Characterization of Macromolecules by FFF and Light Scattering

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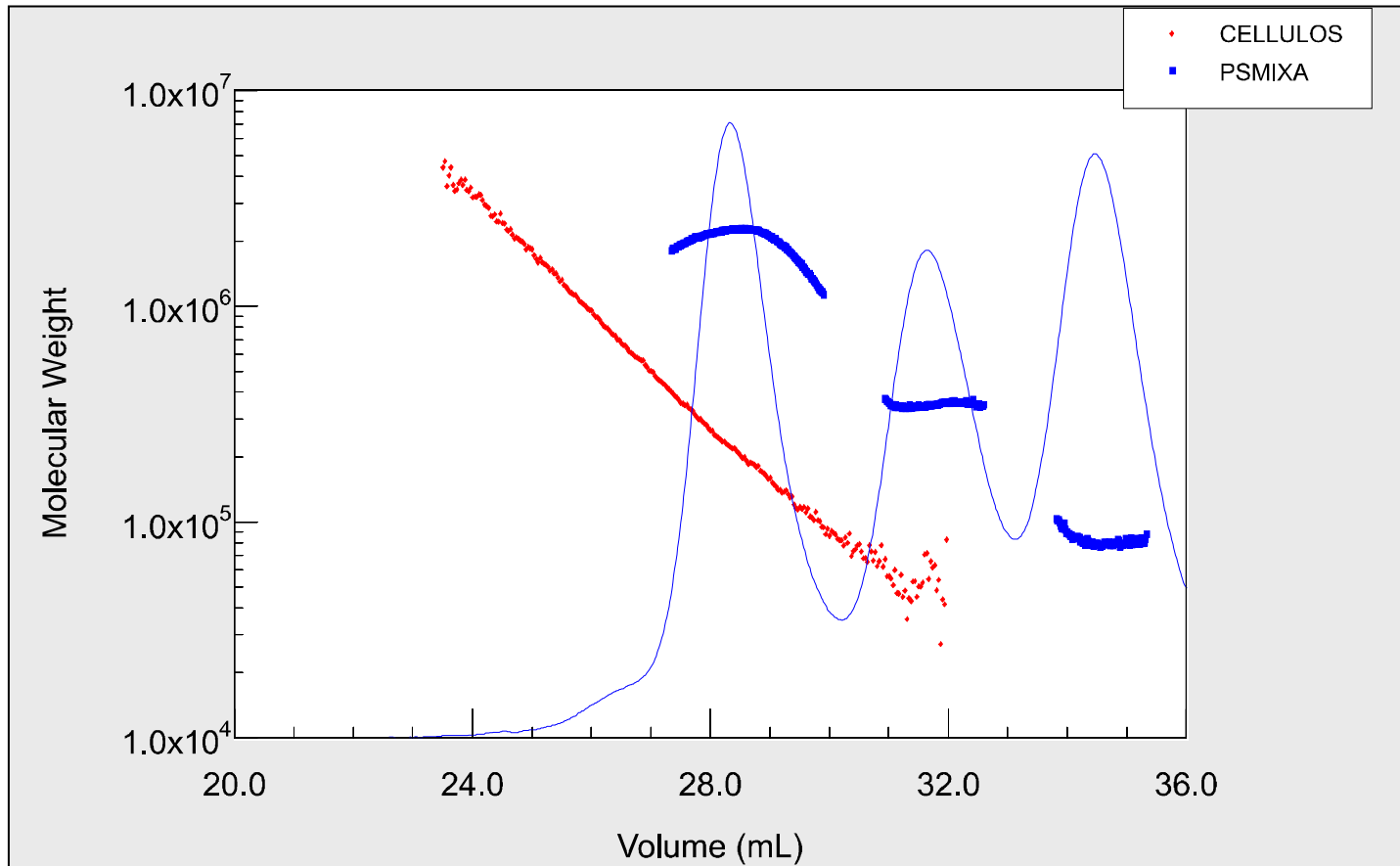
Overview

- **Introduction**

- **The Nature of Scattered Light:**
    - Intensity of scattered light
    - Angular dependence of scattered light
    - Time dependent fluctuations

- **Multi angle light scattering:**
    - Absolute molar masses
    - Radius of gyration

- **Field-Flow Fractionation:**
    - Basic theory
    - Asymmetrical Flow Field-Flow Fractionation (AF4)

