The Postnova Characterization Platform

Characterization of Polymers, Proteins and Particles by FFF and SEC with Advanced Detection

Paul Clarke

Postnova Analytics UK

International Symposium on GPC/SEC and Related Techniques

Amsterdam, September 26th-29th, 2016

Agenda

- **Introduction to Field Flow Fractionation**
- **The Postnova characterization approach**
  - Separation modules
  - Detection modules
- **Application examples**

Field Flow Fractionation

FFF for polymers

FFF is a complementary technique to GPC/SEC

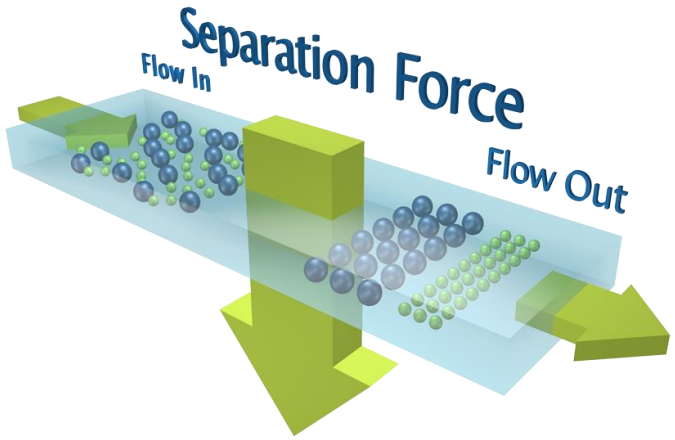
It can separate things SEC cannot (particles, gels, emulsions etc.)

Repeatability good but SEC still better with ‘easy’ soluble polymers

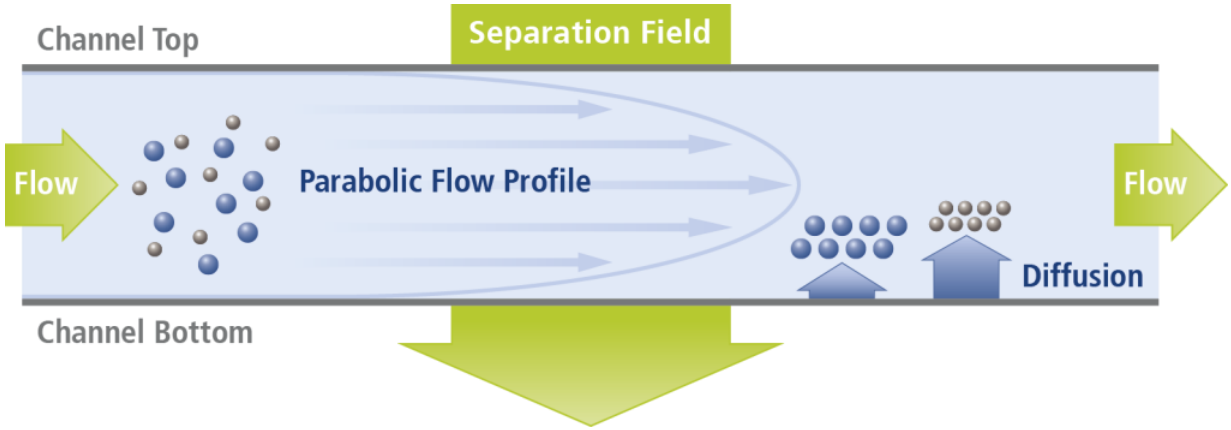
High-molecular weight polymers are always a challenge!

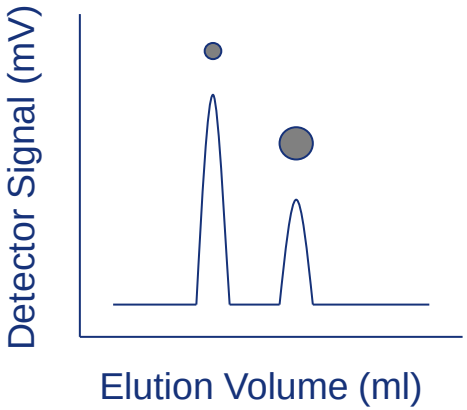
- Sample preparation (but FFF slightly less demanding than SEC)
- Method development

FFF Separation Principle



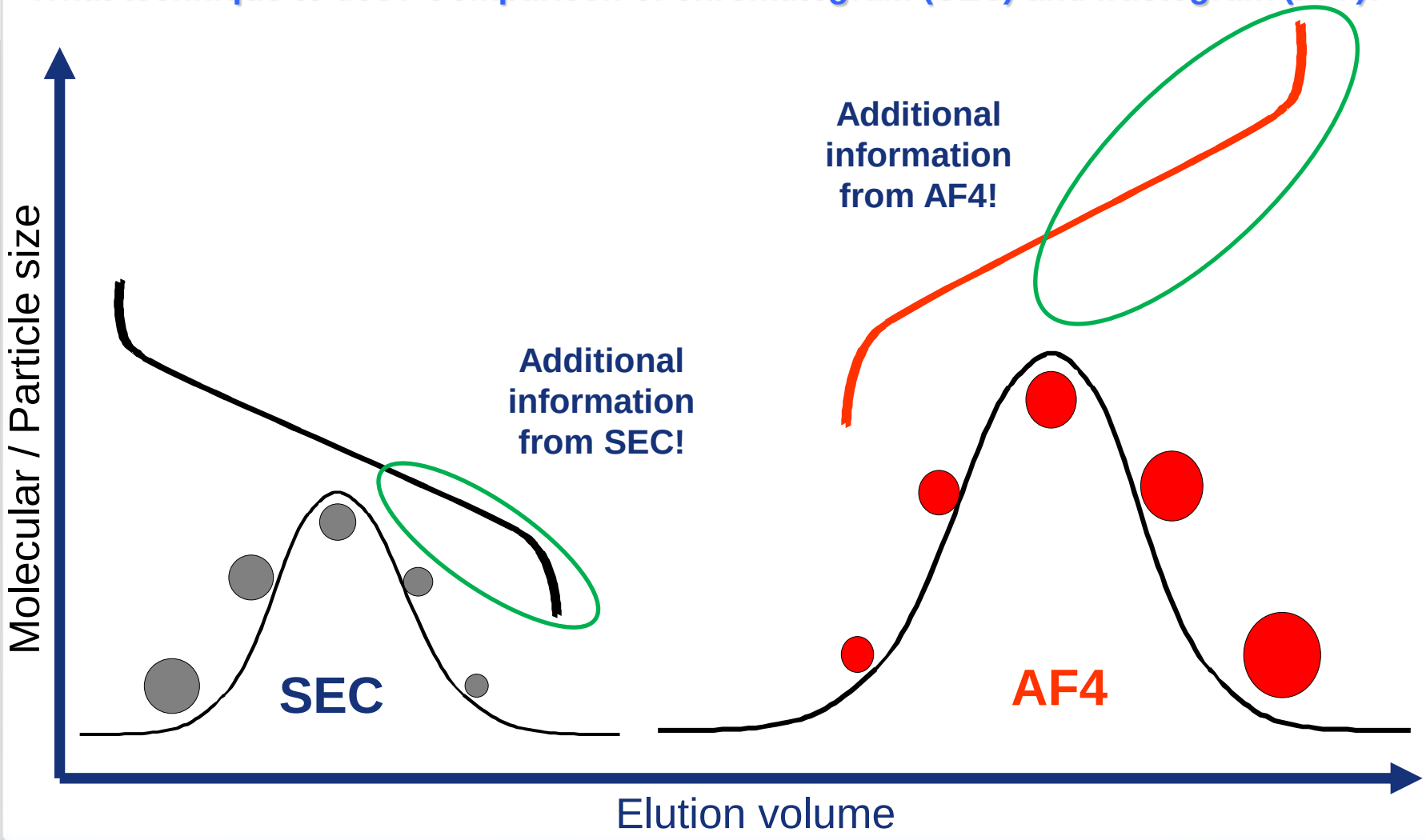
- Separation in a narrow ribbon-like channel
- Laminar flow inside the channel
- External field perpendicular to the solvent flow
  - Flow
  - Centrifugal
  - Thermal
  - Gravitational
  - Electrical



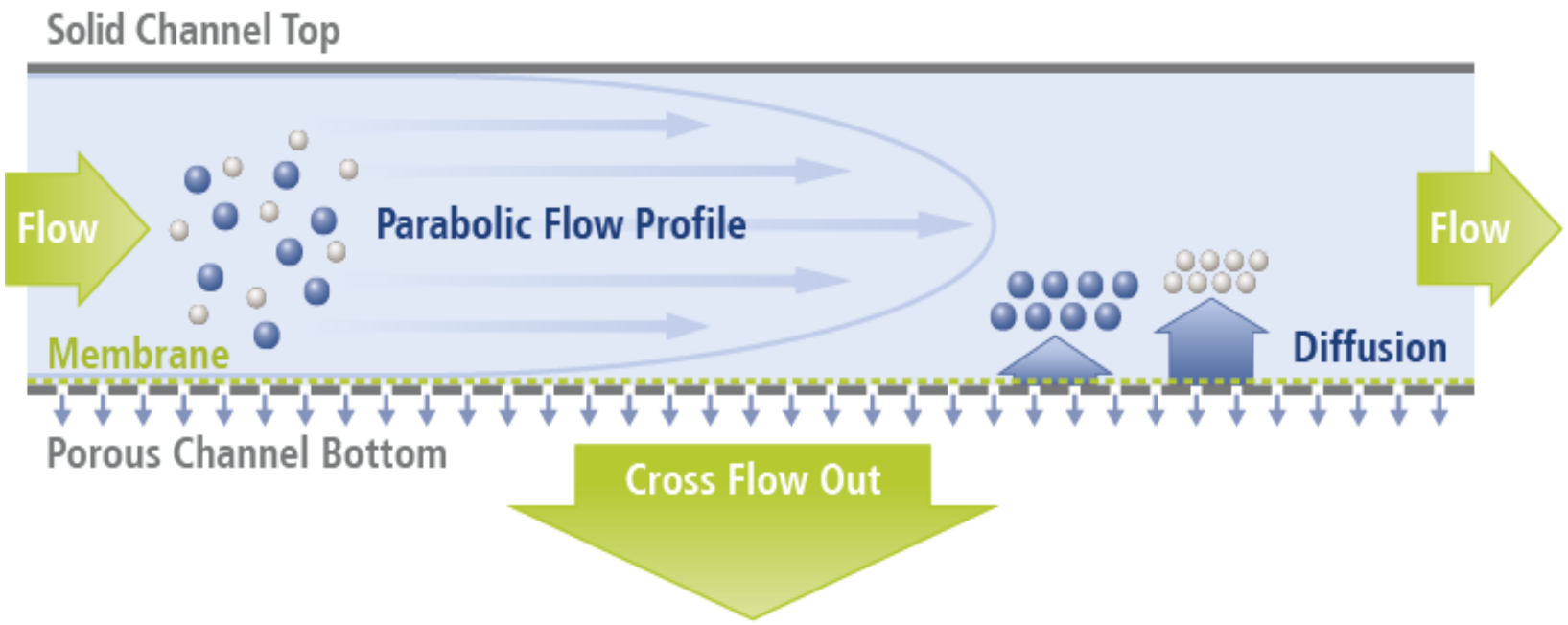


SEC + FFF

What technique to use? Comparison of chromatogram (SEC) and fractogram (FFF):



