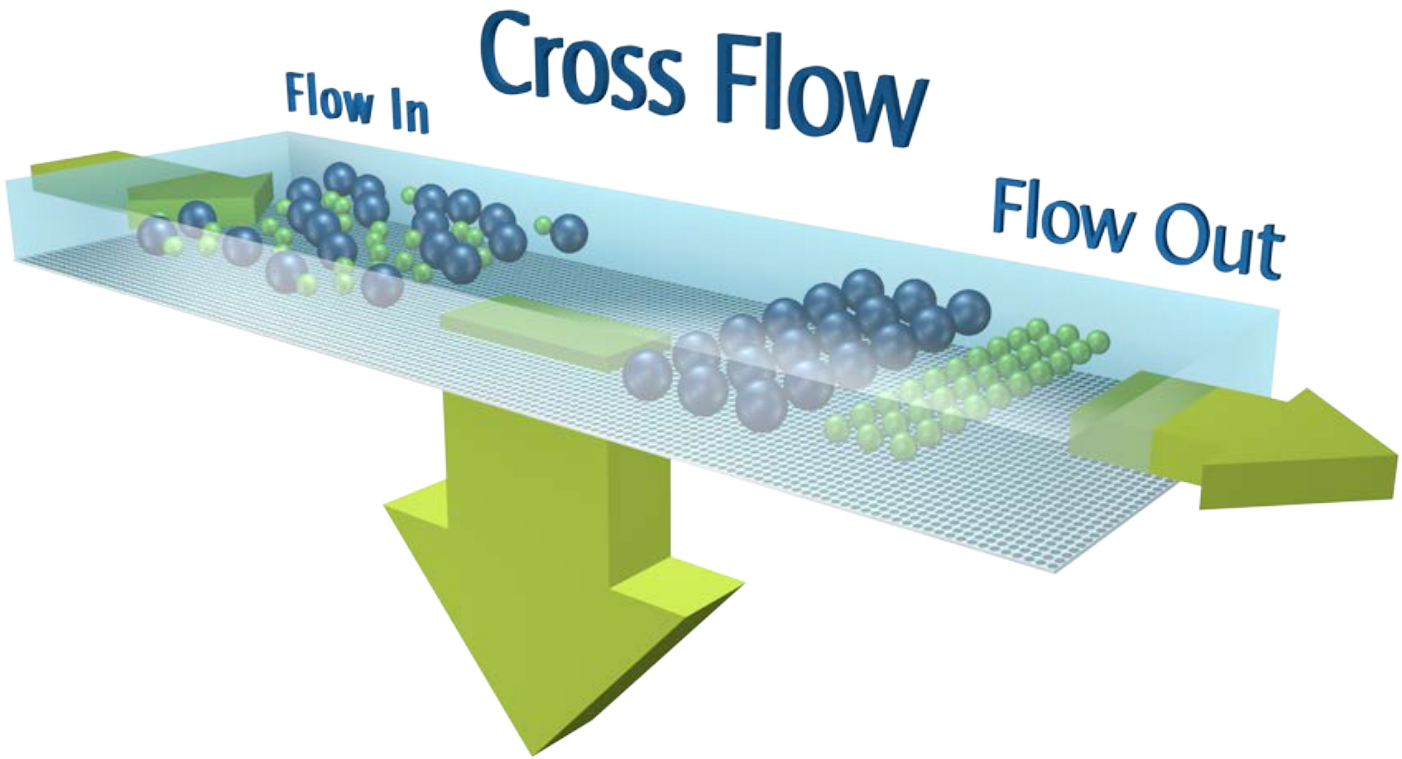
Separation Mechanism



- Centrifugal Field
- Flow Field
- Thermal Field
- Gravitational Field

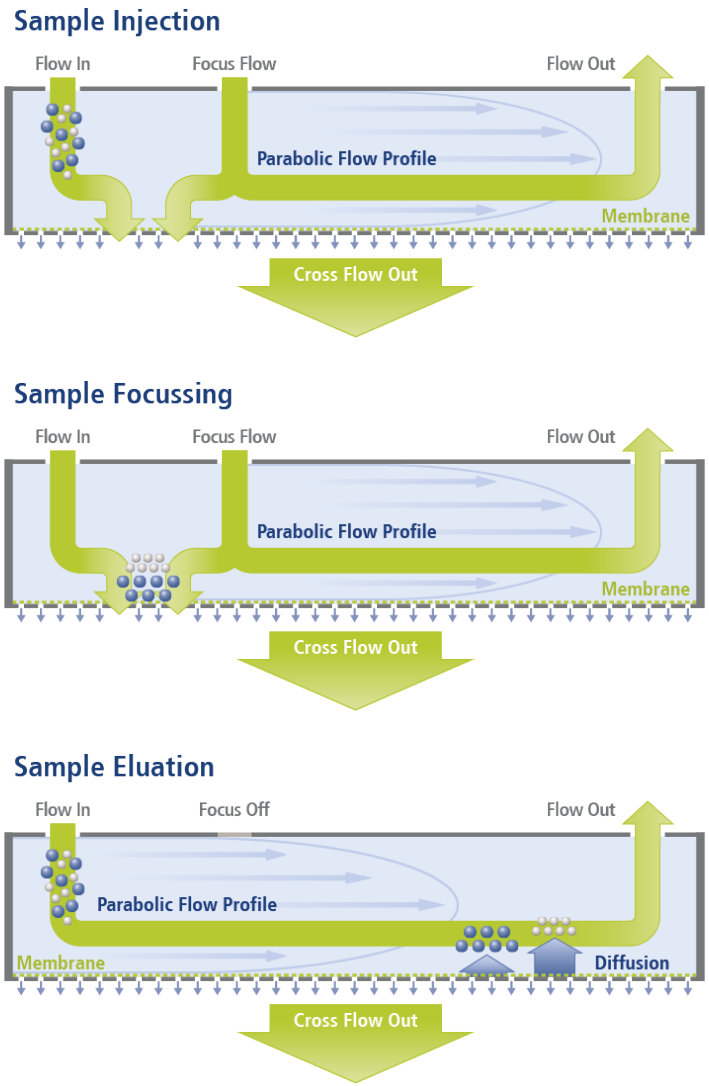
Flow FFF

Asymmetric Flow FFF - Principle



- Hydraulic Pressure Gradient Field (Cross-Flow) for Separation
- Separation based on Size
- Channel at Ambient, Mid or High Temperature

Asymmetric Flow FFF - Principle



Step 1

- Sample is injected directly into the flow stream
- A second pump provides focus flow
- Cross flow is achieved by a precise syringe pump
- Constant flow to the detectors, no valves

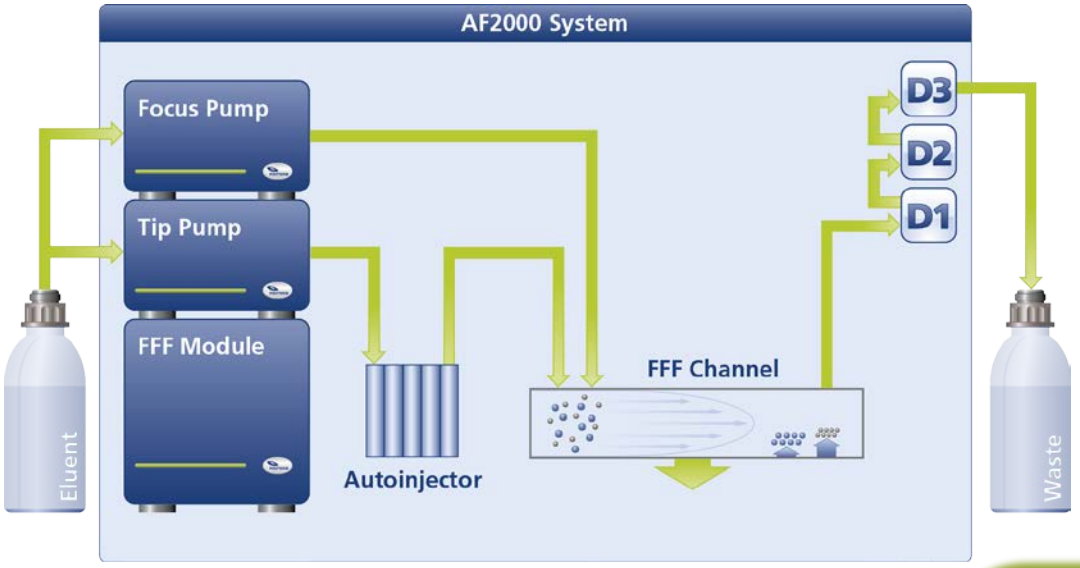
Step 2

- Sample is focused to narrow band
- Improved resolution and sample washing
- Enrichment of very low concentrations

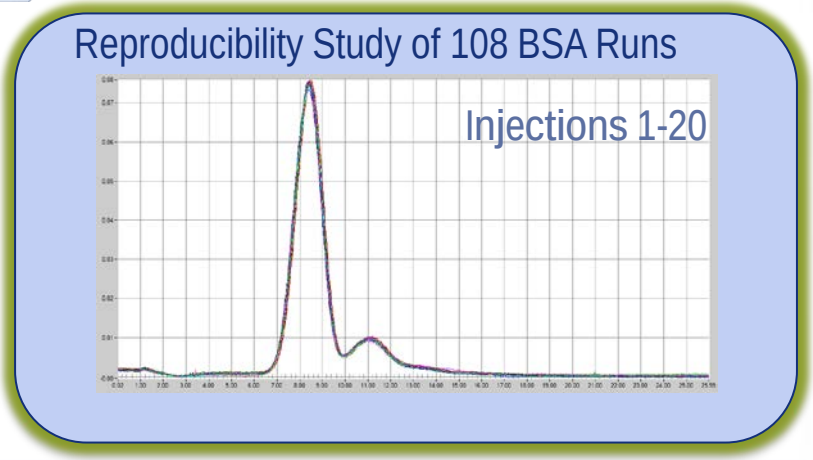
Step 3

- Focus flow stops and main pump elutes the sample
- Variable cross flow
- Detector flow stays constant

Asymmetric Flow FFF - Principle



- Tip Pump for Solvent Transport & Sample Injection
- Focus Pump for Sample Focusing
- X-Flow Pump for Sample Separation
- No Switching, Needle Valves In Flow Path
- Continuous Detector Flow without Interruption
- The most robust and stable AF4 Flow Path
- Patents 6,109,119 and 6,192,764