- **Comparison SEC versus FFF**
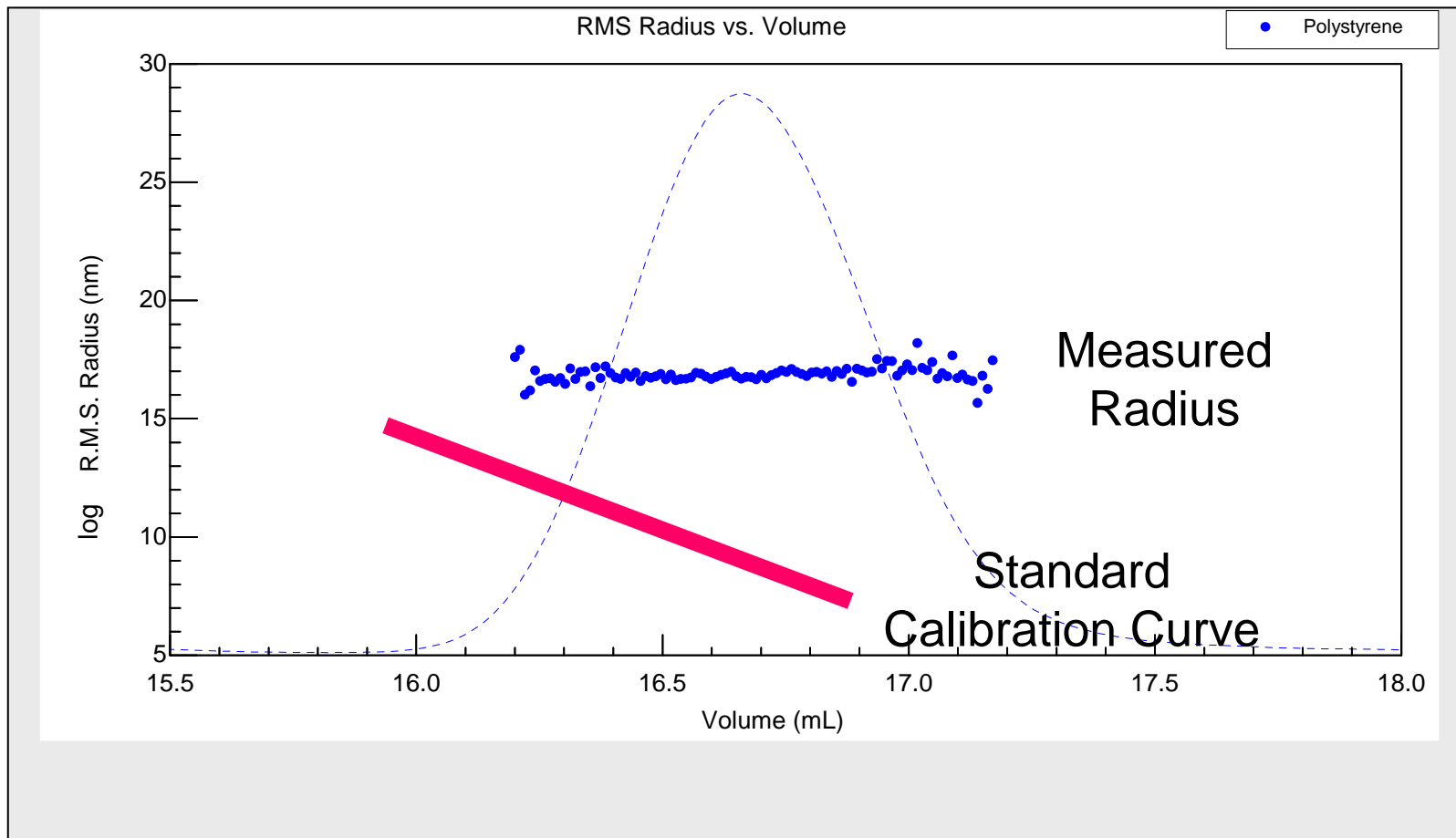
- **Summary**

Absolute MM - Absolute Comparison!

- absolute molar masses
- no assumptions

- true values,
- absolute comparable
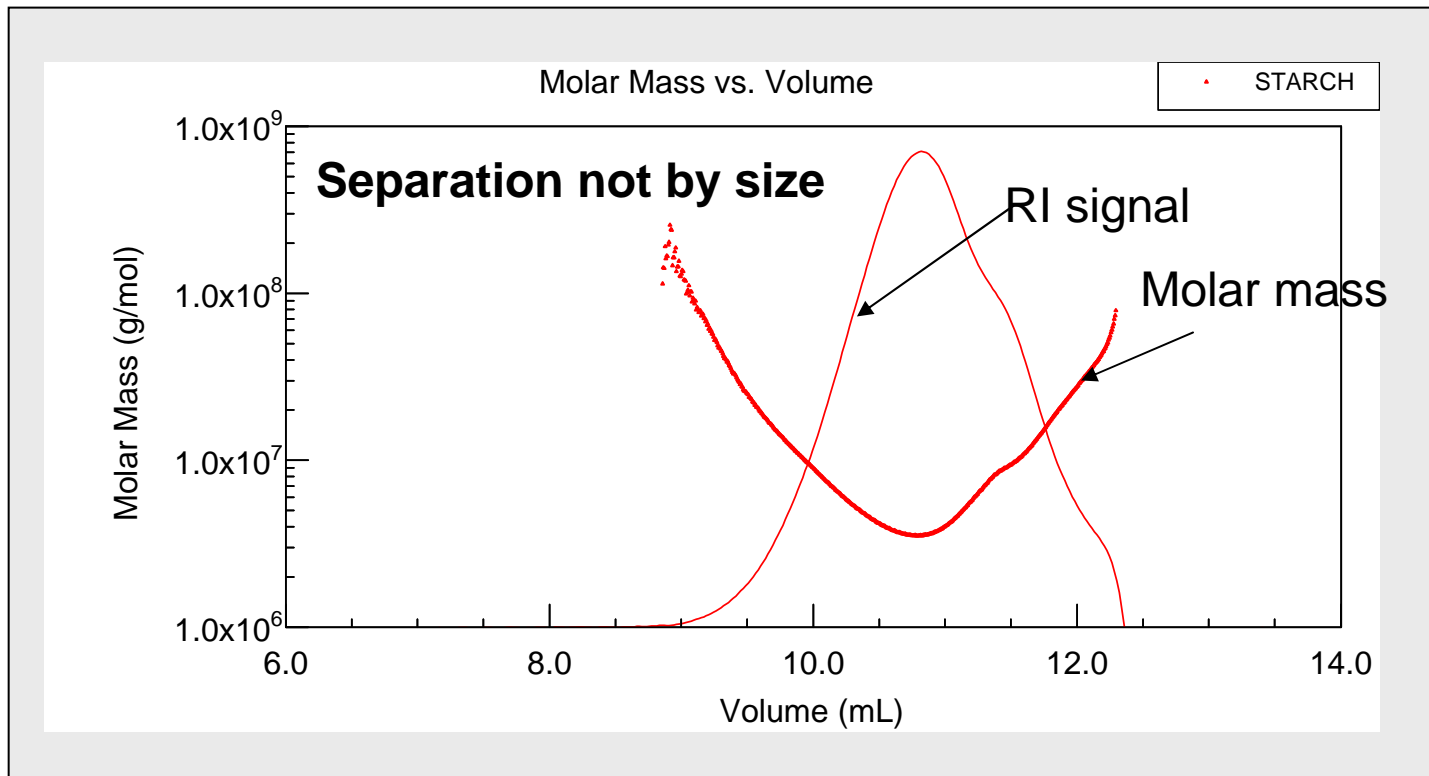
Absolute Detection of Polydispersity