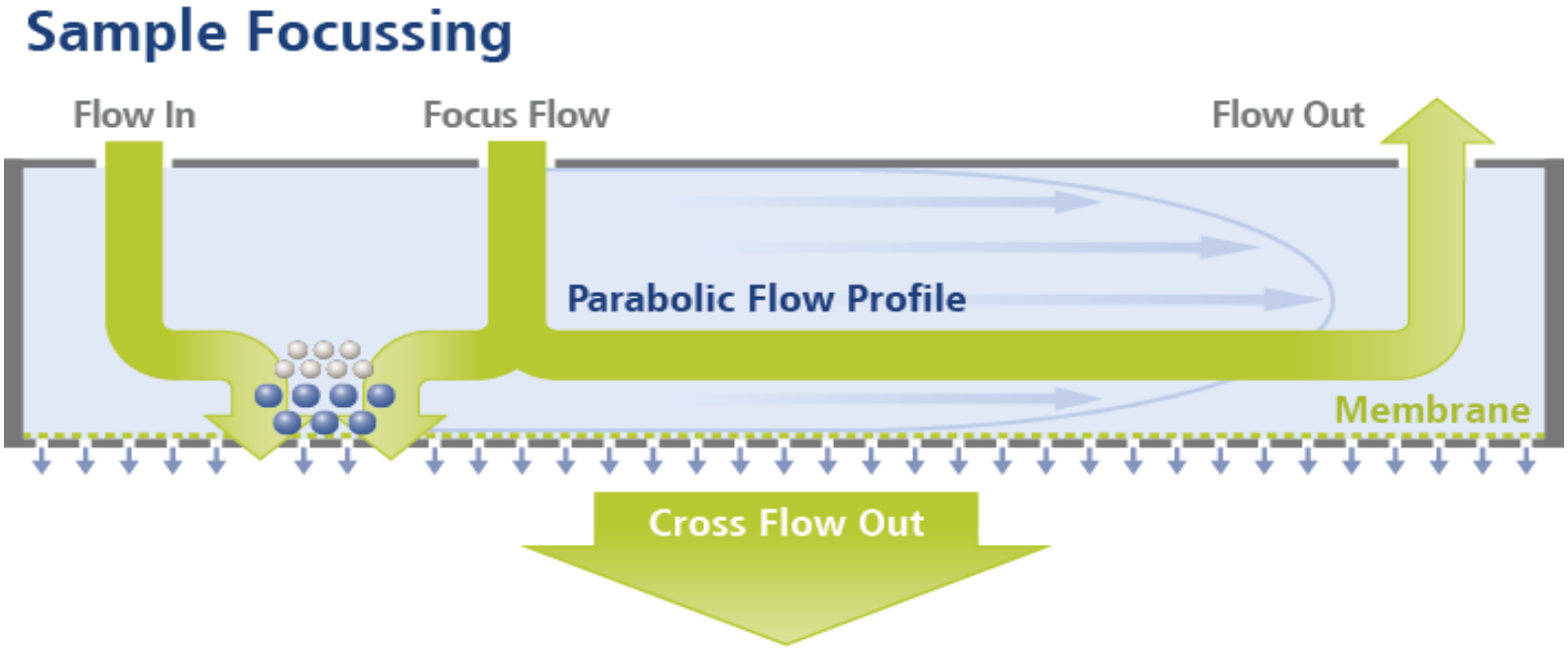
Asymmetric Flow FFF (AF4)



- Hydraulic pressure gradient field (cross-flow) for separation
- Separation based on size

Polymers	Rubbers
Nanoparticles	Polysaccharides
Polymersomes	Liposomes
Proteins	Antibodies
Viruses	Exosomes

Asymmetric Flow FFF (AF4)

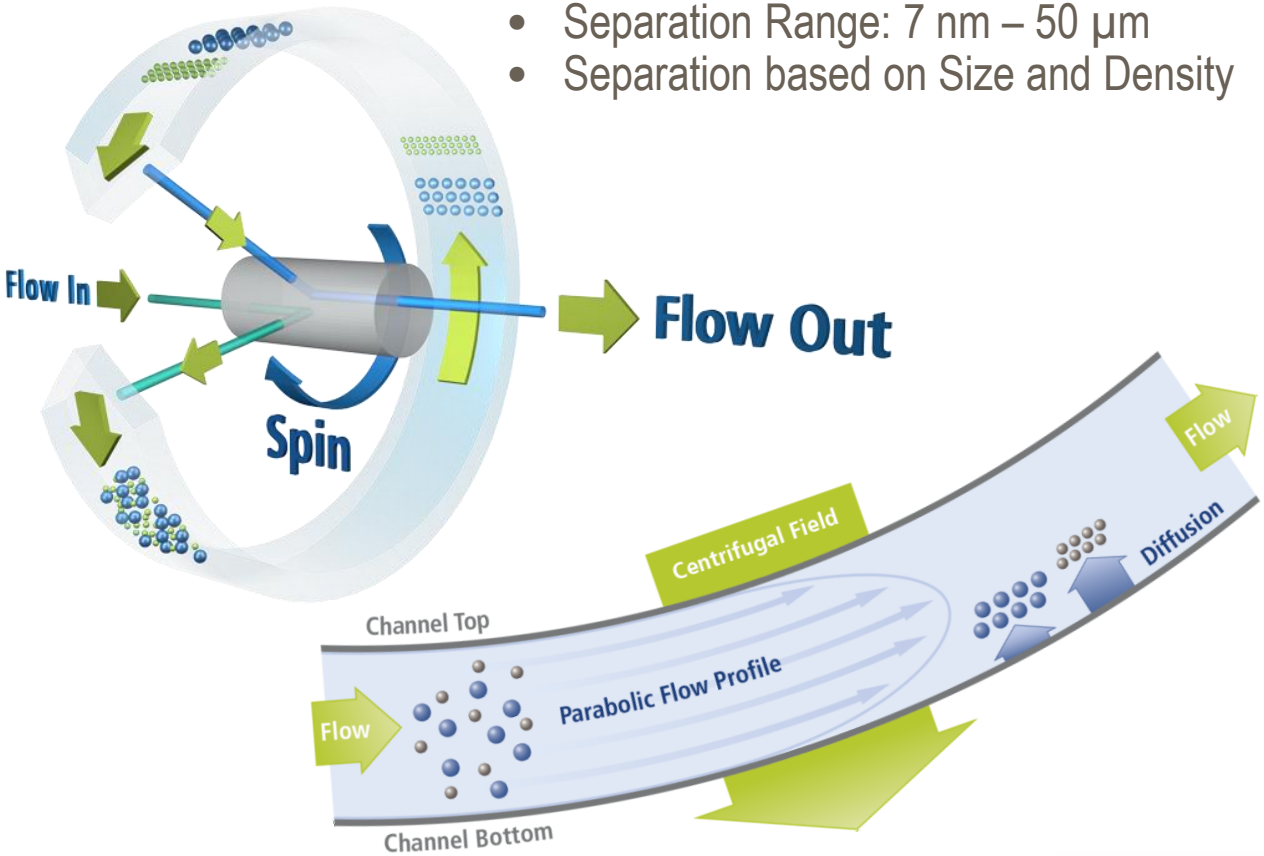


- Focusing of sample
- Cleaning / washing of sample
- Higher resolution / reduced band broadening
- Continuous detector flow
- Increased recovery rate

CF2000 - Centrifugal FFF (CF3)

Centrifugal FFF Channel

- Gravity Separation Field up to 2500 g
- Separation Range: 7 nm – 50 µm
- Separation based on Size and Density