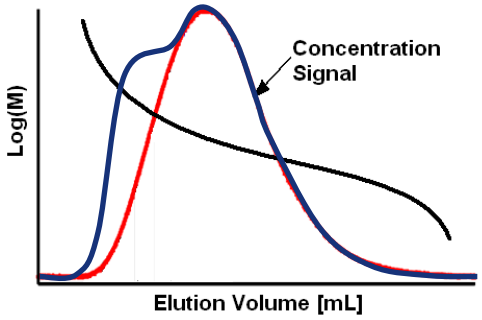
Why FFF versus SEC

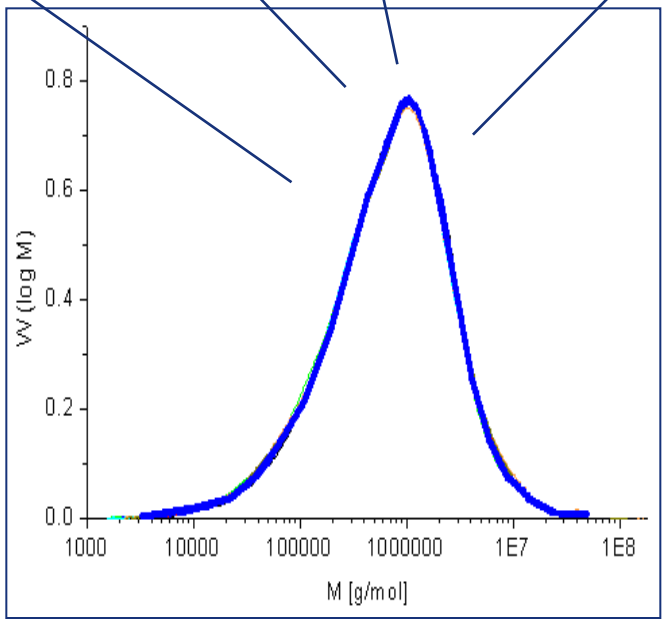
Shear degradation in porous columns / frits
Detected mass too low