- light scattering yields right polydispersity

More Information for Trouble Shooting

- adsorption phenomenon
- bad columns

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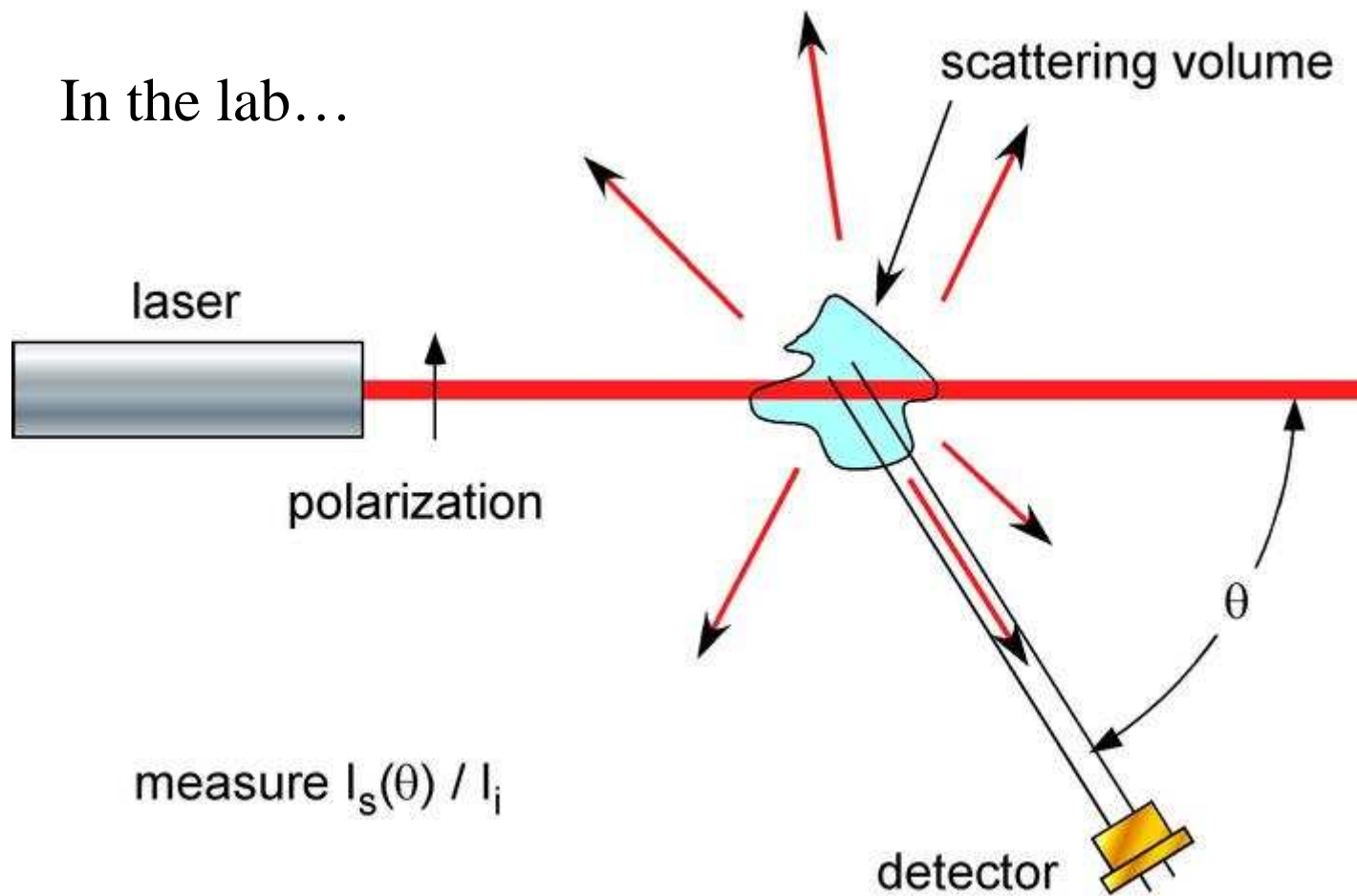
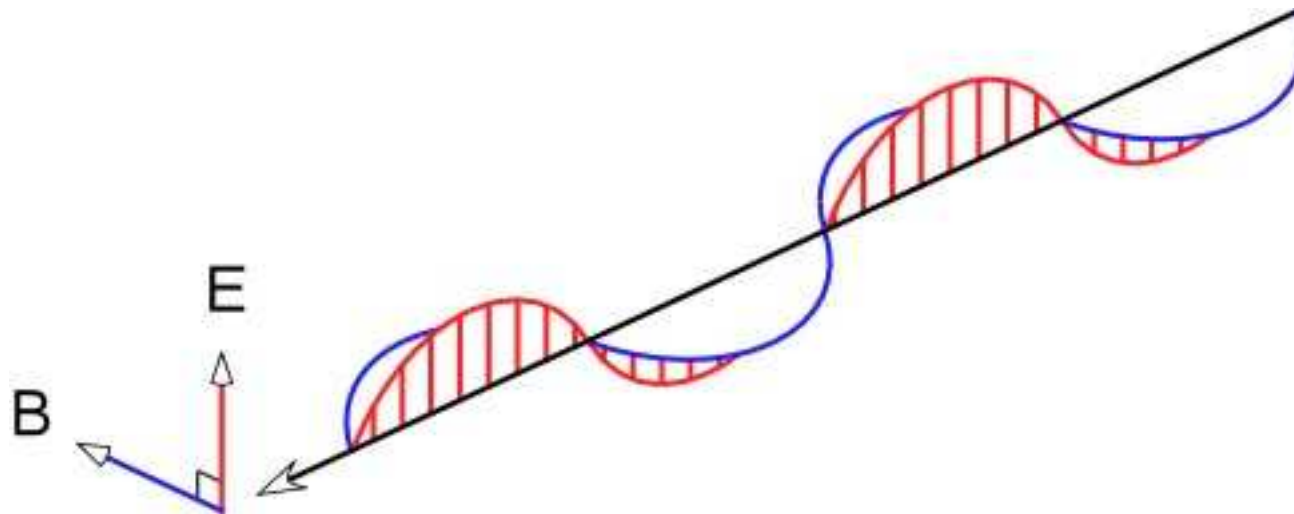- **Comparison SEC versus FFF**