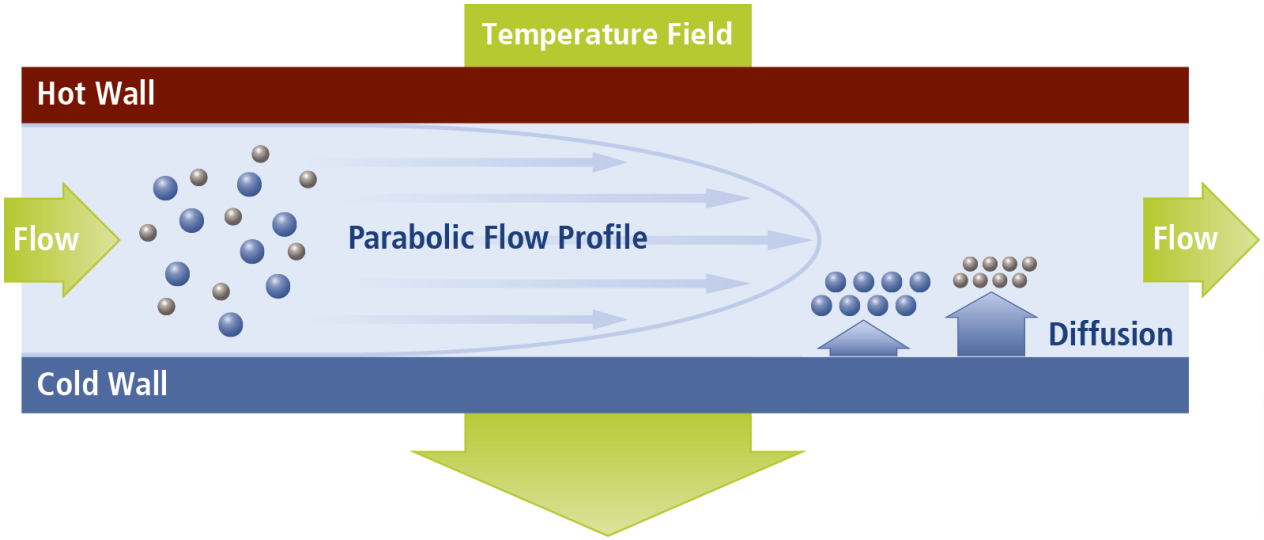


Applications

- Nanoparticle
- Microparticle
- Latices
- Gold / Silver Nano
- Liposomes
- Emulsions

TF2000 - Thermal FFF (TF3)

Principle



- Thermal gradient up to Δ120°C
- Separation 10^3 Da up to >10^7 Da
- Separation depends on D and DT
  (size, chemical composition, micro structure)

Applications

- Rubbers Polymers
- Gels / Latexes
- Cross-linked Polymers

The Postnova Characterization Platform

Light Scattering
Detector
MALS 21

Particle
Size Rg /
Molar Mass

DLS
Malvern
Zetasizer

Particle
Size Rh

Size Separation

AF2000 Flow FFF (AF4)

CF2000 Centri FFF (CF3)

SC2000 SEC

TF2000 Thermal FFF (TF3)

Intrinsic
Viscosity /
Branching

Viscometer detector

Agilent 7900 ICP-MS

Concentration

UV detector
DAD detector
Fluorescence detector
ELS detector
...

RI detector

Polymer characterization by FFF/SEC

What information do the detectors contribute?

Refractive Index (RI) detector – Concentration of the sample

Ultra Violet (UV) detector – Concentration of sample with a given chromophore

Viscometer Detector (Visc) – Intrinsic Viscosity (IV) of the sample (inverse density)

Multi-Angle Light Scattering (MALS) – Molecular weight (MW) and molecular size (Rg)

By combining the detectors (RI, UV, MALS, Visc), we can enhance the data further:

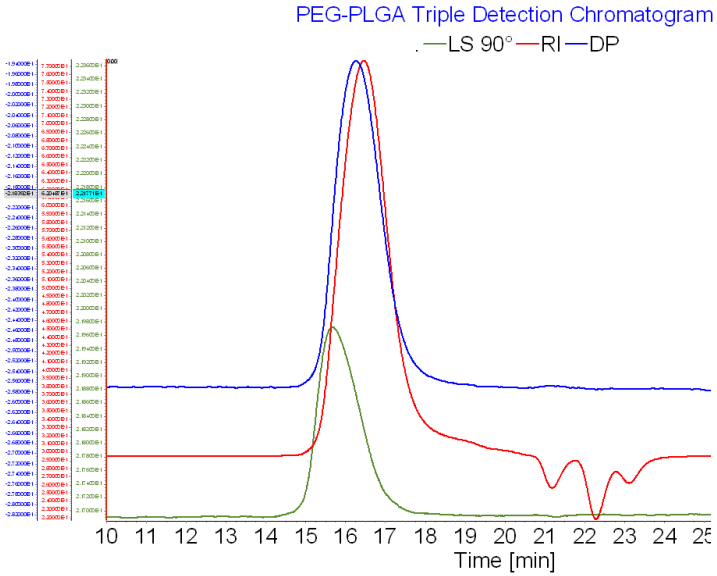
MALS+Visc = Rh, $Rg_{(ff)}$, Mark Houwink data (structure), Branching

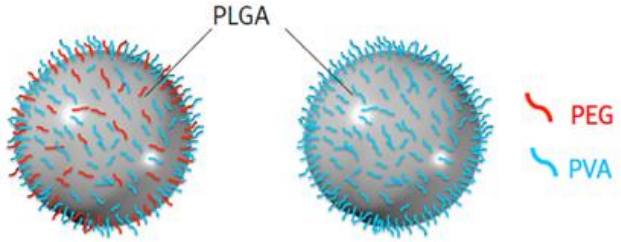
RI+UV = protein conjugate ratio or copolymer content

Application Examples

1. SEC Applications

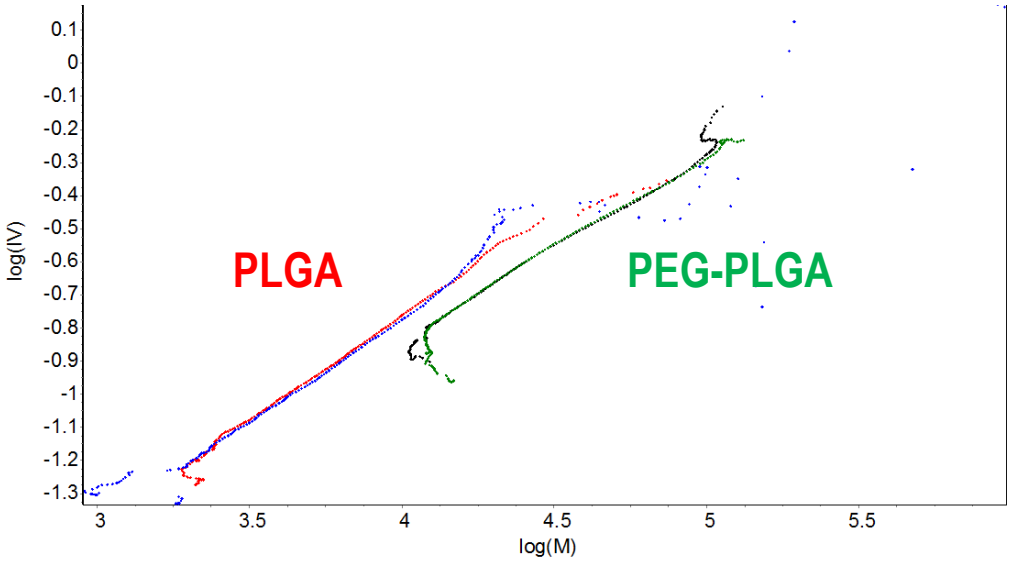
PLGA polymers for nanoparticles





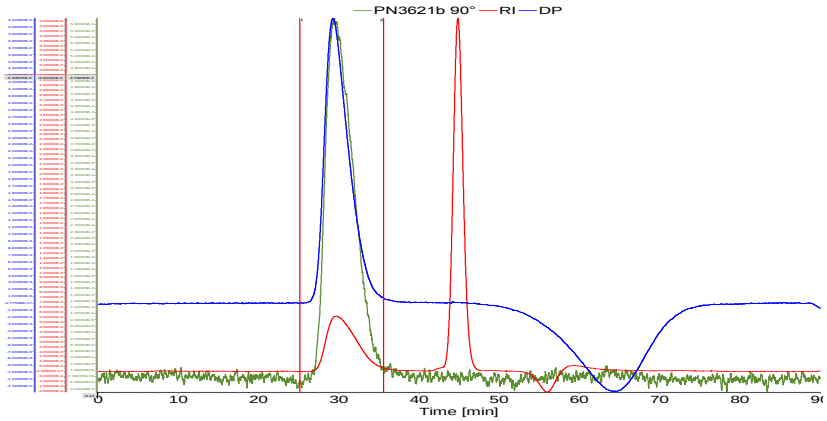
NovaSEC 2000 System
THF @ 1ml/min

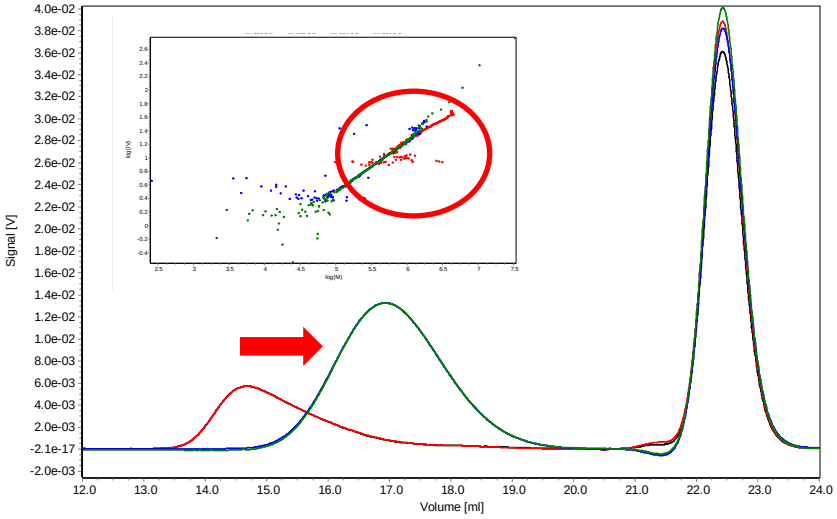
Mark-Houwink plot showing structure difference in solution