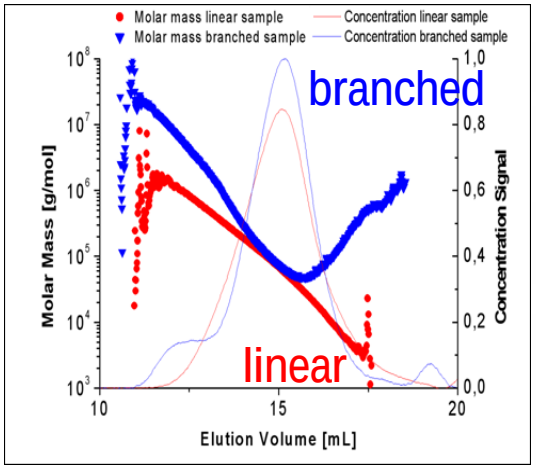
Filtration of gel, clogging of columns

Unwanted interaction with stationary phase
Wrong branching/MMD

Low separation for high M_w
Exclusion Limit

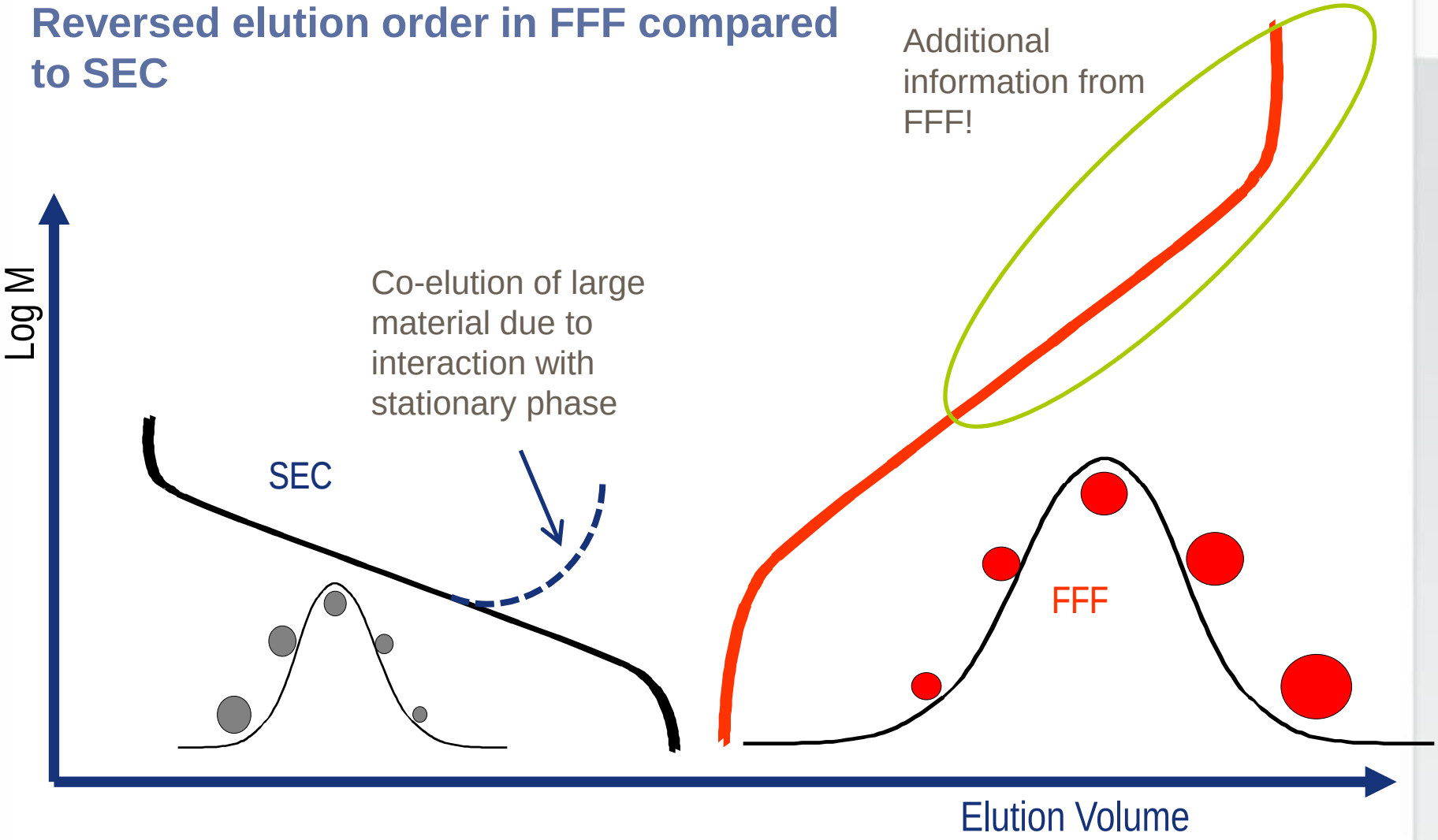






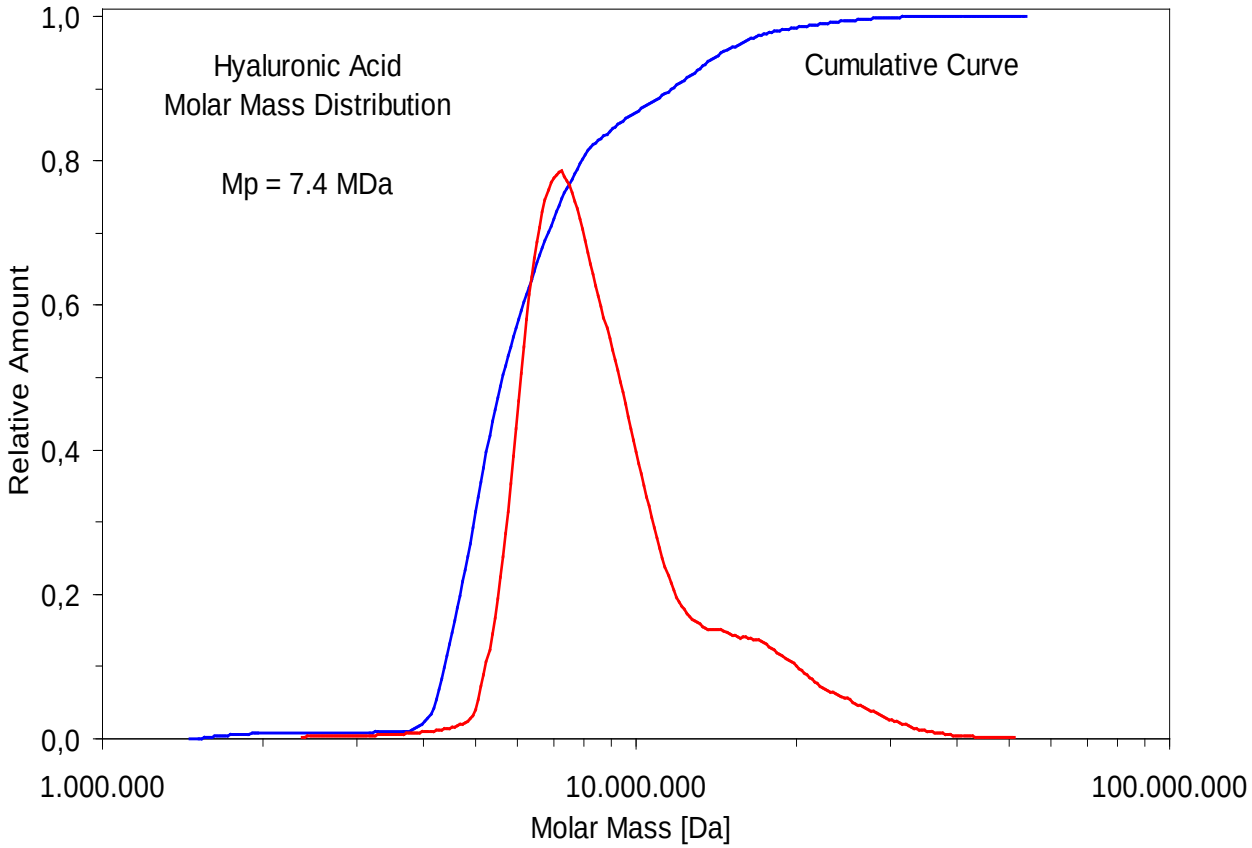
FFF versus SEC

Reversed elution order in FFF compared to SEC



AF2000 – Flow FFF

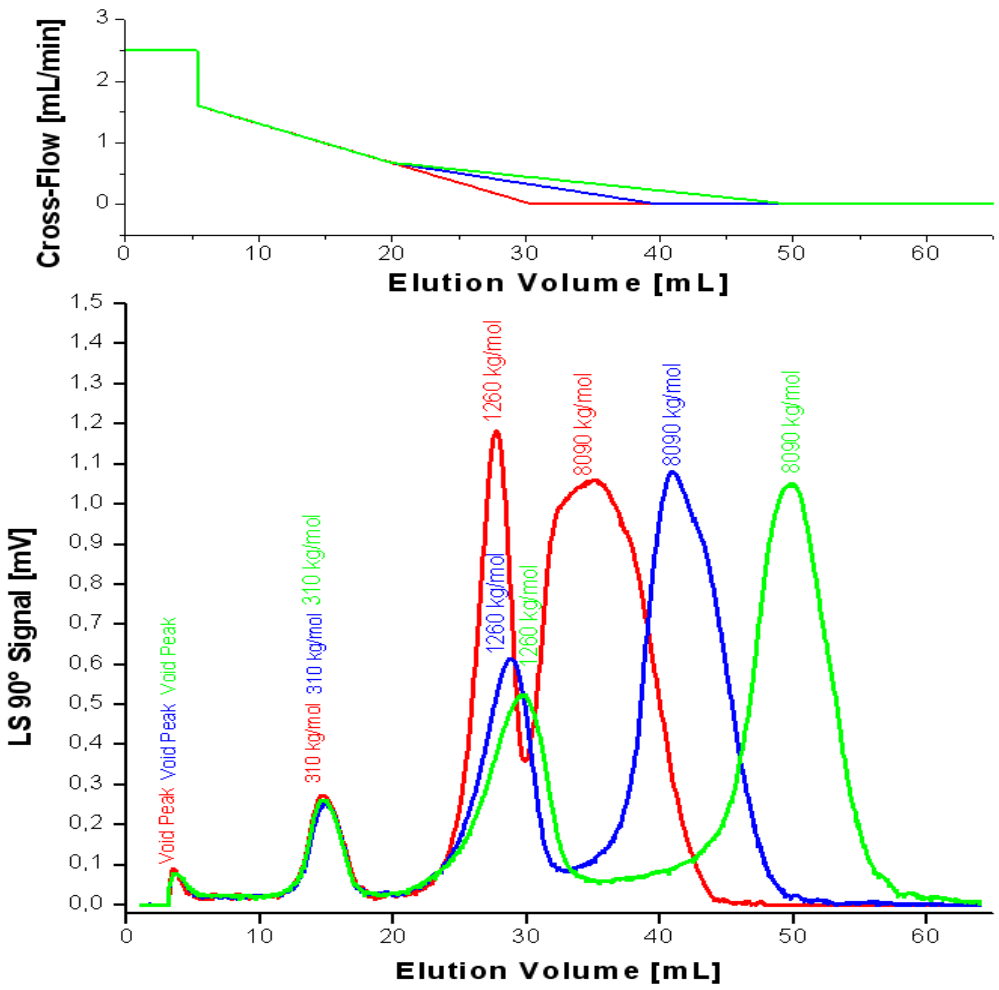
Separation and M_W of Hyaluronic Acid



Flexibility of the Cross-Flow Gradient

“Tailor-made” separation in AF4 realized by cross-flow-adjustment

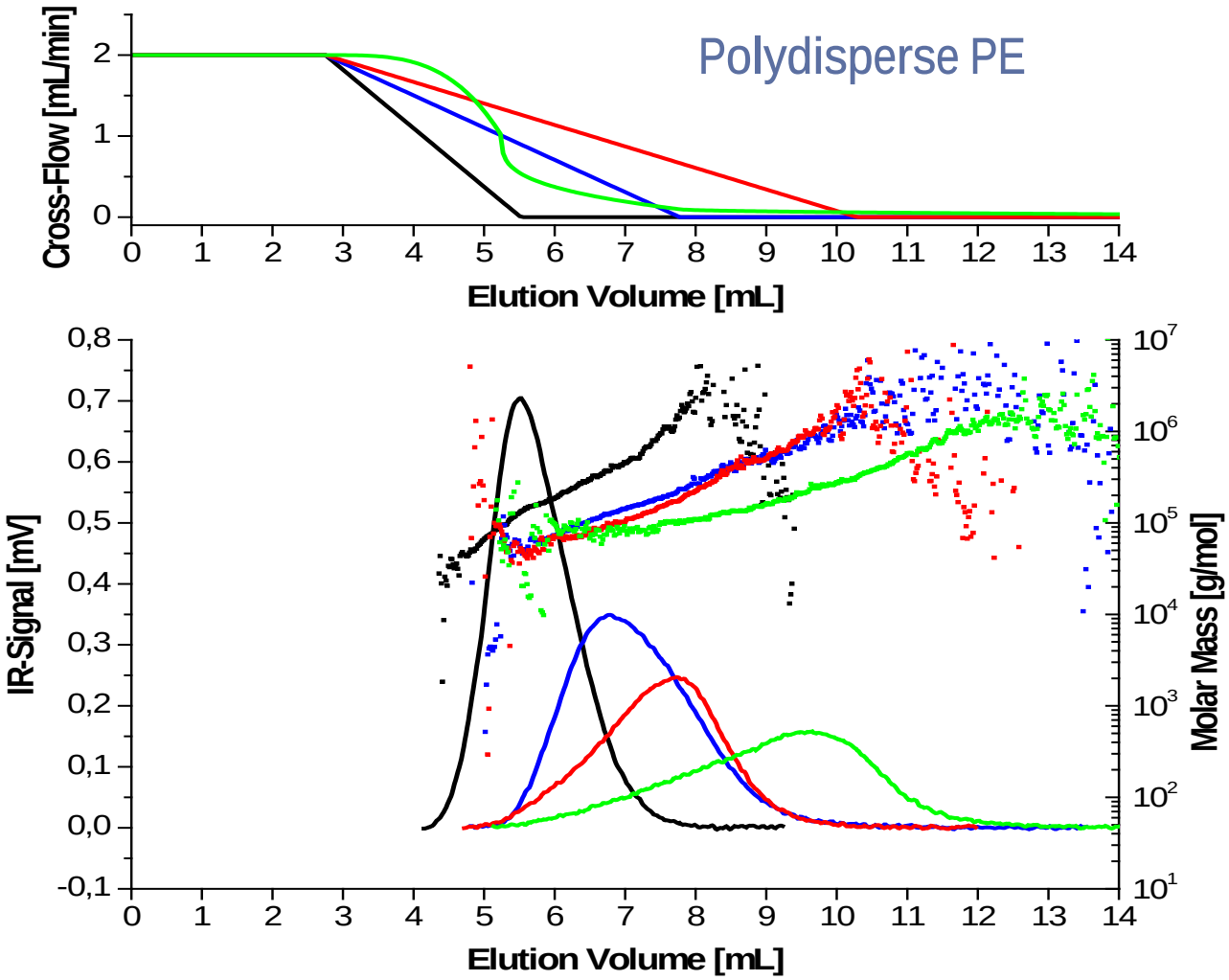
- Extension of Cross-Flow causes better separation
- Selective enhancement of separation
- In SEC not possible!
  - → Column change is time consuming and expensive
  - → Calibration of column determines separation



Mixture of narrow PS: 300, 1200 & 8000 kg/mol

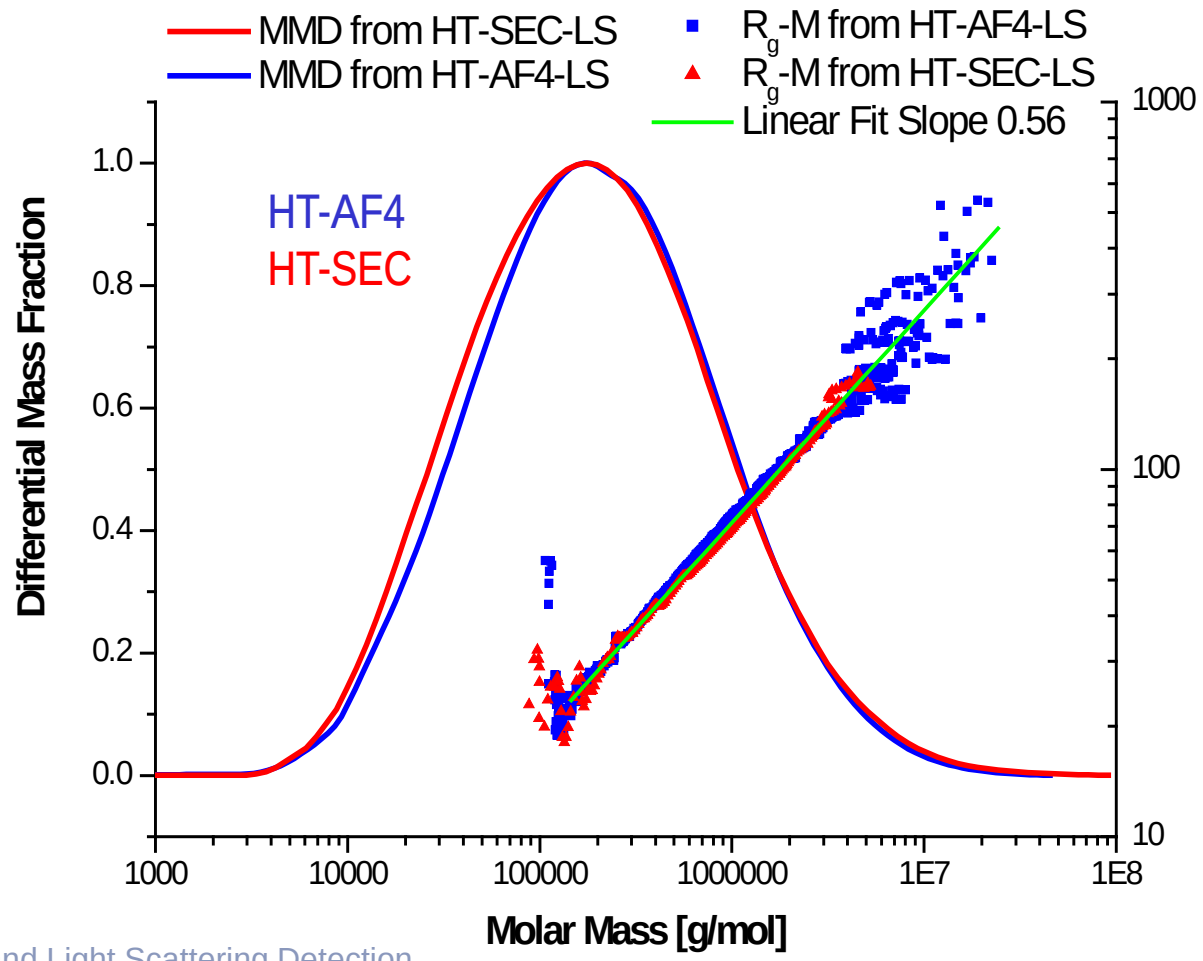
Flexibility of the Cross-Flow Gradient

- Gradient of any shape can be used
- Adjustment of separation according to special requirements of the sample



HT-AF4 vs. HT-SEC in Overlap Size Range

Both separation methods deliver the same molar mass distribution!