- **Summary**

Light Scattering
In Nature …

**Light Scattering
In the Lab …**

In the lab…

scattering volume

laser

polarization

measure $I_s(\theta) / I_i$

θ

detector

8

Light Scattering
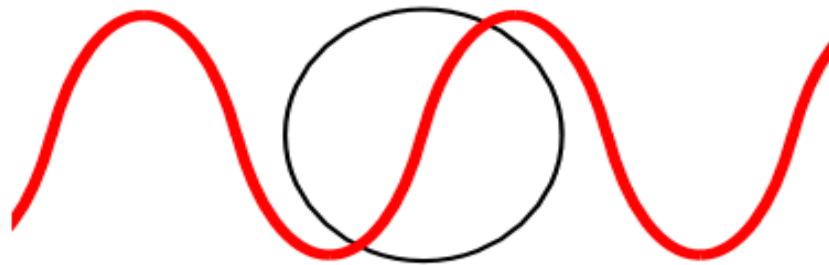# Light and it's Properties

- Polarization: direction of electric field oscillation
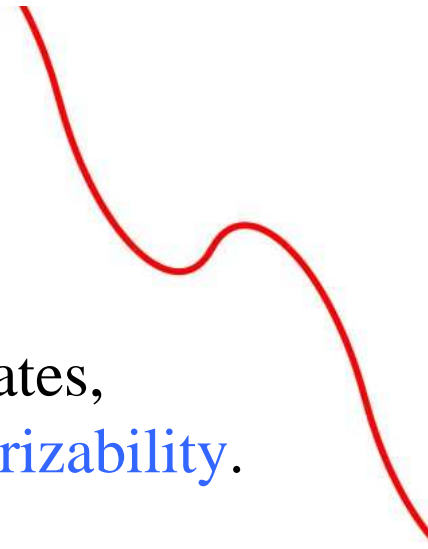
- $$I \propto \left| E \right|^2$$

Light Scattering
How does Light scatter?

When light interacts with matter, it causes charges to polarize.

The oscillating charges
radiate light.

How much the charges move,
and hence how much light radiates,
depends upon the matter's polarizability.

Light Scattering
Index of Refraction n

The polarizability of a material is directly related to its index of refraction *n*.

$a \approx n^2 - 1$

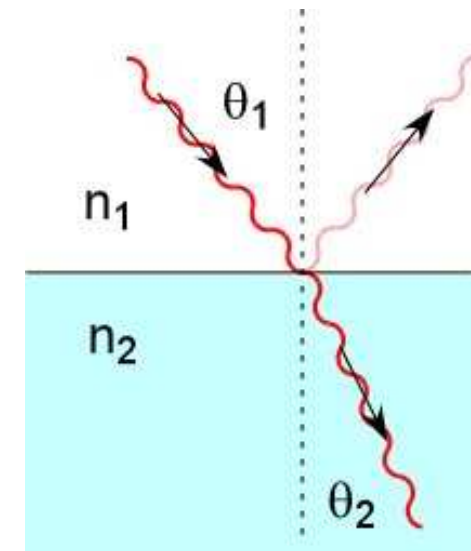The index of refraction is a measure of the velocity of light in a material.