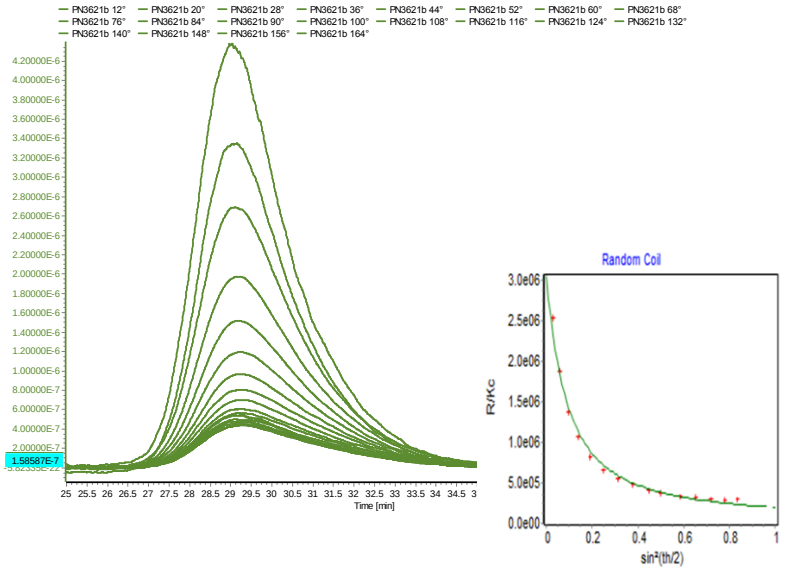
Hyaluronic Acid by SEC

Triple Detection Chromatogram





Angular Dependence – MALS View

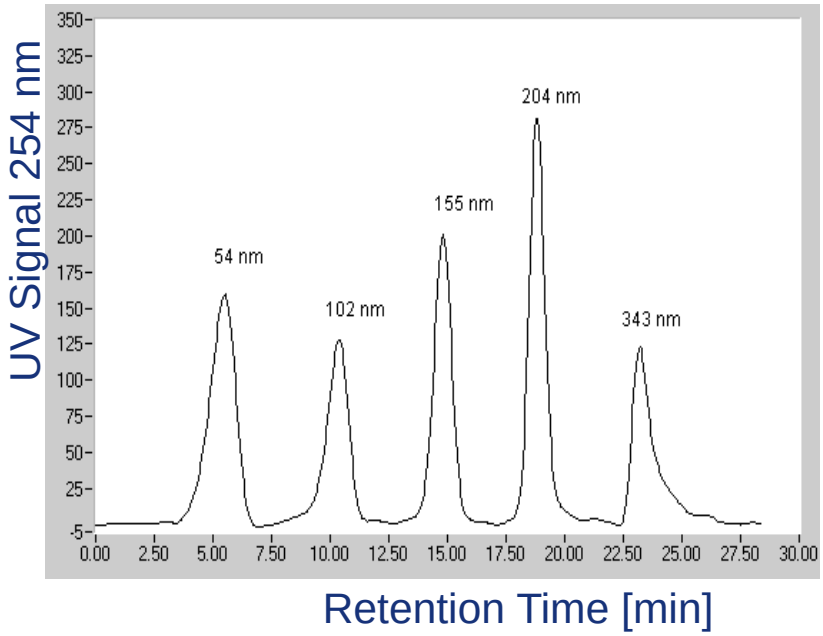


HA sample of ~2 MDa reduced to <0.5 MDa on sterilization.
RI overlay shows effect of sterilization on distribution. Note also MH slope change

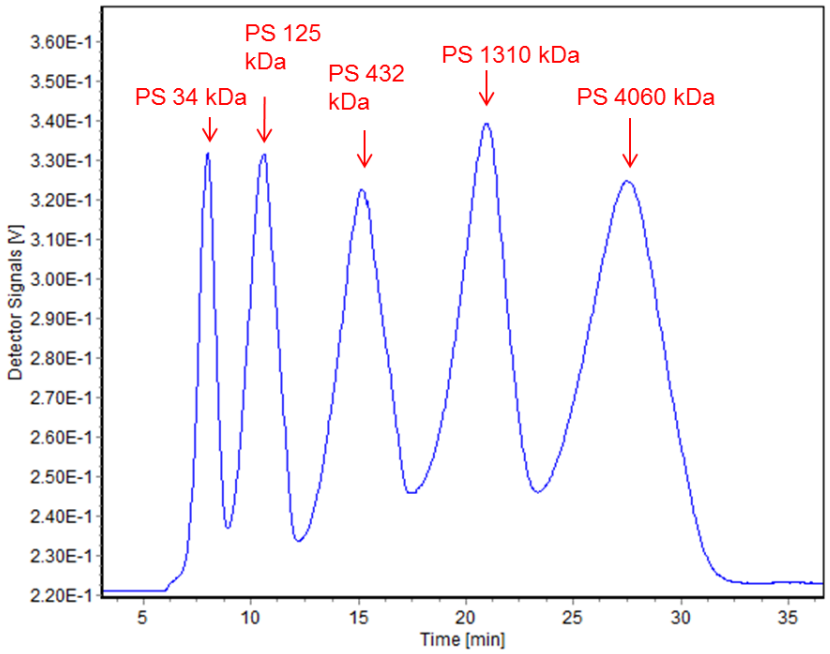
2. AF4 Applications (Flow FFF)

AF2000 – Flow FFF (AF4)

Polystyrene Latex Nanoparticle Mix



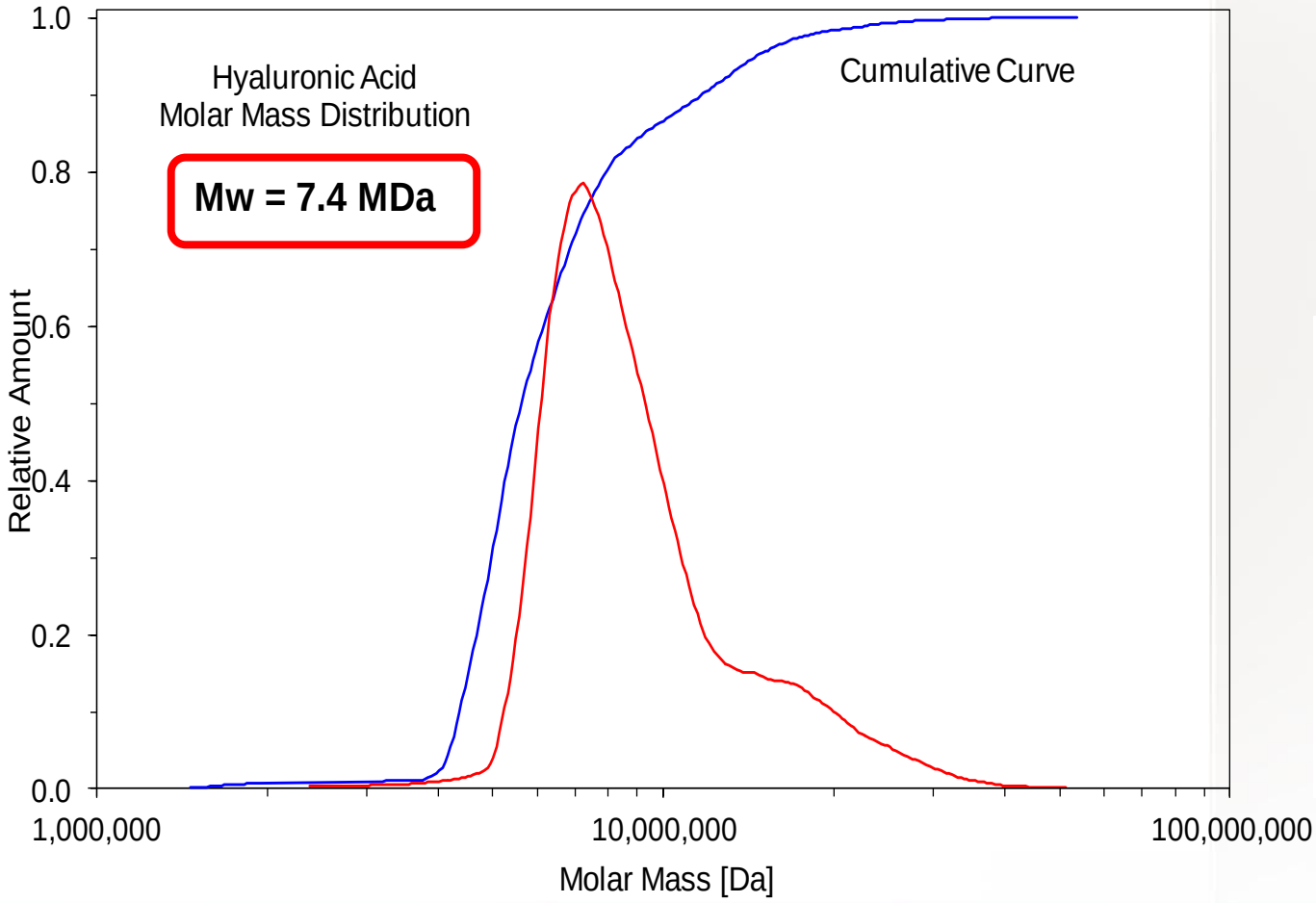
Polystyrene Standards Mix



Same instrument, different flow method!