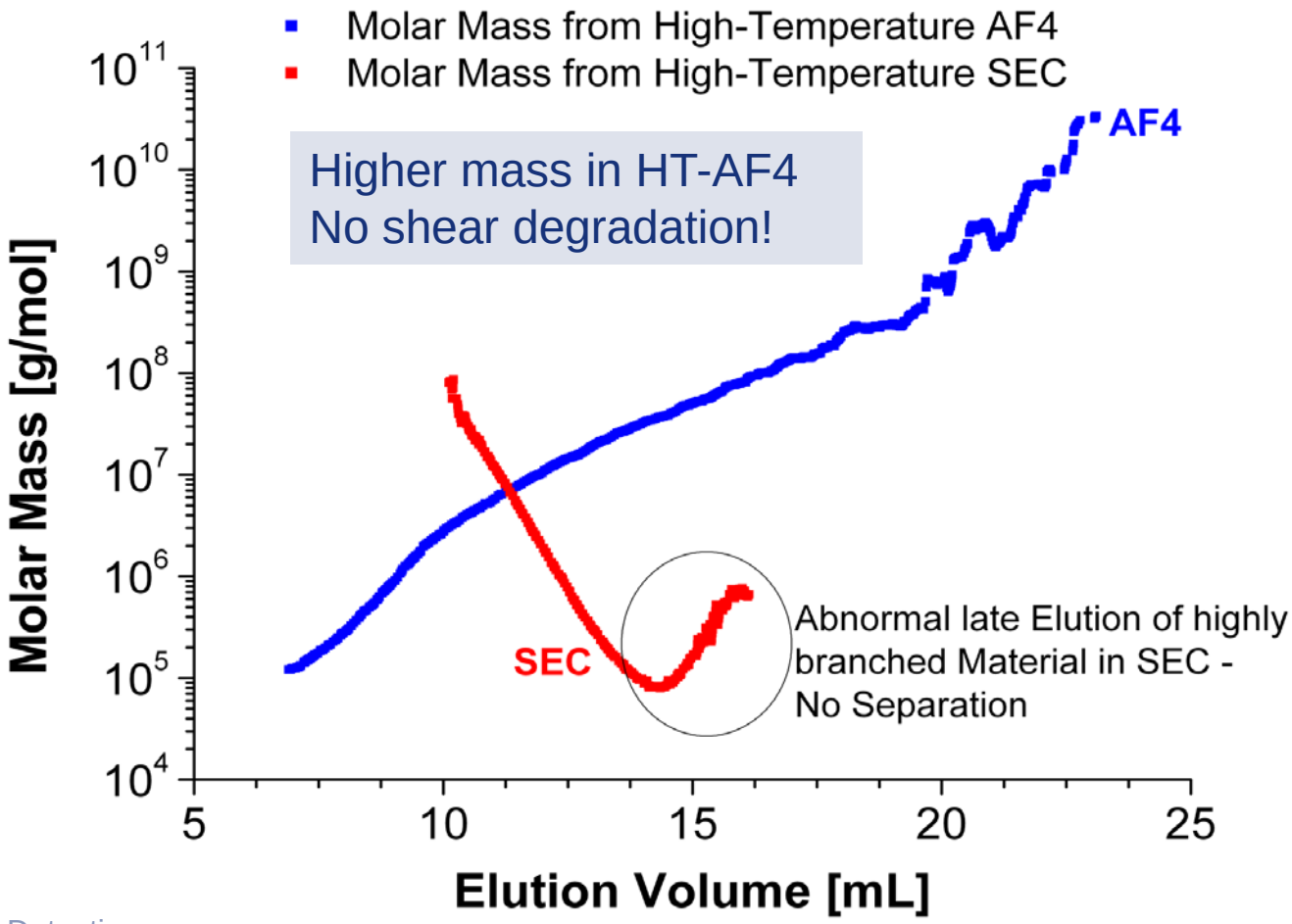


- Slope of R_g-M is similar for AF4 and SEC
- Theoretical value for linear material of 0.56-0.58 indicates proper separation with both methods

IR4 and Light Scattering Detection

Problematic Material: LDPE
HT-AF4 vs. HT-SEC

AF4 vs. SEC separation of highly branched LDPE



IR4 and Light Scattering Detection

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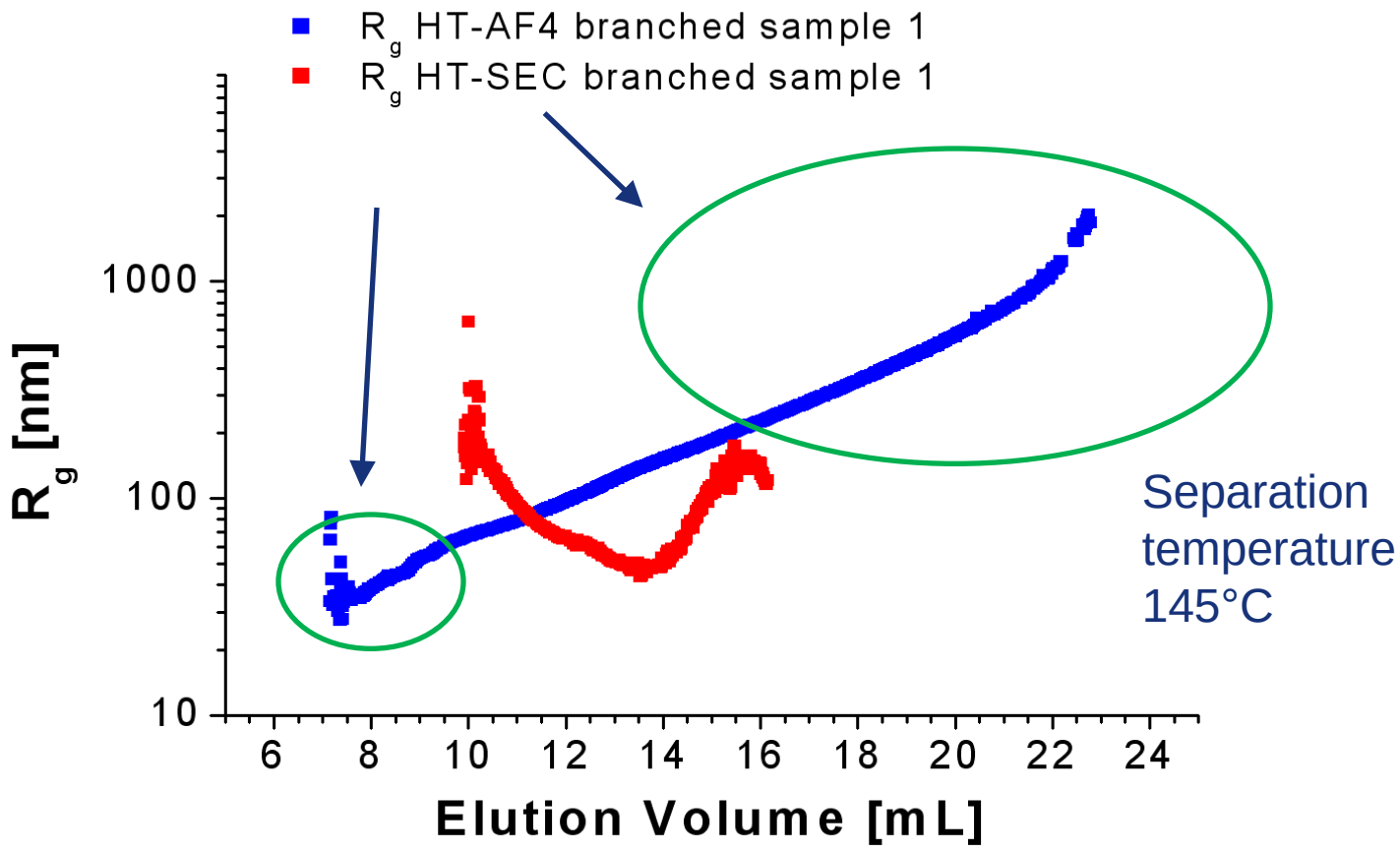


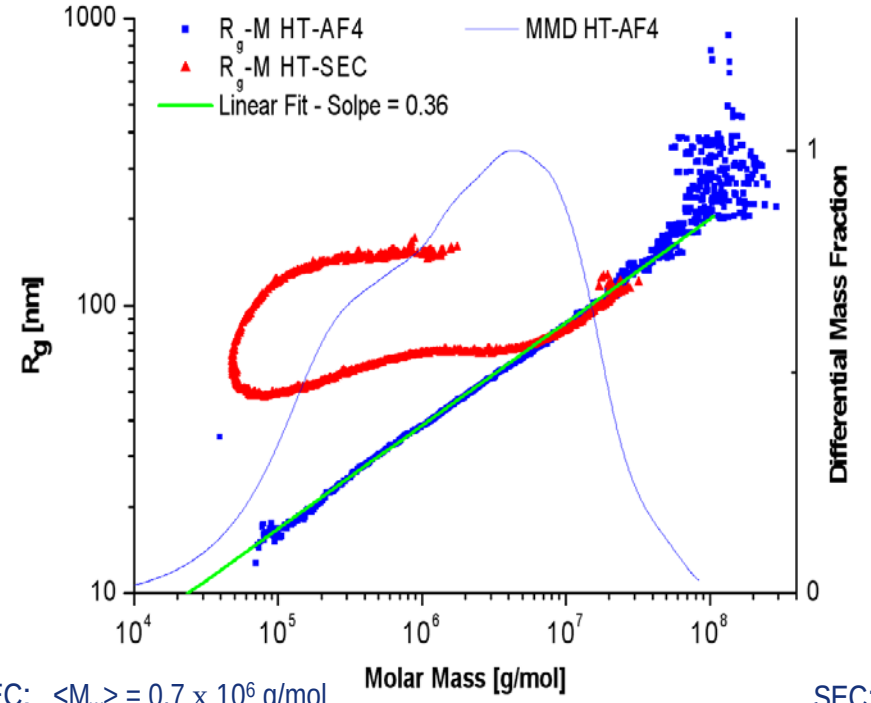
IR4 and Light Scattering Detection

FFF Applications - LDPE

Conformation plot AF4 vs. SEC - LDPE

- Correct branching calc. possible in AF4 → highly branched species
- Extremely high molar mass material separated without shear degradation