e.g., speed of light
$$V_{liquid} = \frac{V_{vacuum}}{n}$$
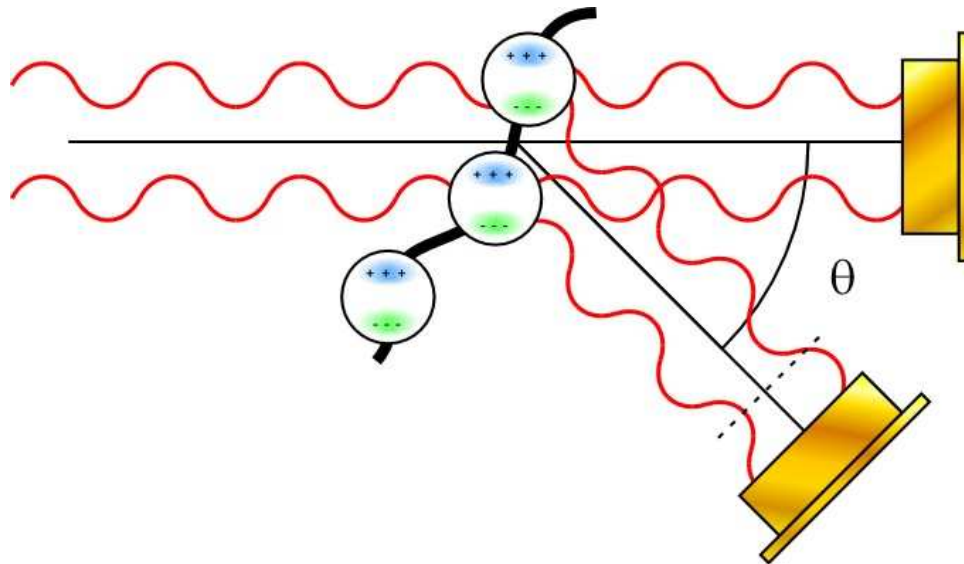
For solutes, the polarizability is expressed as the specific refractive index increment, *dn/dc*.

$$E_{scattered} \propto \frac{dn}{dc}$$

$$I_{scattered} \propto \left(\frac{dn}{dc}\right)^2$$

Light Scattering
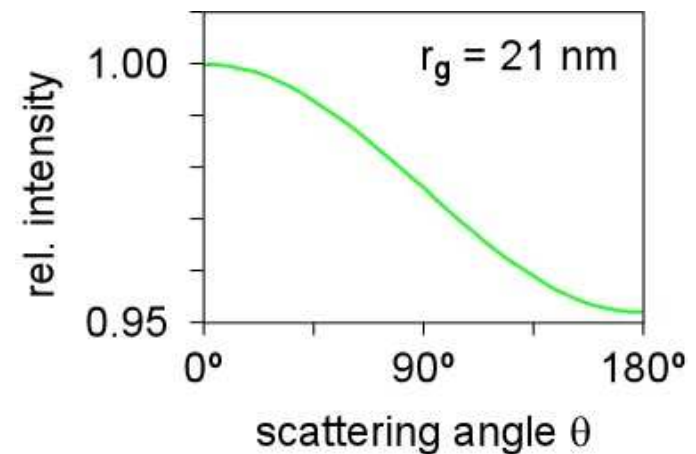## Angular Dependence of Light Scattering

detector at 0°
scattered light
in phase

detector at θ, scattered light
out-of-phase

Intramolecular interference leads to a
reduction in scattering intensity as the
scattering angle increases.

$$I(\theta)_{\text{scattered}} \propto Mc\left(\frac{dn}{dc}\right)^2 P(\theta)$$
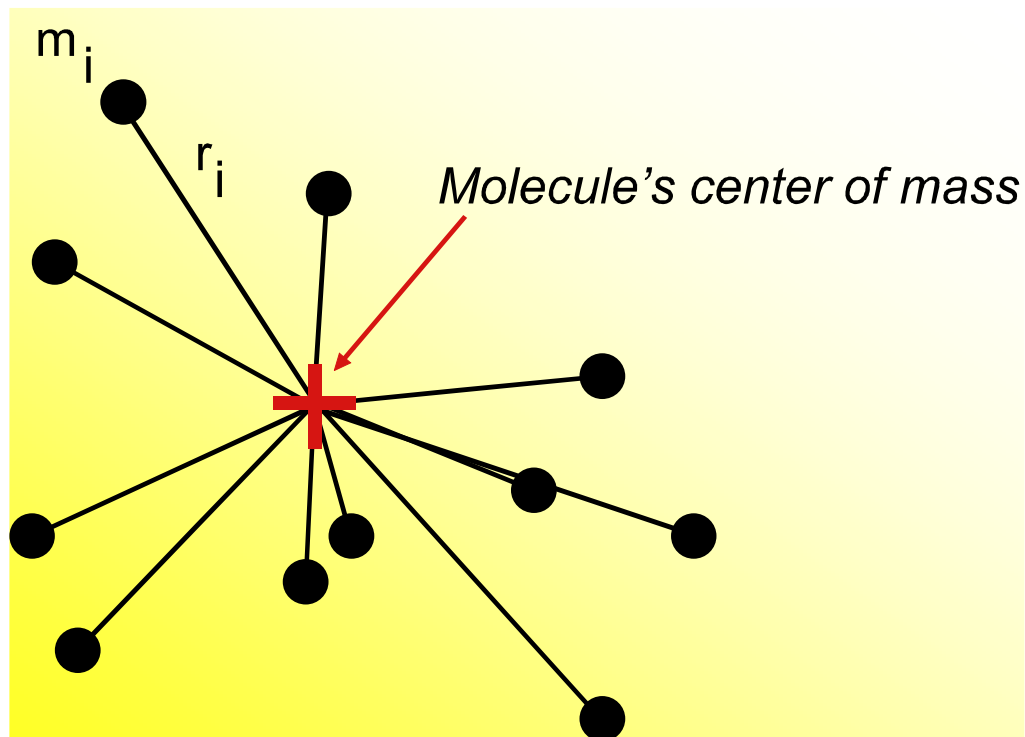
Light Scattering
Scattering Function