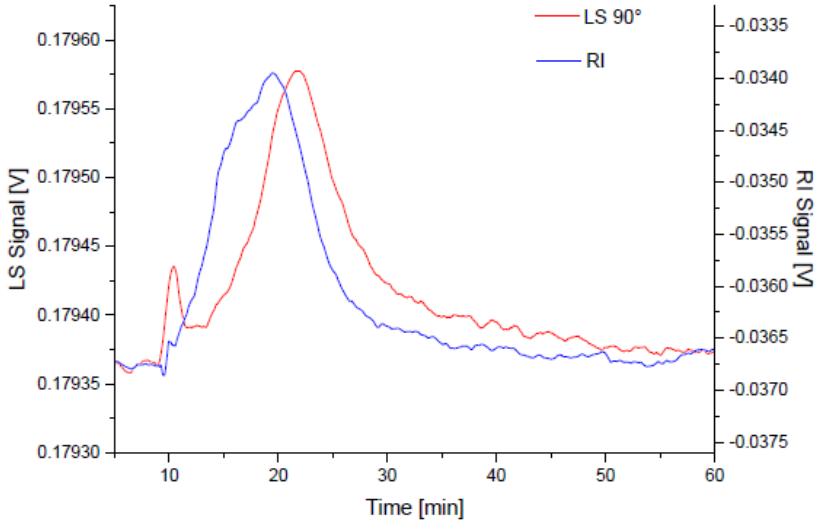
AF4 - High MW Polymers

Separation and MW of Hyaluronic Acid

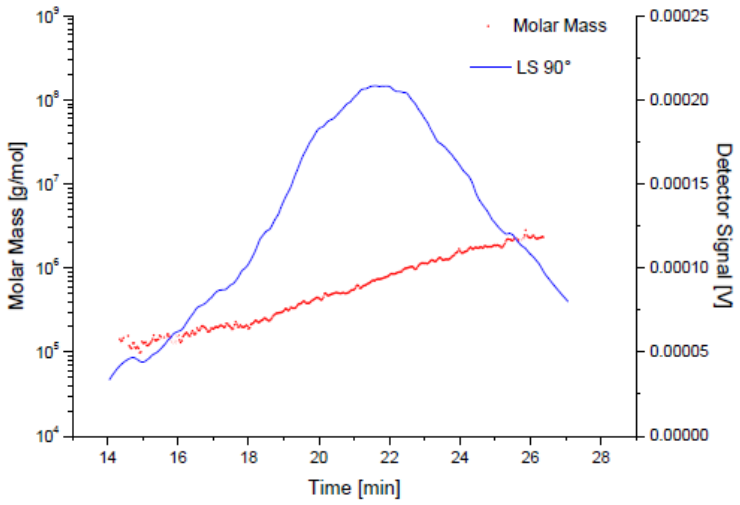


Crosslinked HA Sample by FFF

AF4 – MALS/RI Fractogram



Molecular weight data

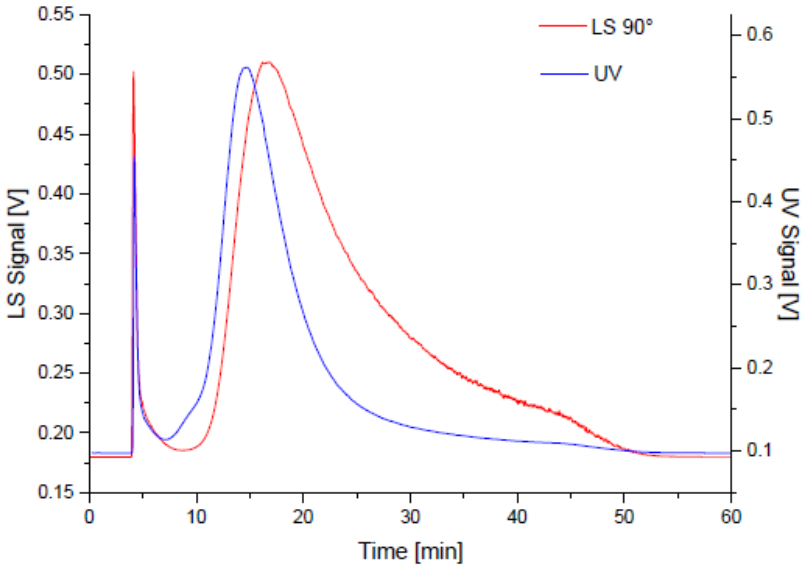


	M_w [g/mol]
n-Average	$2.8 \times 10^5 \pm 9.1$ %
w-Average	**$5.3 \times 10^5 \pm 3.9$ %**
z-Average	$1.0 \times 10^6 \pm 10.2$ %

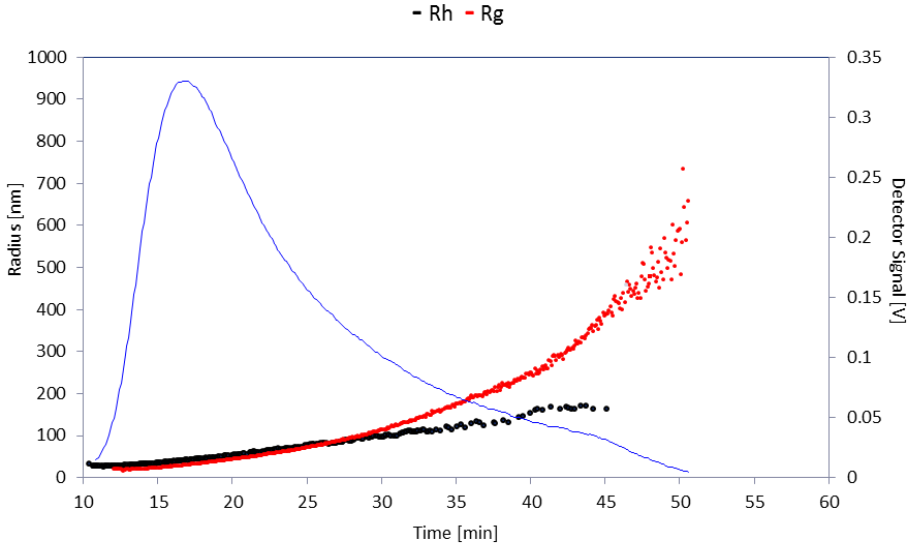
Crosslinked HA showed <10% recovery on SEC but >80% recovery on FFF

Dextran Coated Magnetic Nanoparticles

Raw Data Fractogram of AF4 - MALS and UV

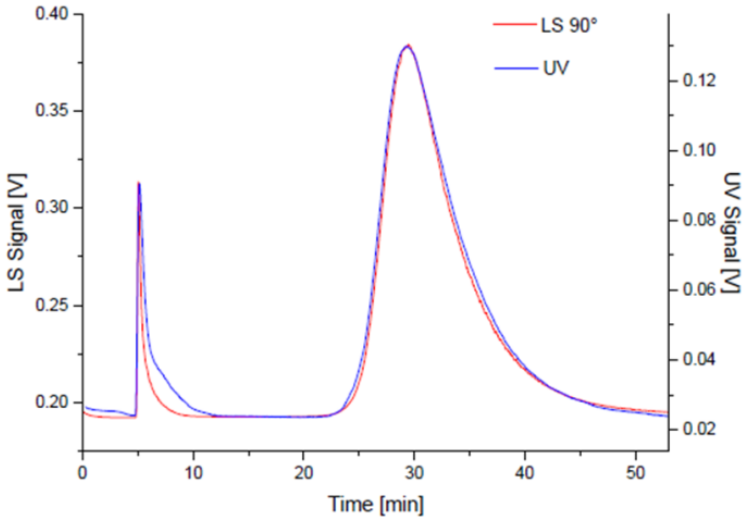


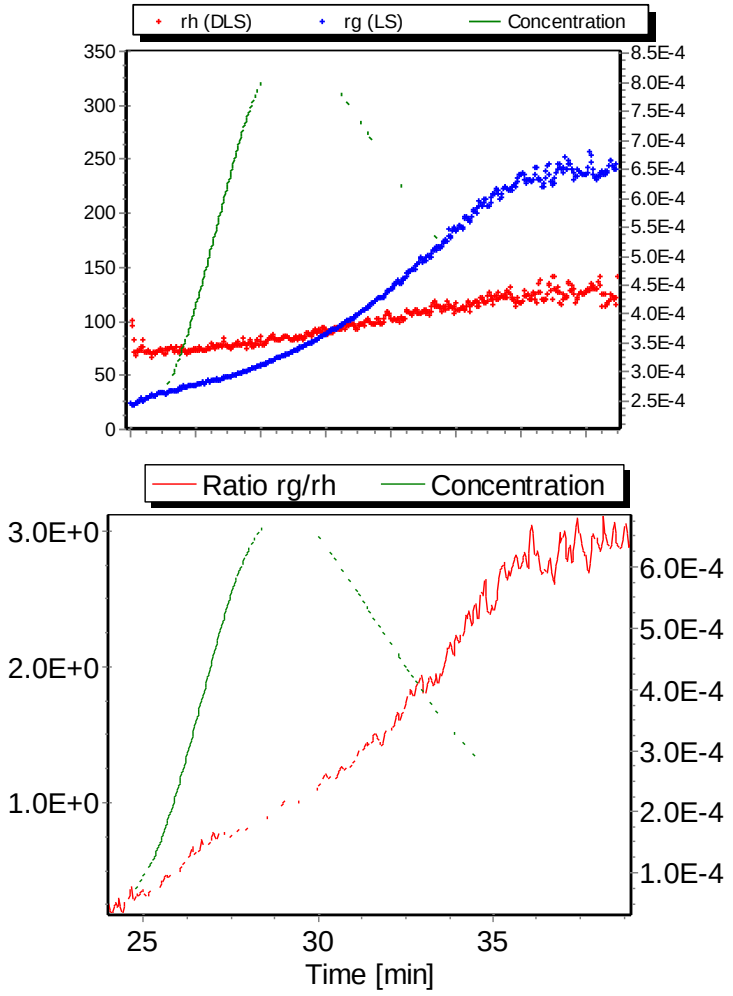
Rg/Rh ratio indicates aggregation which is confirmed by TEM



Polymersome structure

Radius of Gyration (MALS) and Hydrodynamic Radius (DLS)