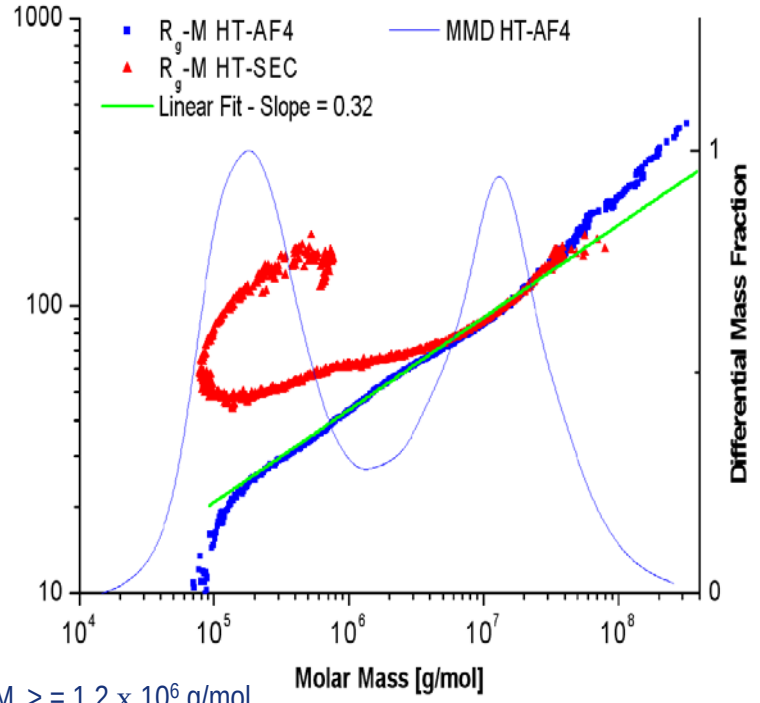
Sample 1



SEC: $<M_w>$ = 0.7 x 10^6 g/mol

AF4: $<M_w>$ = 5.7 x 10^6 g/mol

Sample 2



SEC: $<M_w>$ = 1.2 x 10^6 g/mol

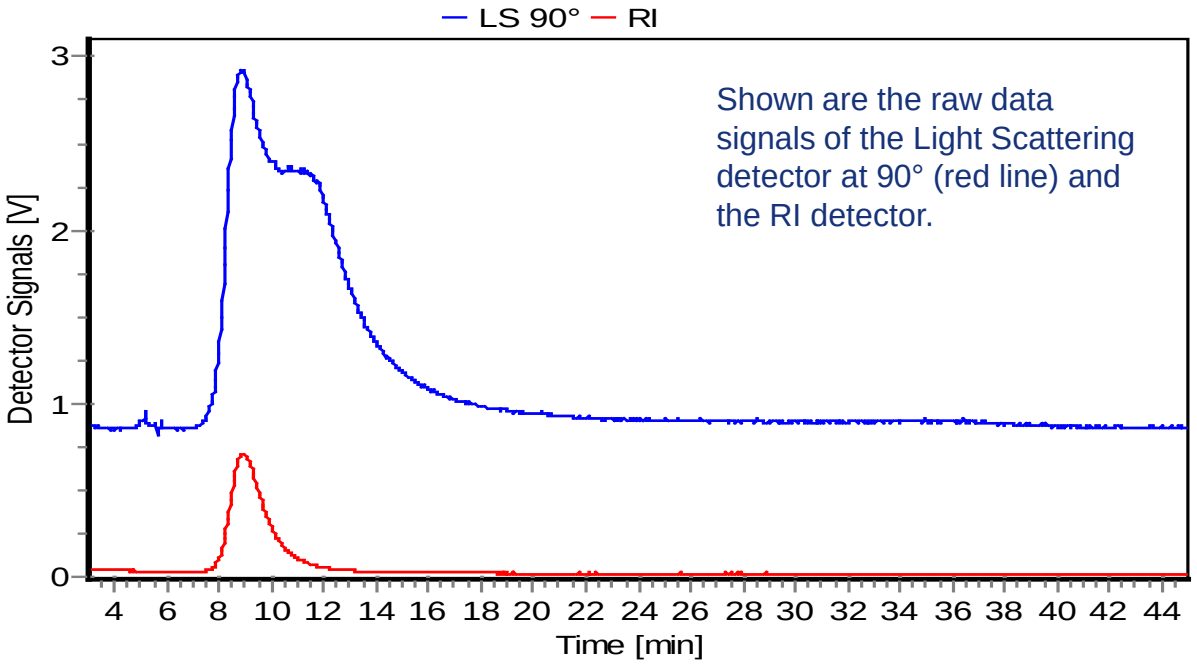
AF4: $<M_w>$ = 10.2 x 10^6 g/mol

Polyelectrolyte Analysis

Strong Polyelectrolyte Free Polyelectrolyte Prior to Crosslinking

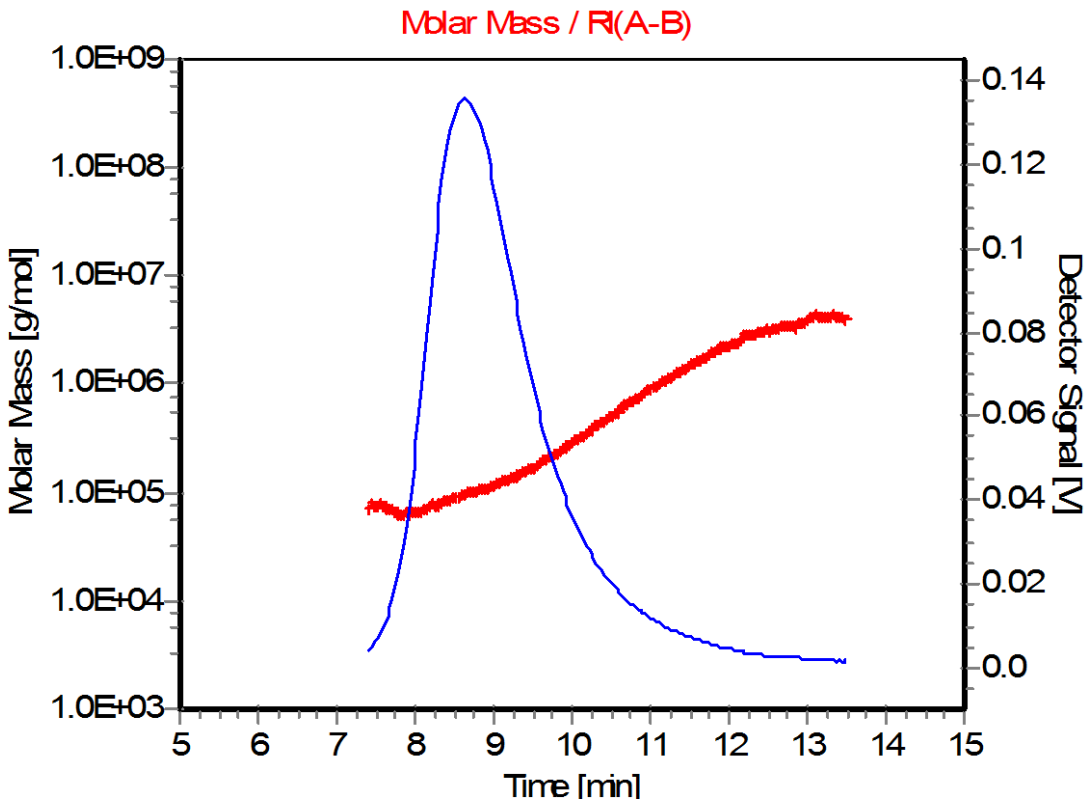
Sample PA1 this sample is extremely difficult to elute from Aqueous SEC columns and Polymers of this type are often eluted using organic solvents such as DMF/DMSO/DMA with salts

This separation was achieved using adjusted pH and low salt solution on a AF4 membrane



Aggregation

The Polyelectrolyte distribution via AF4 provides aggregation state of the polymer in aqueous solutions



Calculated Molar Mass of sample PA1(red crosses) and RI detector signal (blue line) plotted against elution time.

The Molar Mass calculation was performed using the Light Scattering and the RI

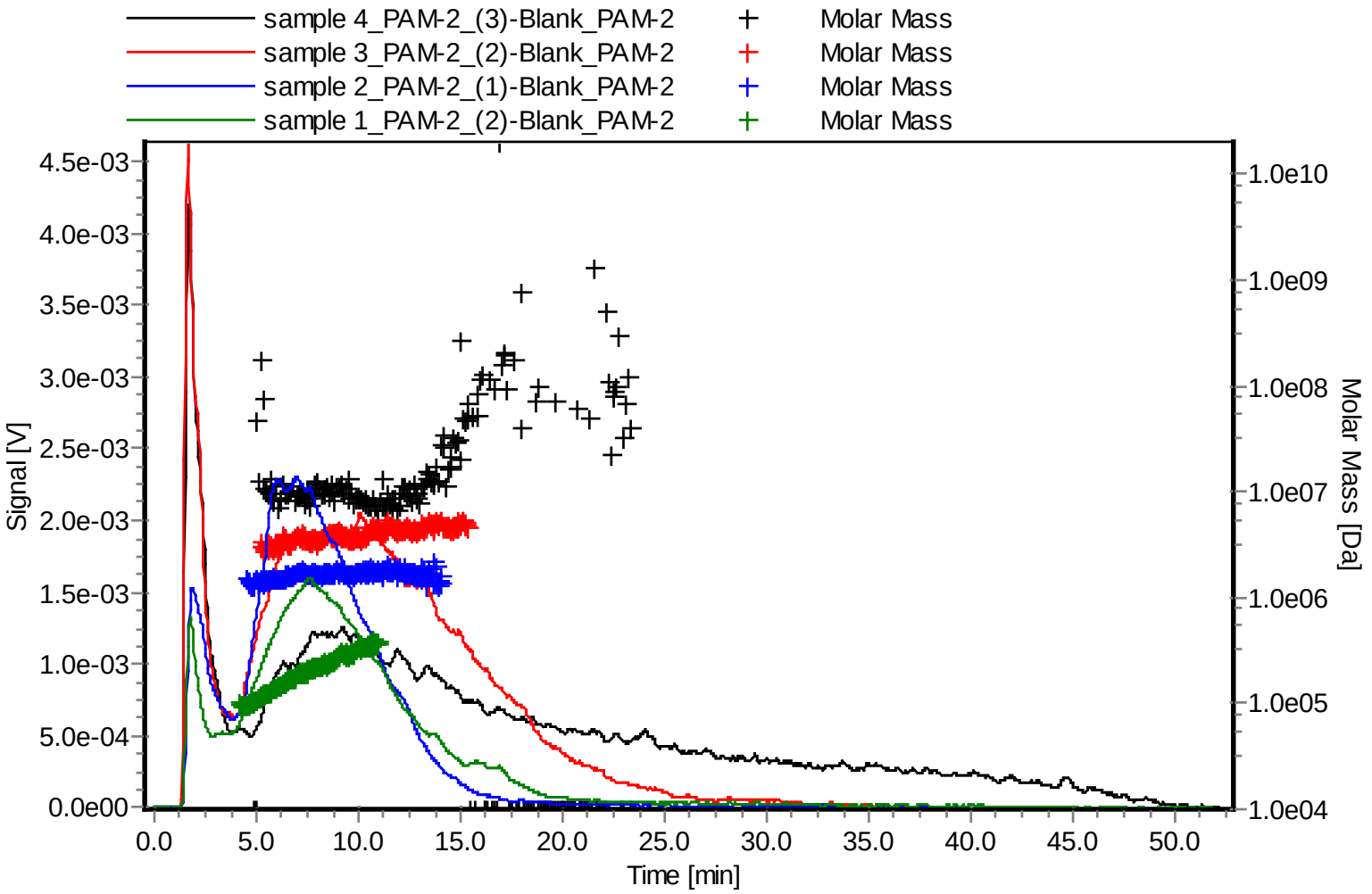
Characterization of Polyacrylamide samples using AF4-MALS for Crude Oil Recovery Wells