■ *P(θ)* is the form factor or "scattering function", which describes how the amount of scattered light varies with scattering angle. This variation is affected by polydispersity, conformation, and $<r_g^2>$, the mean square radius. The greater $< r_g^2 >$, the larger the angular variation. Note that $P(0°) = 1$
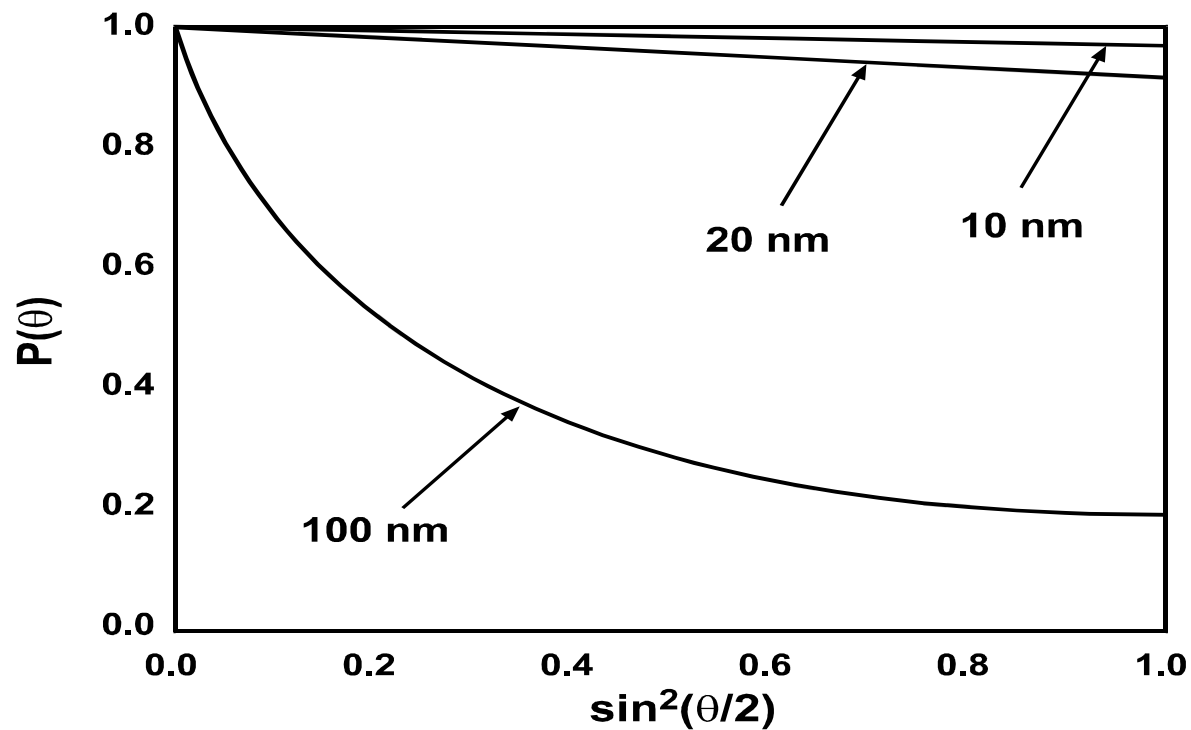
Light Scattering
Radius of Gyration

$<r_g^2>$ is the mean square radius, relating to the distribution of mass within the molecule, given by:

m_i

r_i

Molecule's center of mass

$$<r_g^{\ 2}> = \frac{\Sigma r_i^{\ 2} m_i}{M}$$
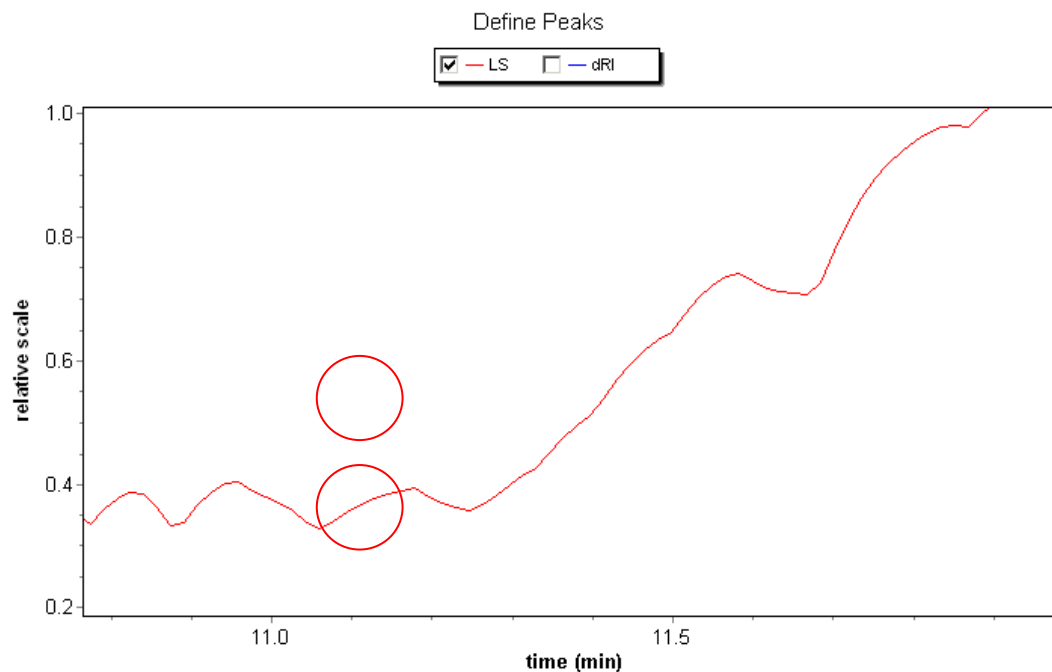
Light Scattering
Basic Principles