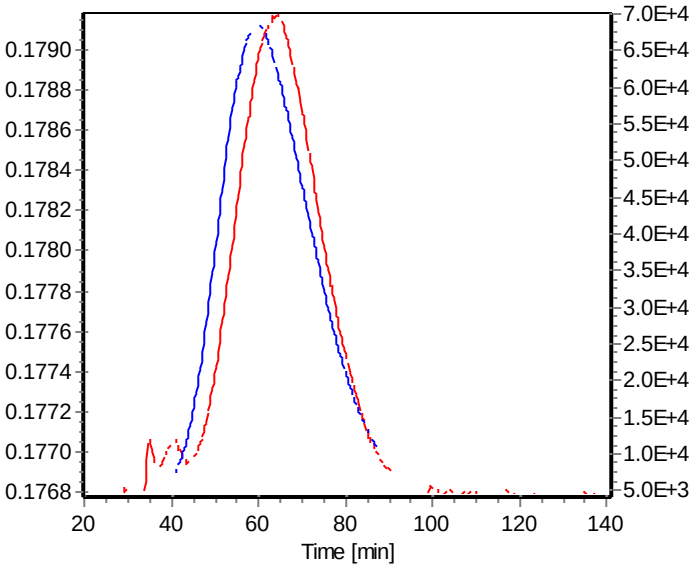


Data indicates mixture of structures (spheres and tubes) also seen on TEM

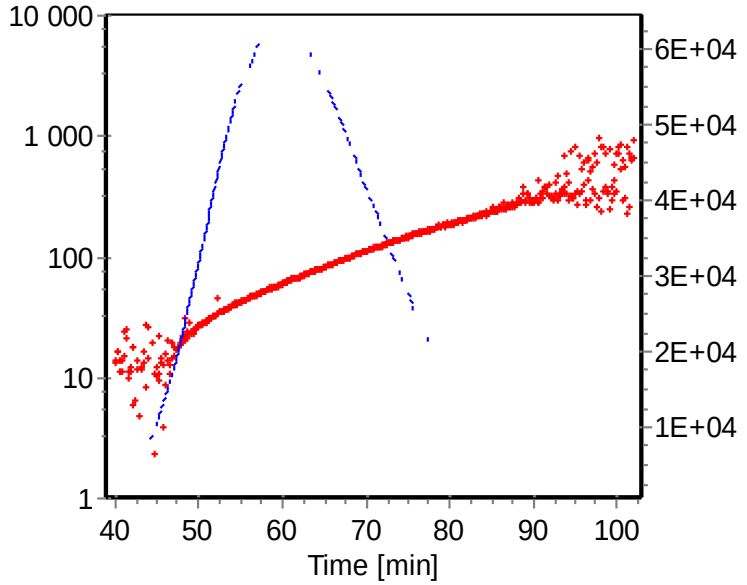
SiO_2 Sample AF4-MALS-ICP-MS

SiO_2 Standard, Conc. ~2 mg/L

90° LS Signal (red trace) and Si 28 (blue trace)



Radius / Si28



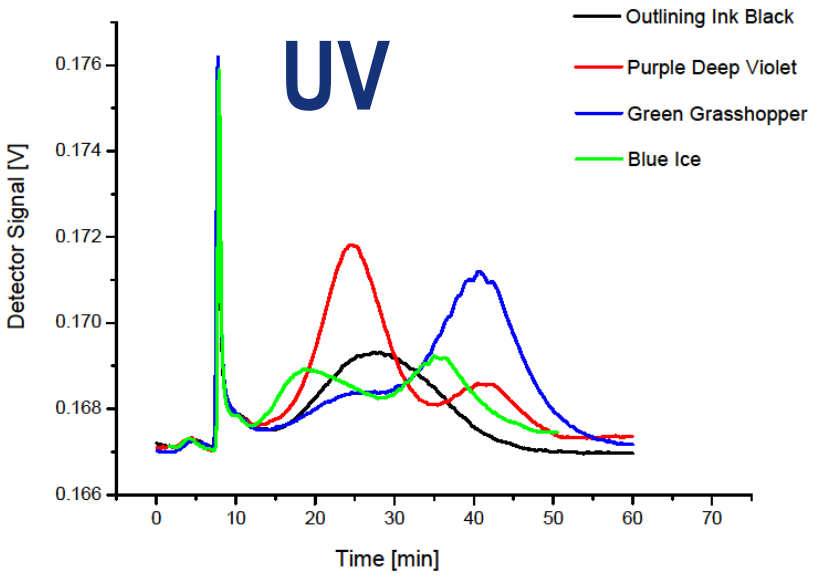
Conditions

- Injection Volume: 2000 µL
- Concentration:~ 2 mg/L
- LS 90°(red trace)
- Si 28 Signal (blue trace)

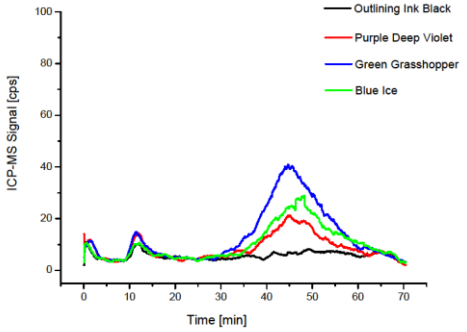
Overlay: Radius of Gyration and Si 28 trace

- Calculated from MALS angular data
- Broad distribution starting from 7 nm up to large particles (998 nm).

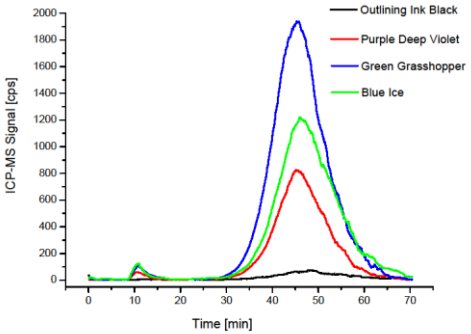
AF4-UV-ICP-MS – Tattoo ink analysis



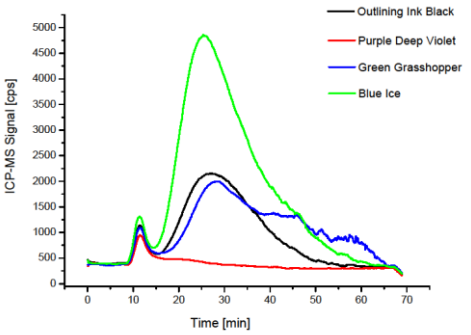
Al^{27}



Ti^{47}



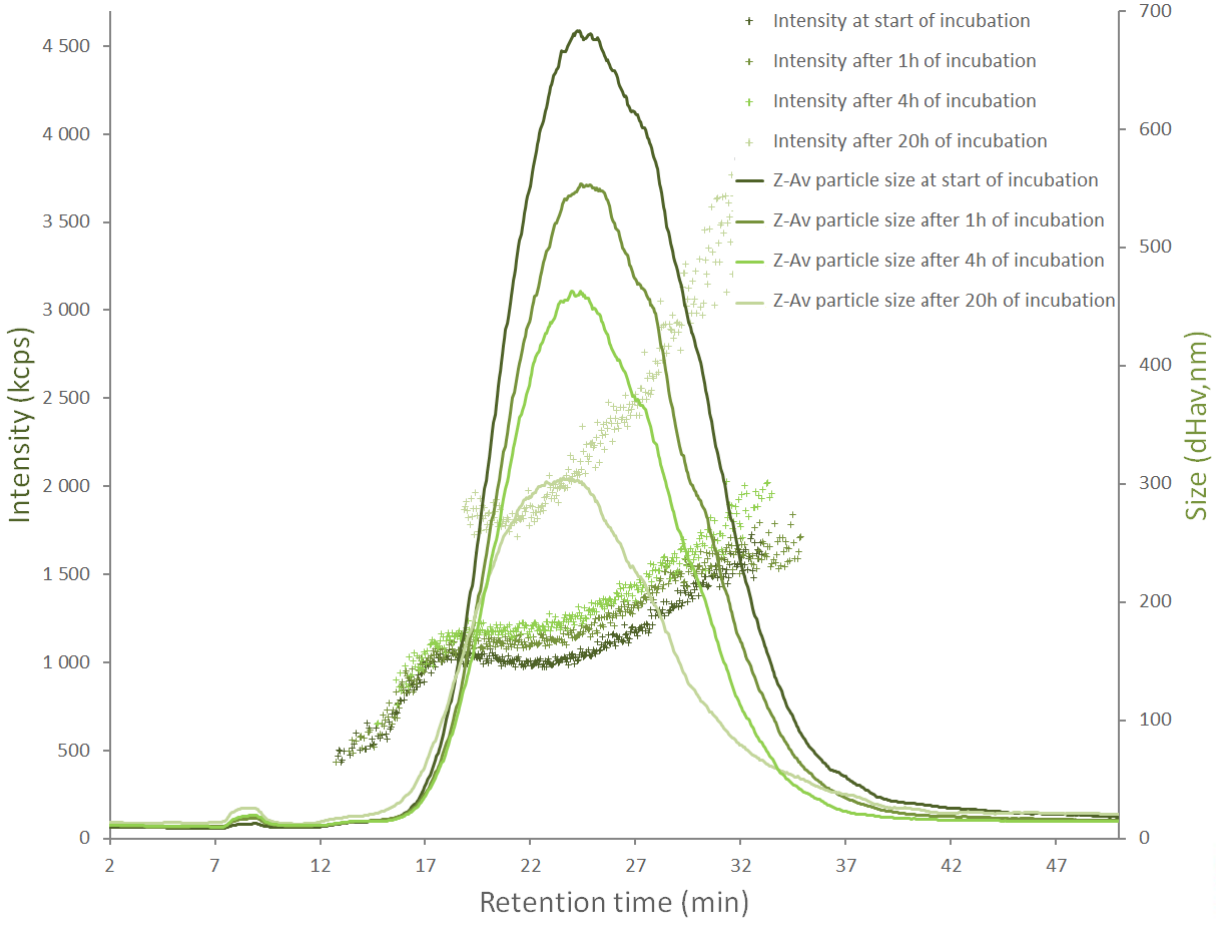
Cu^{63}



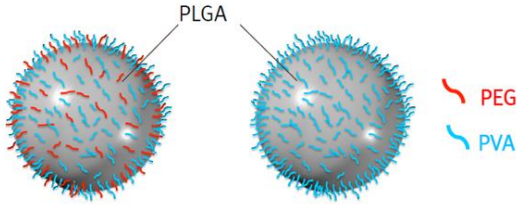
3. CF3 Applications (Centrifugal FFF)