System Characteristics	
Asymmetrical Flow FFF	AF2000 MT
Refractive Index detector	PN3140
Multi angle Light Scattering	PN3621

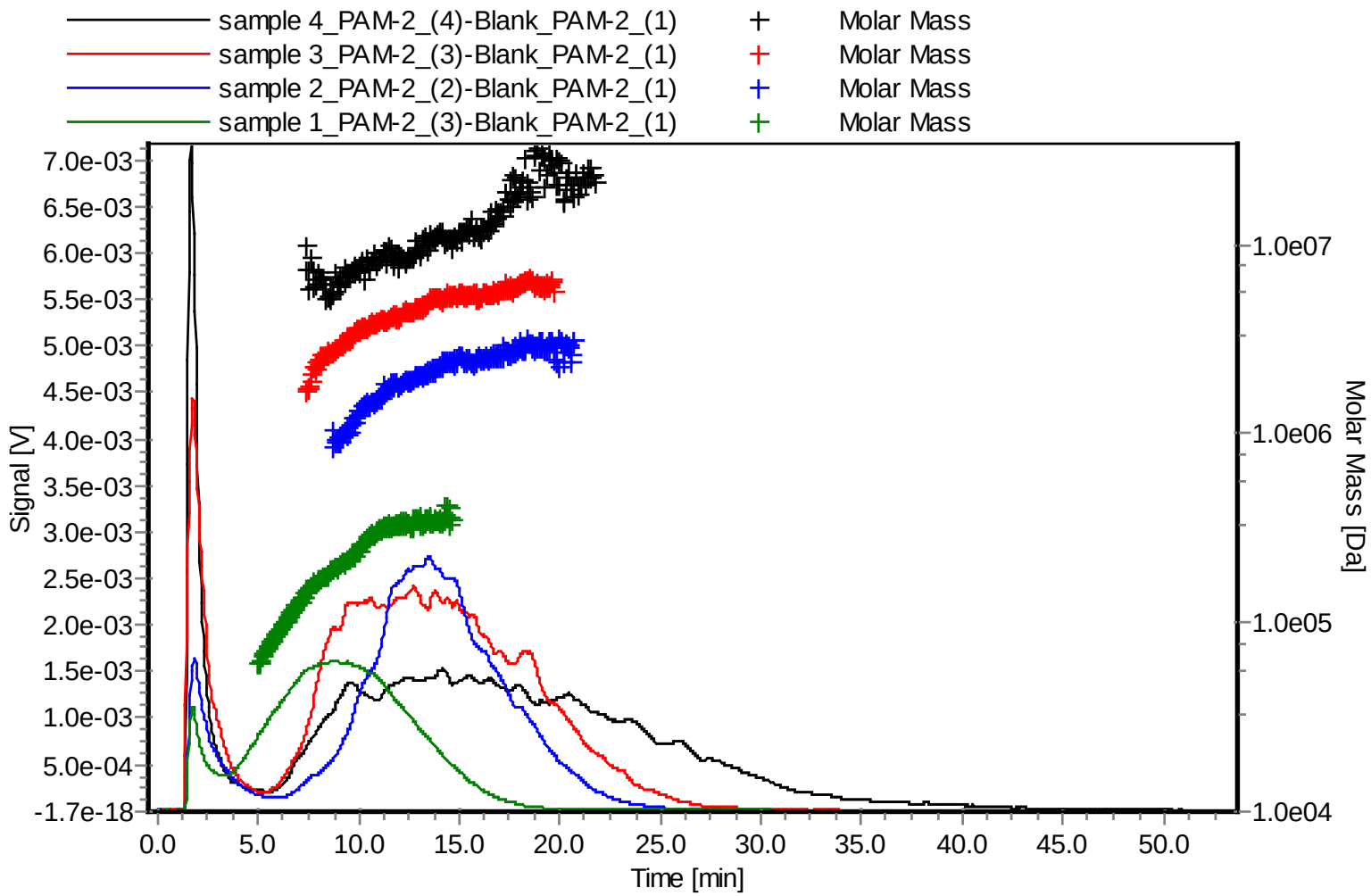
Sample	Conc. (g/mL)	Injected mass (µg)
1	3.0	150
2	2.4	120
3	0.4	40
4	1.0	50

Method	
Cross flow rate	0.5 mL/min decay
Channel flow rate	0.5 mL/min
Focus flow rate	0.8 mL/min
Injection flow rate	0.2 mL/min
Focusing time	3 min
Focusing position	8.9 cm
Channel thickness	300 µm
Membrane	10 kDa RC

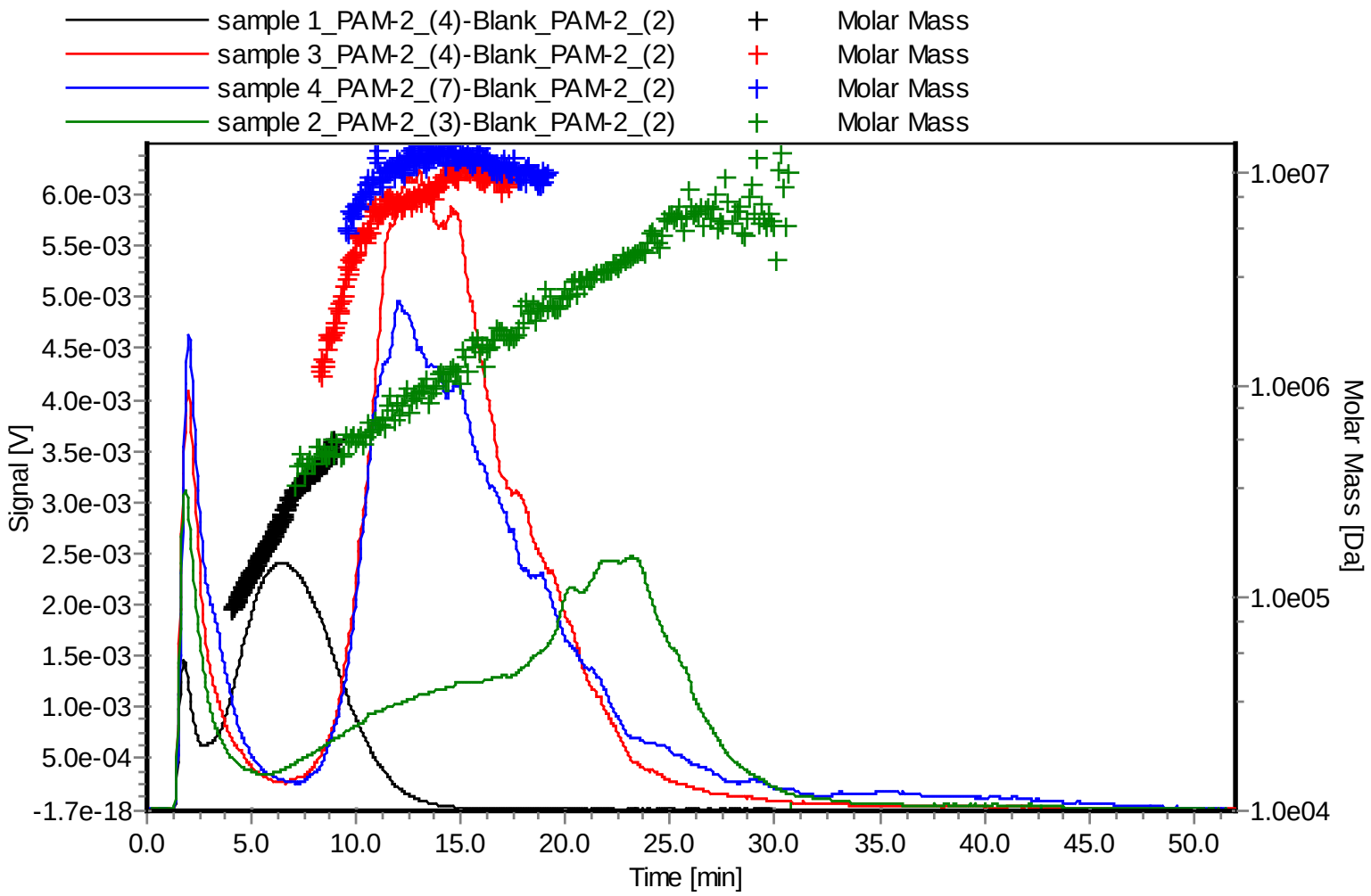
Polyacrylamide run in 1000 ppm NaOH solution pH13



Polyacrylamide run in 150 mM NaCl solution at pH 7.4



Polyacrylamide run 3 mM NaN_3 solution with a pH of 3



Observed Changes in Molar Mass and Radius of Gyration Calculations using SLS theory

The running buffer was a 3 mM NaN_3 solution with a pH of 3. A dn/dc value of 1.87 was assumed for all samples.

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	R_n (nm)	R_w (nm)	R_z (nm)	PDI
1	1.71E+5	2.14E+5	2.68E+5	22.6	23.4	24.7	1.25
2	1.30E+6	2.58E+6	4.00E+6	67.9	96.4	125.1	1.98
3	6.27E+6	7.63E+6	8.31E+6	170.4	179.8	183.7	1.21
4	1.04E+7	1.08E+7	1.11E+7	205.8	207.4	208.7	1.03

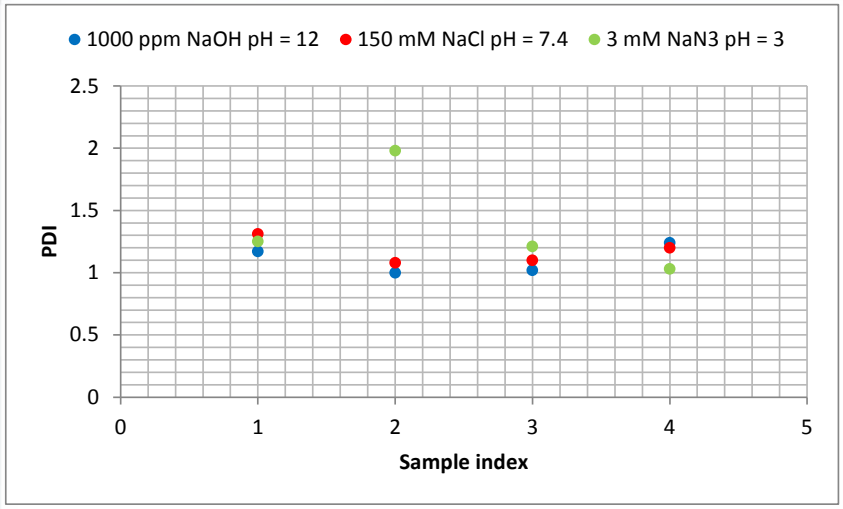
The running buffer was a 150 mM NaOH solution with a pH of 7.4.