1. The amount of light scattered is directly proportional to the product of the molar mass and the molecular concentration

   ▪ [The amount of light scattered (divided by the incident light intensity) by a solution into a particular direction per unit solid angle in excess of the amount scattered by the pure solvent is directly proportional to the product of the weight-average molar mass and the molecular concentration. $R(\theta)$, in limit as $\theta \rightarrow 0$, $\propto Mc$]

2. The variation of scattered light with scattering angle is proportional to the average size of the scattering molecules.

   ▪ [The variation of light scattered with respect to $\sin^2\theta/2$, in the limit as $\theta \rightarrow 0$, is directly proportional to the average molecular mean square radius. $dR(\theta)/d\sin^2\theta/2 \propto <r_g^2>$]
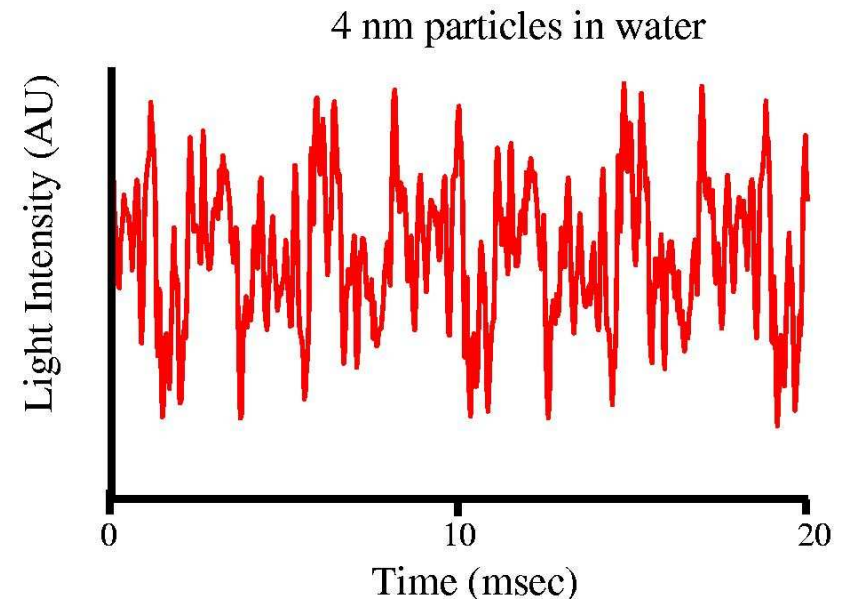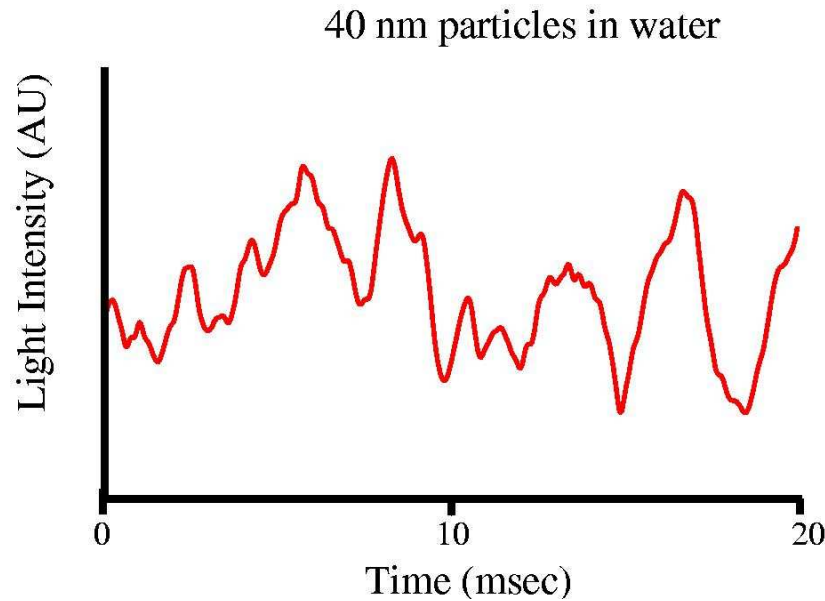
Light Scattering
Time dependent Intensity Fluctuations

- Averaged over longer time (seconds) the intensity of scattered light is constant and a function of molar mass.

- Fast measurement (µs) shows fluctuations in the intensity of the scattered light:

Light Scattering
Time dependent Intensity Fluctuations

40 nm particles in water

4 nm particles in water

- The rate at which particles diffuse is related to their size, provided all other parameters are constant.

- Brownian motion results in light intensities which fluctuate in time. The hydrodynamic radius is a function of Brownian motion.