Nanoparticle drug delivery

CF3-DLS – Detection of PEG-PLGA-Nanoparticles in cell medium

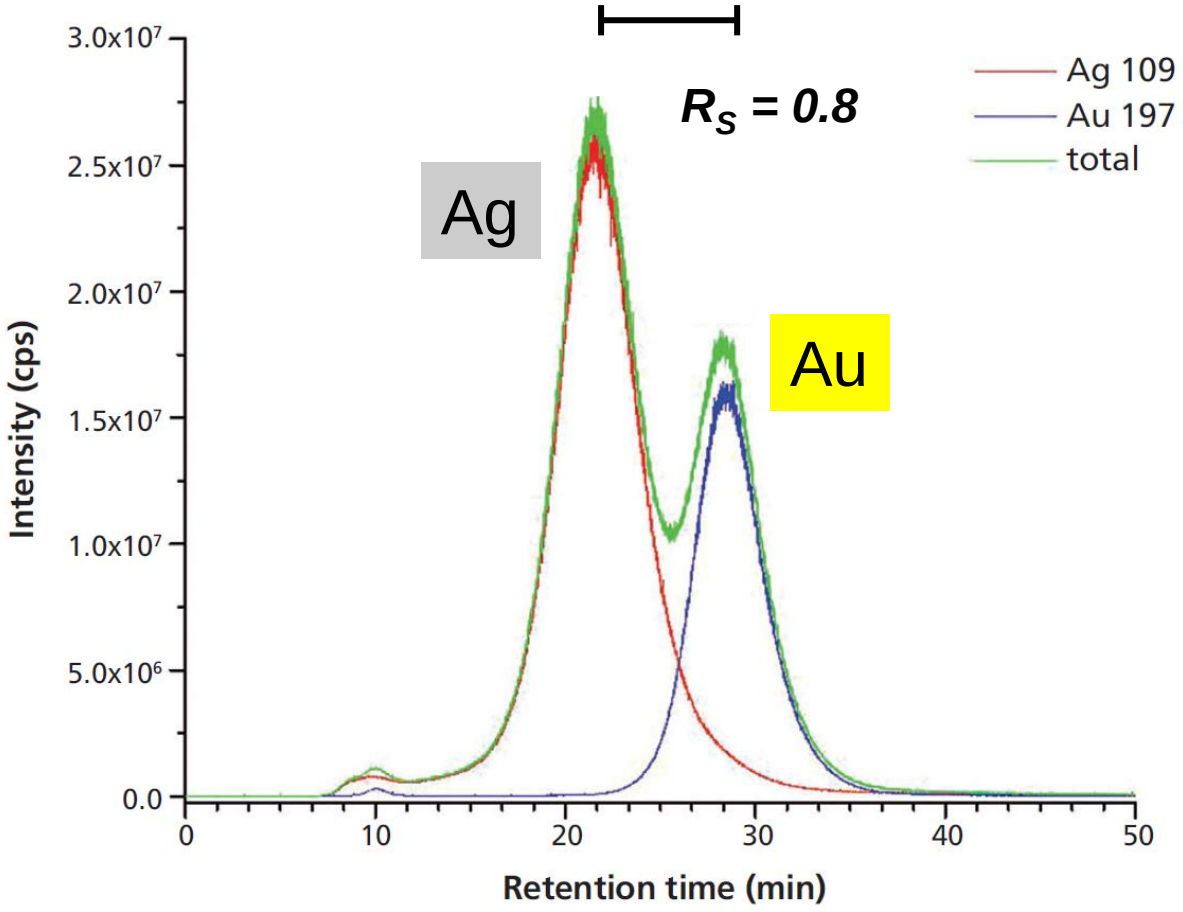


- Incubation of the nanoparticles in cell media was performed at 98 °F.
- Detected NP size increased over time
- The decreasing overall particle numbers observed after prolonged incubation shows agglomeration/ aggregation of nanoparticles



Kohl Y., et al, Nanosafety Conference 2013, Biopolymer Nanoparticles for Therapeutic Applications: Synthesis, Characterization and Assessment of Biocompatibility..

Mass Discrimination: Au- and Ag Nanoparticles (d = 20 nm)

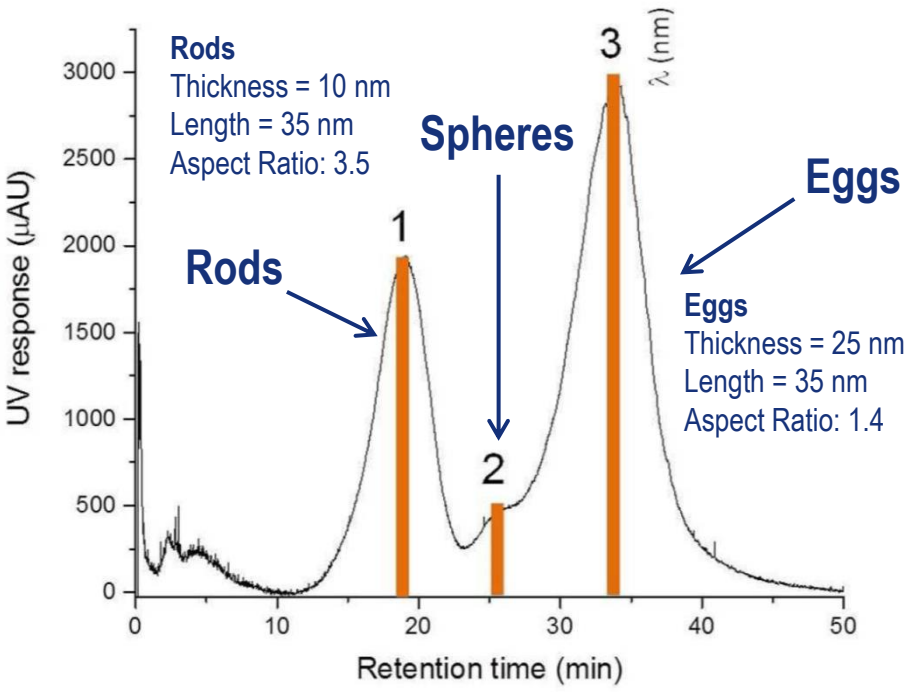


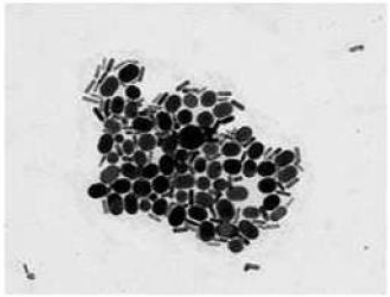
- Density-based separation (CF3) + information about the chemical composition of the particles (ICP-MS) in one single run

Tadjiki and Klein, *The Column*, **2014**, 10(14), 11-16.

CF2000-UV + TEM

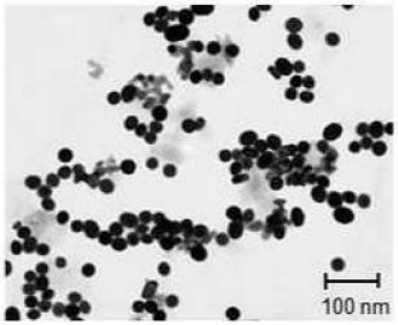
Fractionation of Au Nano Rods from Au Nano Eggs



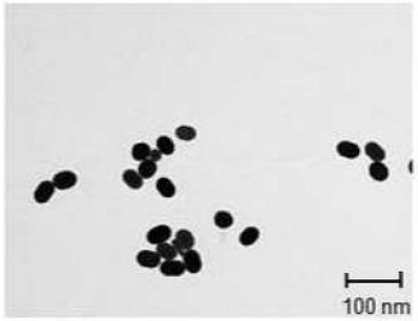


Original

Fraction 1



Fraction 2



Fraction 3

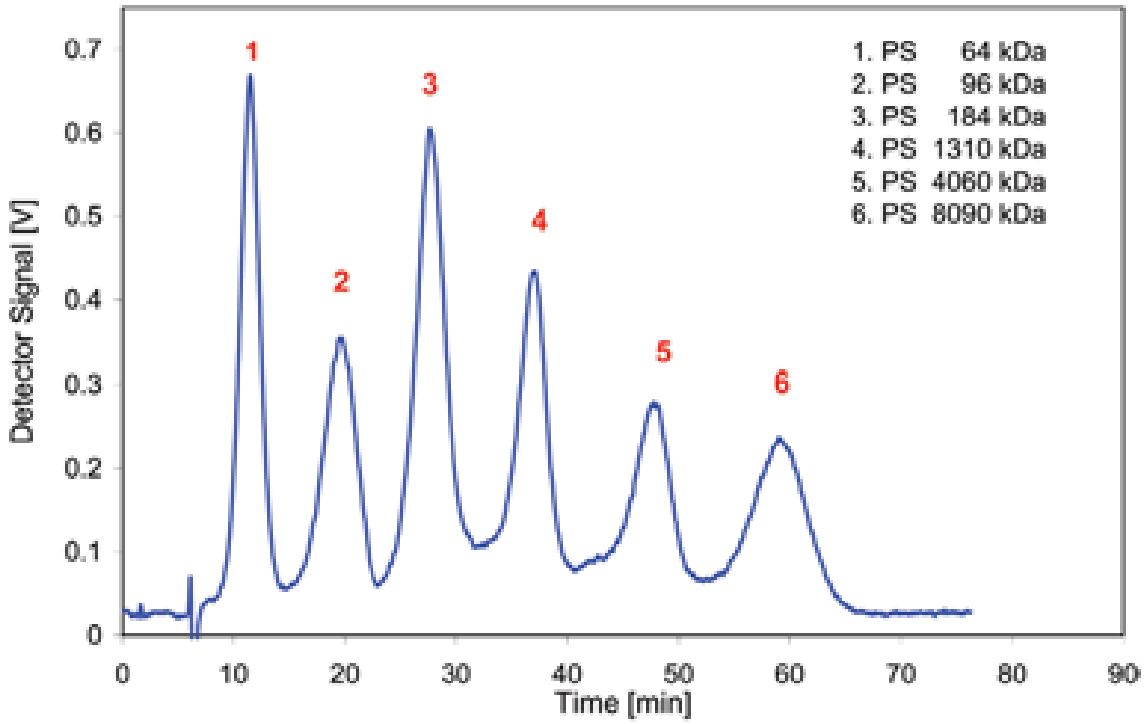
- Separation according to mass and hydrodynamic diameter

Tadjiki and Klein, *The Column*, **2014**, 10(14), 11-16.