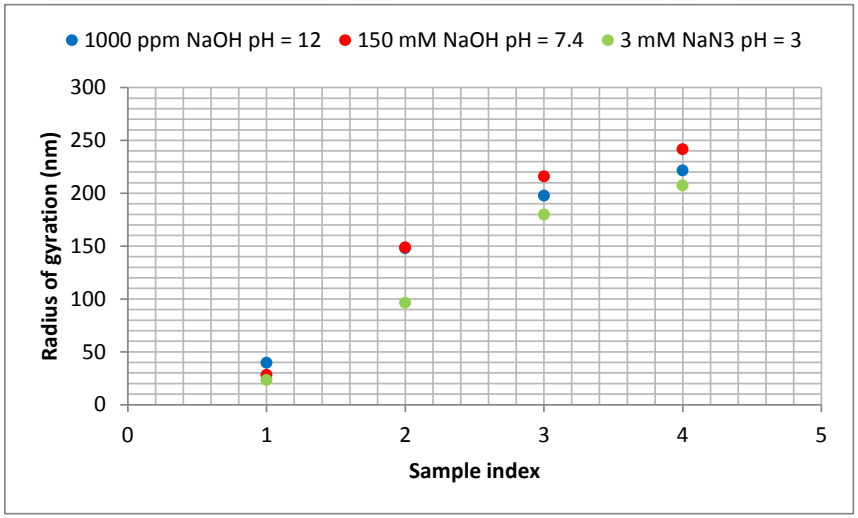
1	1.43E+5	1.88E+5	2.34E+5	23.0	28.1	33.4	1.31
2	1.97E+6	2.13E+6	2.26E+6	143.9	148.7	152.6	1.08
3	4.12E+6	4.53E+6	4.86E+6	208.9	215.9	221.9	1.10
4	9.86E+6	1.18E+7	1.45E+7	234.5	241.5	249.4	1.20

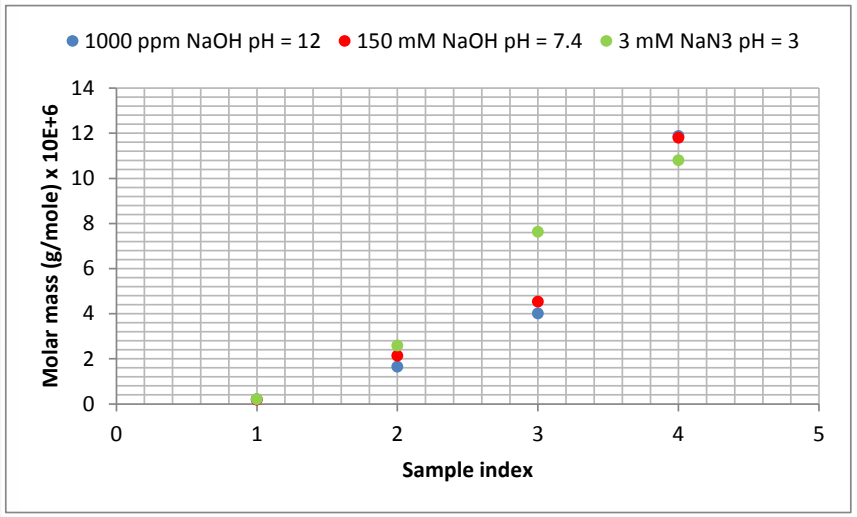
The running buffer was a 1000 ppm NaOH solution pH 13

1	1.69E+5	1.98E+5	2.29E+5	38.2	39.5	42.8	1.17
2	1.64E+6	1.645E+6	1.66E+6	148.9	148.9	149.7	1.00
3	3.92E+6	4.01E+6	4.10E+7	196.4	197.7	199.3	1.02
4	9.55E+6	1.187E+7	1.35E+8	221.7	221.5	219.8	1.24

The effect of pH on Aggregation states of Poly-Acrylamide UHMWP







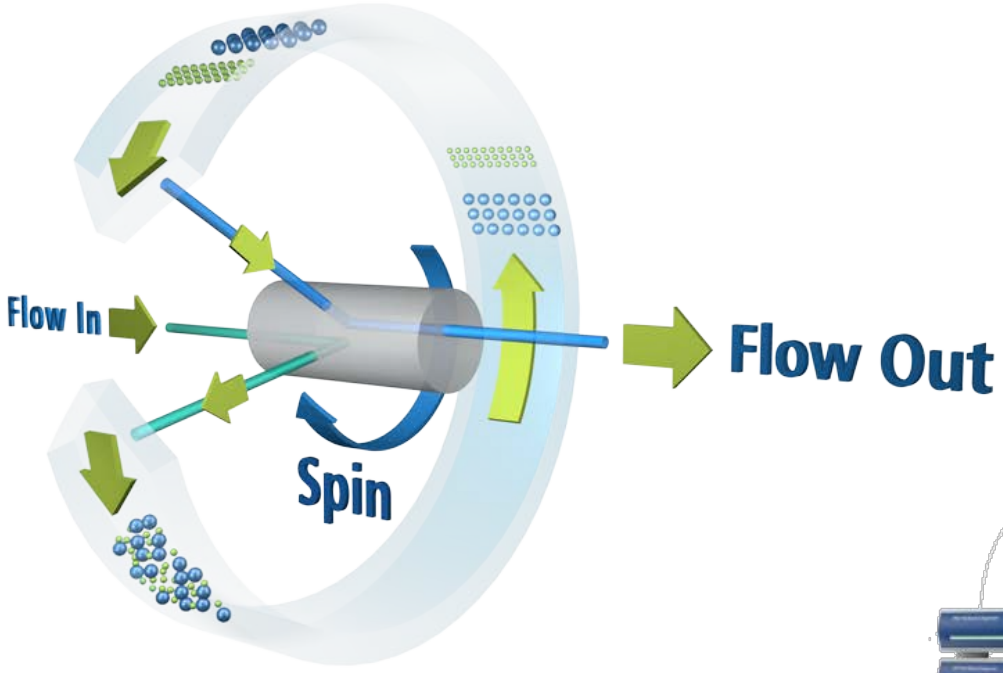
FFF excels well beyond the capabilities of SEC with the ability to run a wide range of pH states, temperature and cross flow conditions to characterize the UHMWP polymers behavior in solution. This capability is unique to FFF and is what sets the technique apart from SEC.

Analysis carried out by Postnova USA, Dr. Soheyl Tadjiki

Centrifugal FFF

Centrifugal FFF – Principle

Separation Principle



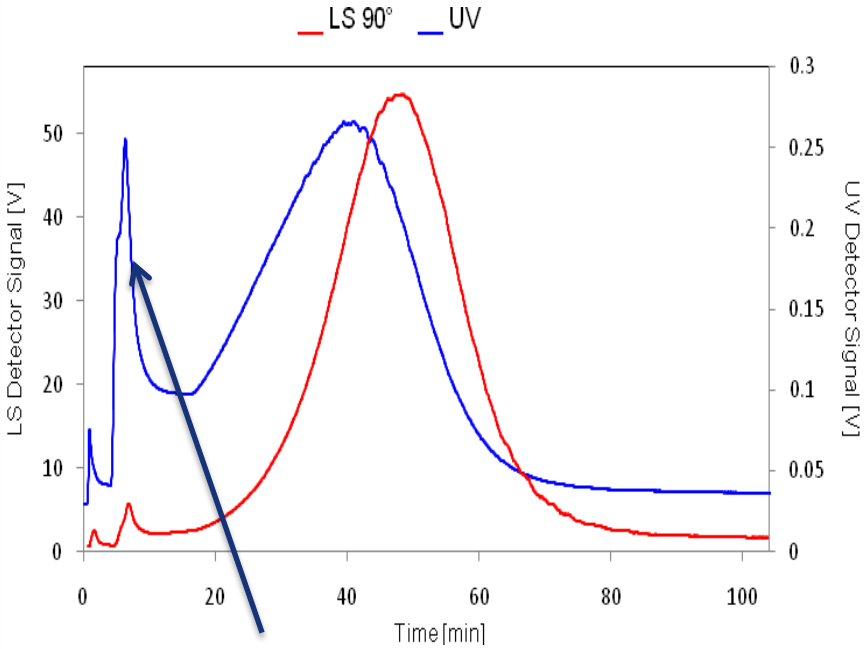
Content

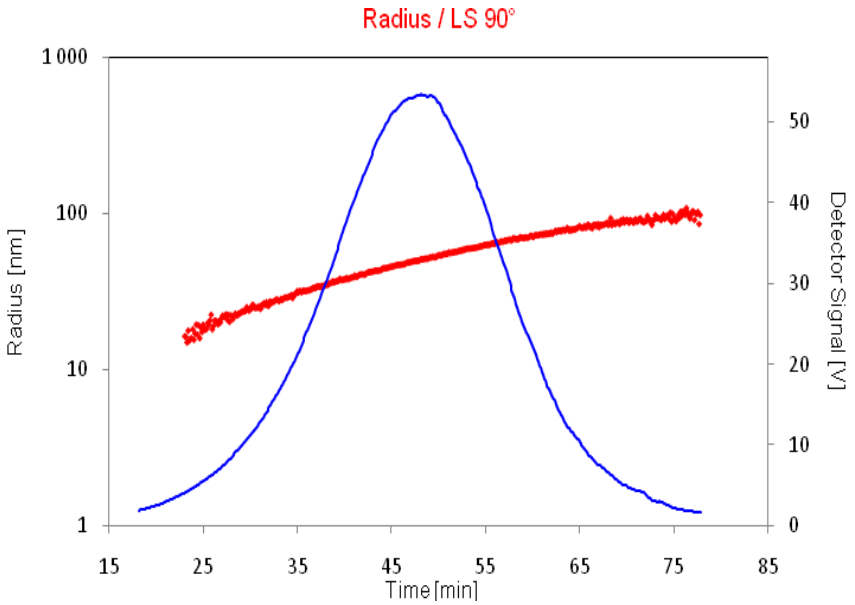
- FFF Principle
- Systems
- Applications
- Summary

- Gravity Separation Field up to 2.500 g
- Size Separation Range: Particles 5 nm – 100 µm
- Separation based on Size and Density

Polyelectrolyte Encapsulated Around NanoParticle

Shown are the raw data signals of the Light Scattering detector at 90° (red line) and the UV detector at 254 nm using Centrifugal Field Flow Fractionation CF3

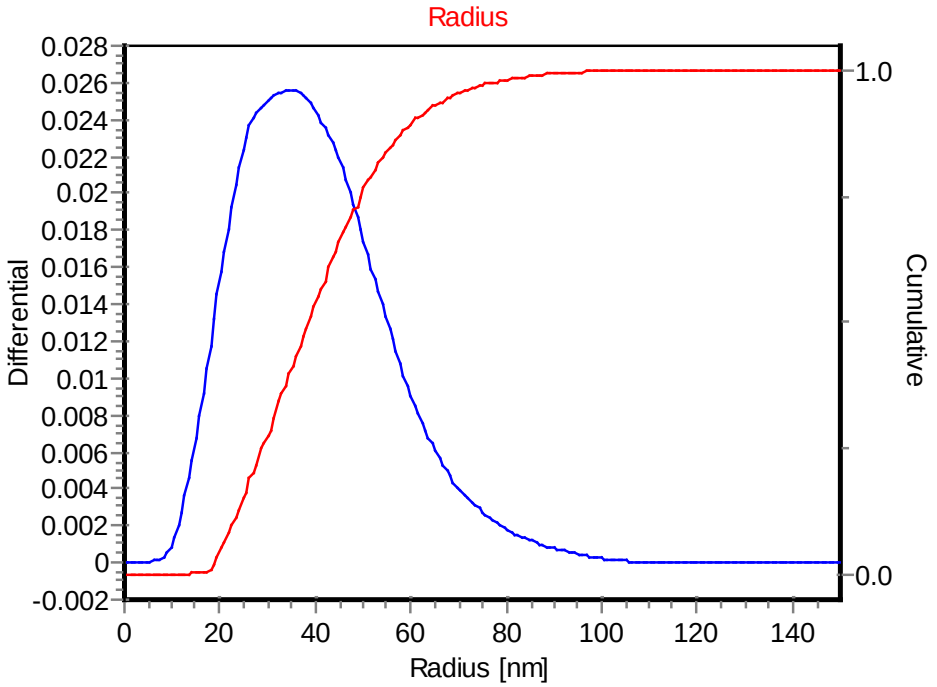




Using Centrifugal FFF (CF3) we can separate the free Polyelectrolyte from the cross-linked encapsulated nanoparticle.

Encapsulated Nanoparticle with Cross-linked Polyelectrolyte Particle Distribution using CF3

In the following figures the particle size distribution is shown:
Differential Radius Distribution (blue line) and Cumulative Radius Distribution (red line) of the second peak of sample.

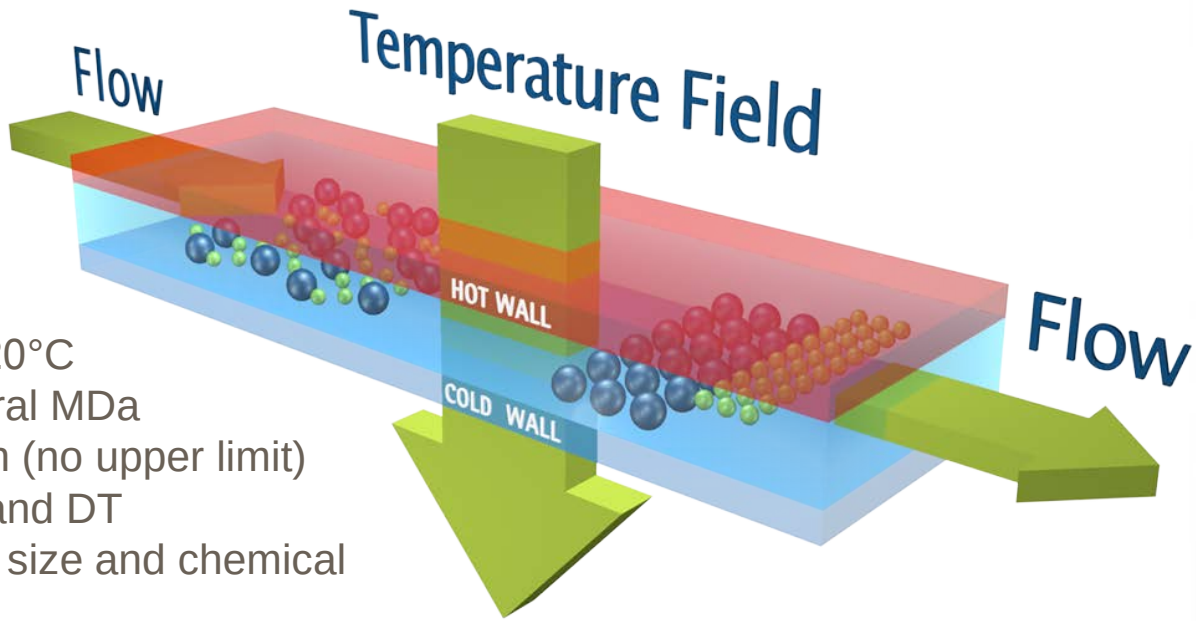


	Rg [nm]
n-Average	30
w-Average	41
z-Average	55

Thermal FFF

Thermal Field-Flow Fractionation

TF3 involves a hot and cold plate to generate a temperature gradient perpendicular to the separation channel. Thermal Diffusion occurs and this us often a property of the polymers morphology.

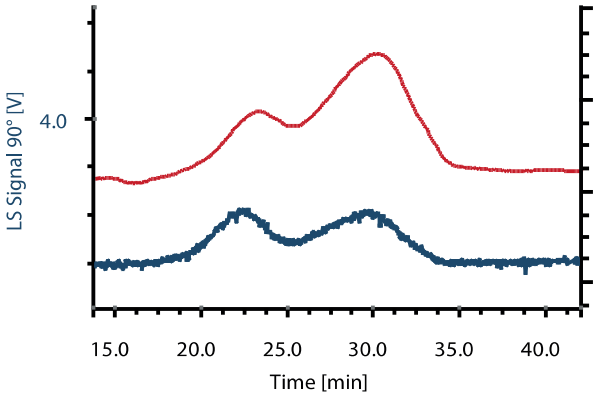


- Thermal gradient up to Δ120°C
- Separation kDa up to several MDa
- Analysis time, 10 – 120 min (no upper limit)
- Separation depends on D and DT
  → Separation according to size and chemical composition

SEC versus Thermal FFF

Analysis of PS and PMMA by SEC and Thermal FFF

PS, PMMA and a mixture of both standards in THF. Taking advantage of the separation by chemical composition in TF3.



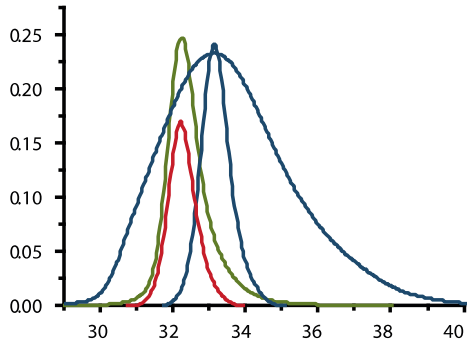
TF3 Fractogram showing RI and LS Signal of mixed PS-PMMA standards.(Δ T = 115 K).

Component	Retention Time (R_T)	Molecular Mass (M_w)
PS	19.1 - 24.7 min	95.7
PMMA	26.2 - 34.5 min	104.4

SEC versus Thermal FFF

Investigation of PEO-PS Homopolymer mix by Thermal FFF

PEO 116g kg/mol
PS 63 kg/mol
PS-co-PEO 92 kg/mol
PS-co-PEO 134 kg/mol



SEC Elugram of PEO, PS and PS-co-PEO polymer standards of comparable random coil volume

TF3 Fractogram of PS and PEO with partial peak separation

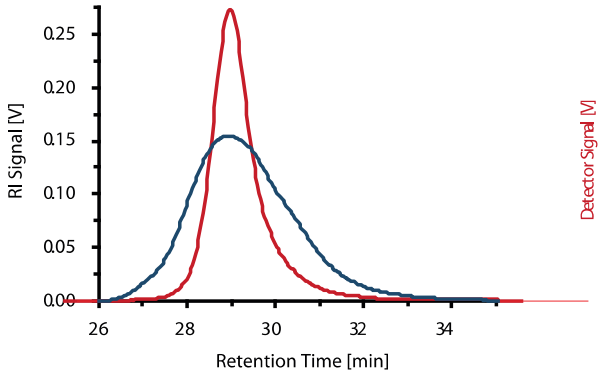
Concentration determination of homopolymer standards by RI using specific dn/dc values

ent [K]

SEC versus Thermal FFF

Investigation of PEO-PS Homopolymer mix by Thermal FFF

PS 319 kg/mol
PEO 496g kg/mol



SEC Chromatogram of PS and PEO showing no separation

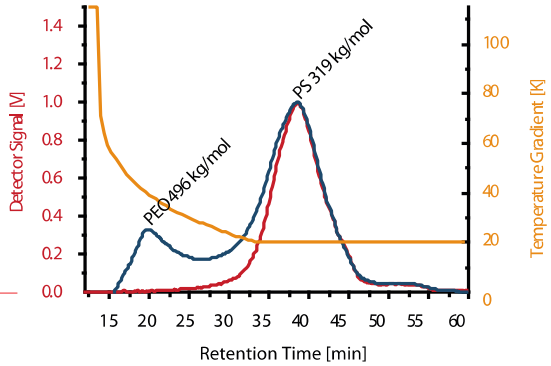


Figure 11: TF3 Fractogram of PS and PEO with partial peak separation

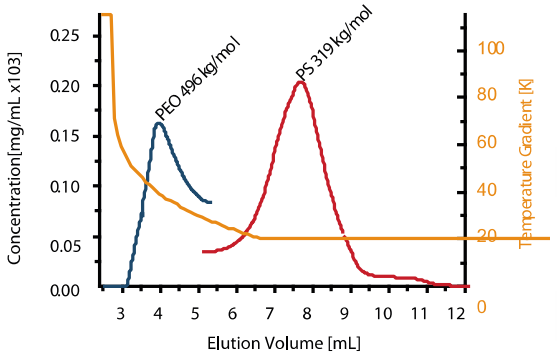


Figure 12: Concentration determination of homopolymer standards by RI using specific dn/dc values

$dn/dc_{PEO} = 0.068$ mL/g
$dn/dc_{PS} = 0.165$ mL/g

Partial Resolution of both PEO – PS homopolymer samples are achieved where The sampled demonstrate comparable hydrodynamic volume

Presentation Ends Here!! Conclusion

- **Field Flow Fractionation Complements SEC/GPC in standard separations**
- **FFF solves problems when the mass or particle size of the macromolecule exceeds the pore size of the column**
- **FFF solves the problem when the interaction between the macromolecule and the packing material limits the solvent and pH choice**
- **It solves the problem when morphology is more important than straight size**
- **FFF solves the problem when the macromolecule is reactive and unstable and the removal of packing and solvent control enables sensible elution characteristics**
- **FFF can be tailored to the application**
- **FFF is Great Fun to do in the lab!!**