4. TF3 Applications (Thermal FFF)

TFFF Applications – PS

Separation of PS standards



System

- TF2000 FFF System
- PN3150 RI Detector

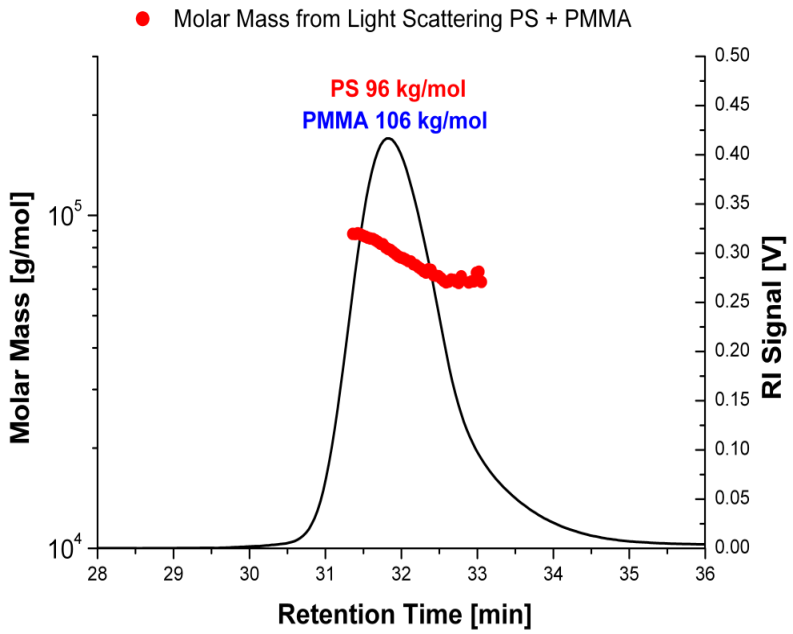
Conditions

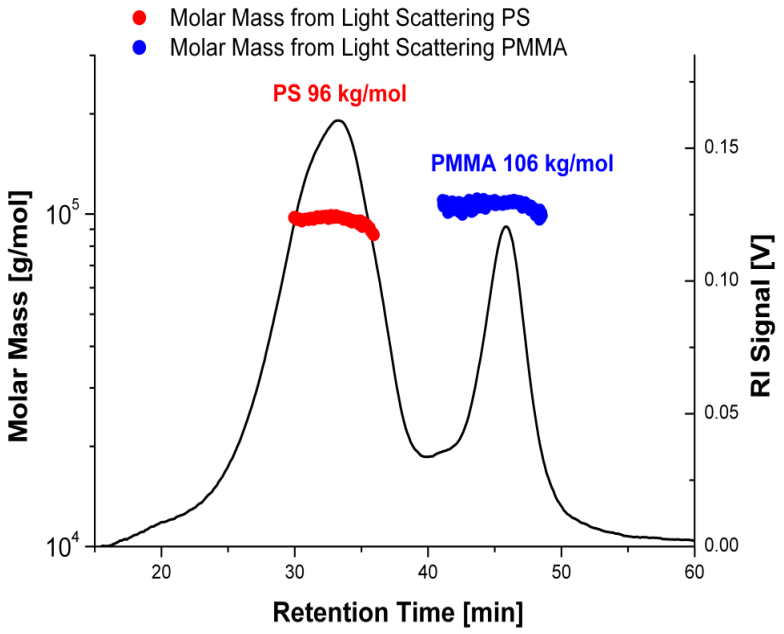
- Injection Volume: 20 µL
- Concentration: 2 mg/mL
- Temp. grad. ΔT = 90°K to 0°K

TF2000 Applications

Separation of PS and PMMA-Standards with same R_h

SEC in THF (SDV Columns) vs. **Thermal FFF in THF**

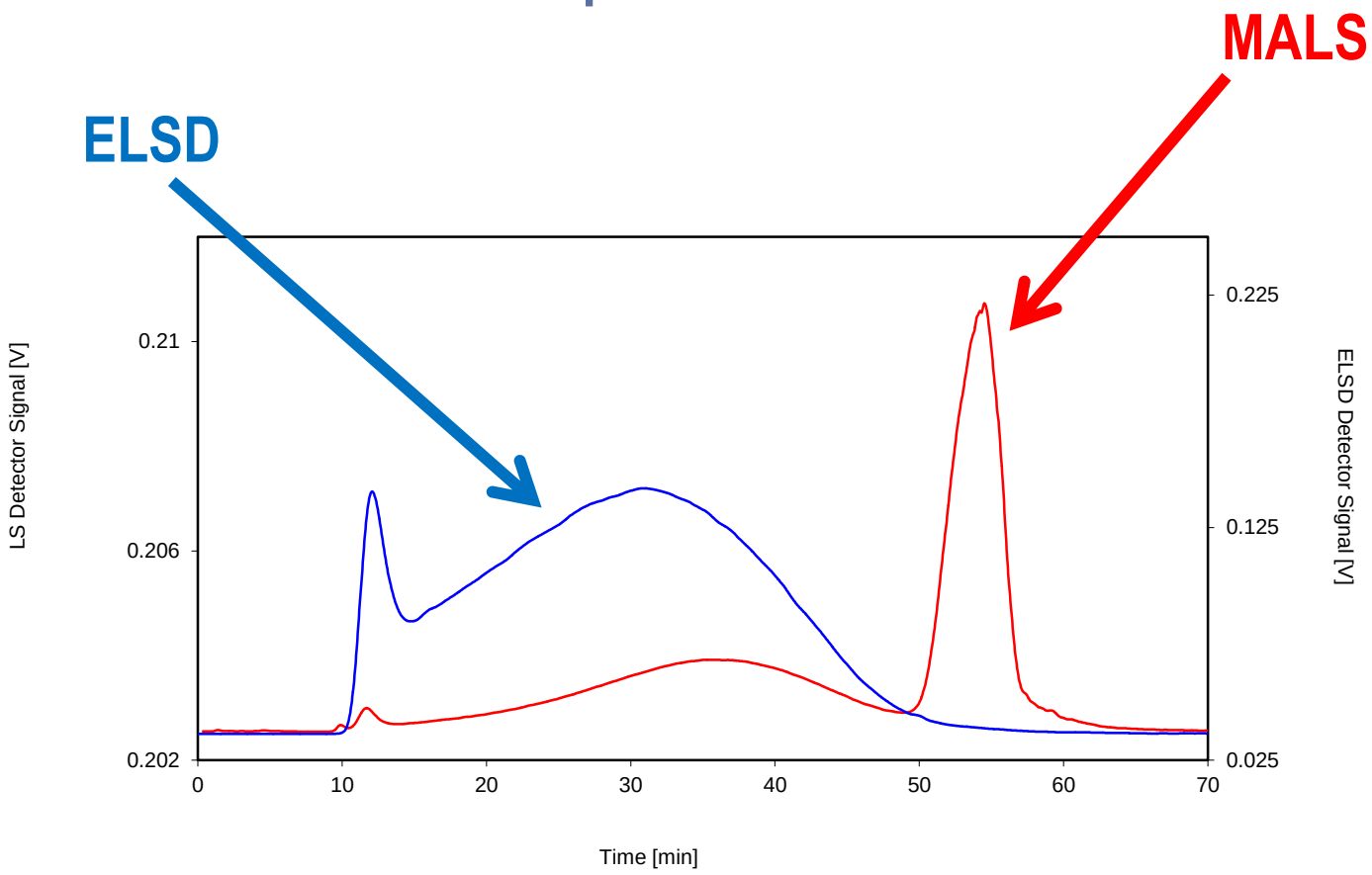




→ **Separation by Hydrodynamic Size and Chemical Composition**

TF2000 Applications

Natural Rubber Sample

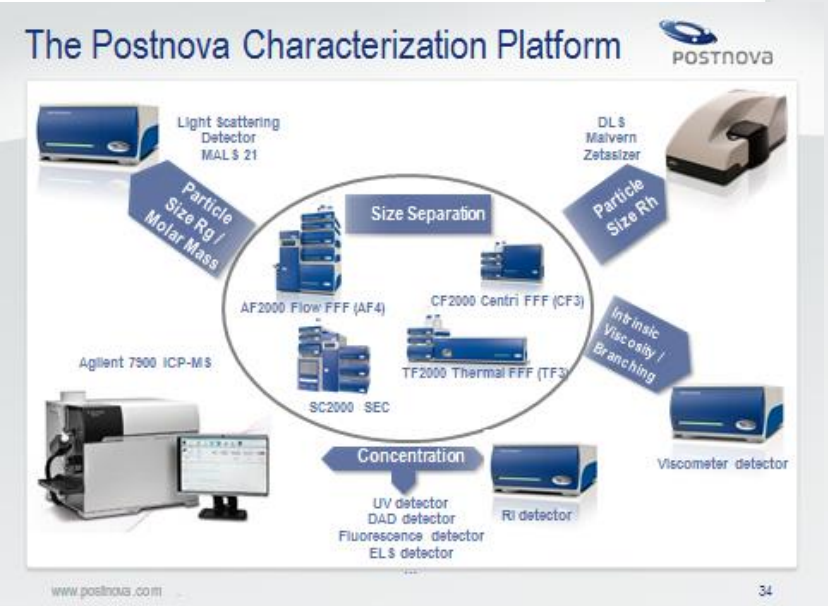


See Gerhard Heinzmann talk tomorrow at 14:00 !

Summary

FFF and GPC/SEC are both well-established techniques for the characterization of macromolecules, with FFF also a valuable tool for separation and characterization of (nano)particles, gels, aggregates and larger complexes.

The overlap between the GPC/SEC and FFF application areas and technique capabilities can now be fully exploited in a single, highly flexible modular instrument platform from Postnova.



Thank you!

paul.clarke@postnova.com