Light Scattering
Timescale of Motion

How does one get from a diffusion coefficient to R_h?
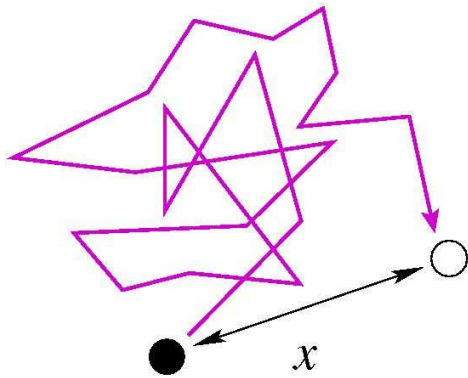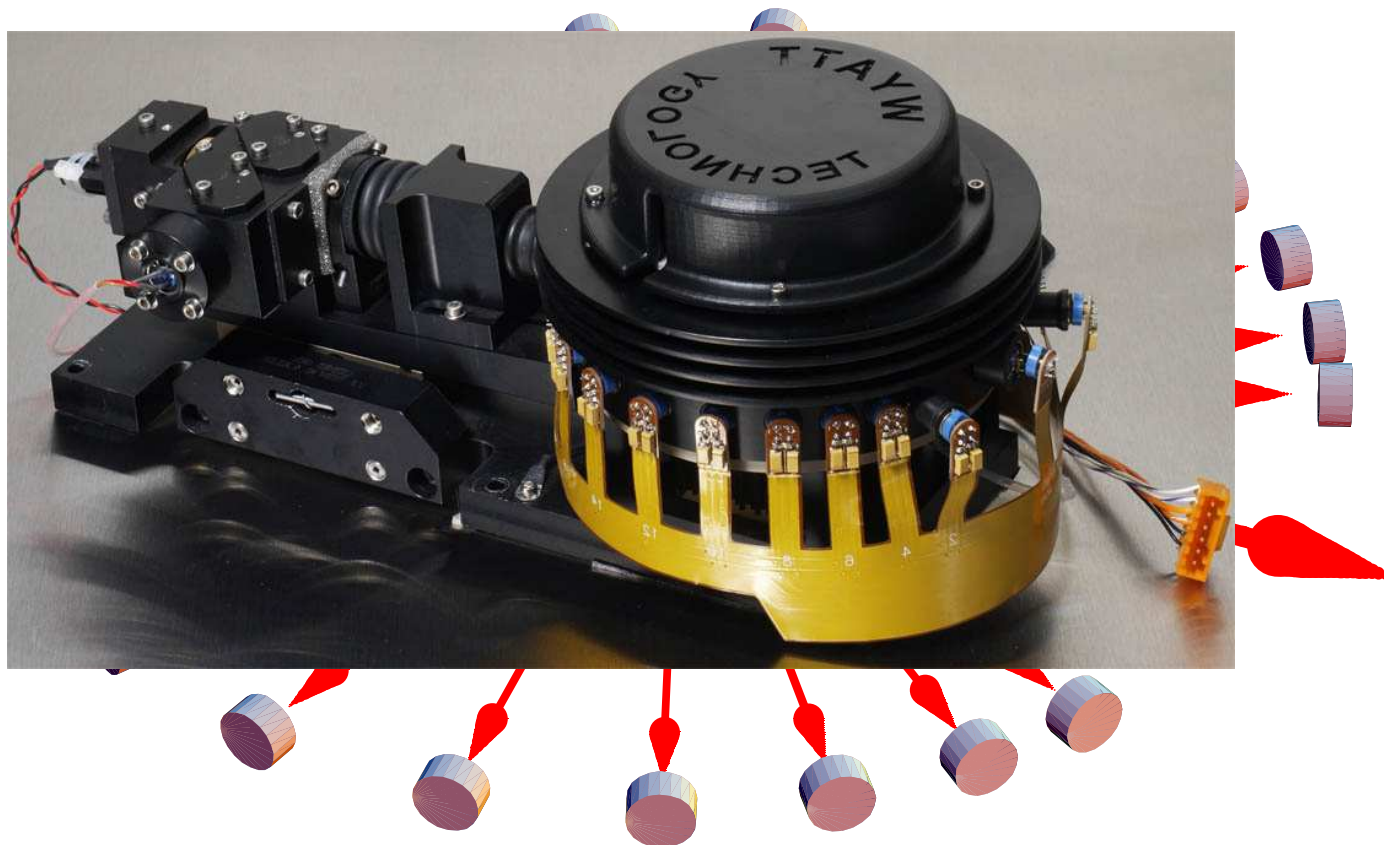
$$R_h = \frac{k_b T}{6\pi\eta D_t}$$

k_B – Boltzmann's constant
T – temperature (Kelvin)
η – viscosity of solvent
R_h – **hydrodynamic radius**

Multi Angle Light Scattering
DAWN Flow Cell & Detector Geometry

Multi Angle Light Scattering
# Basic Light Scattering Equation

$$\frac{K*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c$$

where

$K^* =$	$4\pi^2 \, (dn/dc)^2 \, n_0^2 / (N_A \lambda_0^4)$
n_0	is the refractive index of the solvent.
c	is the concentration of the solute molecules (g/ml).
$R(\theta)$	is the fraction of light scattered per unit solid angle, in excess of the light scattered by the solvent, divided by the incident intensity.
N_A	is Avogodro's number.
l_0	is the vacuum wavelength of the incident light.
dn/dc	is the refractive index increment, which tells how much the refractive index of the solution varies with solute concentration.
M_w	is the weight-average molar mass.

Multi Angle Light Scattering
Data Analysis – Online Experiment

- Perform fit of angular data
- Intercept gives Mw for that slice
- Initial slope gives rms radius for that slice
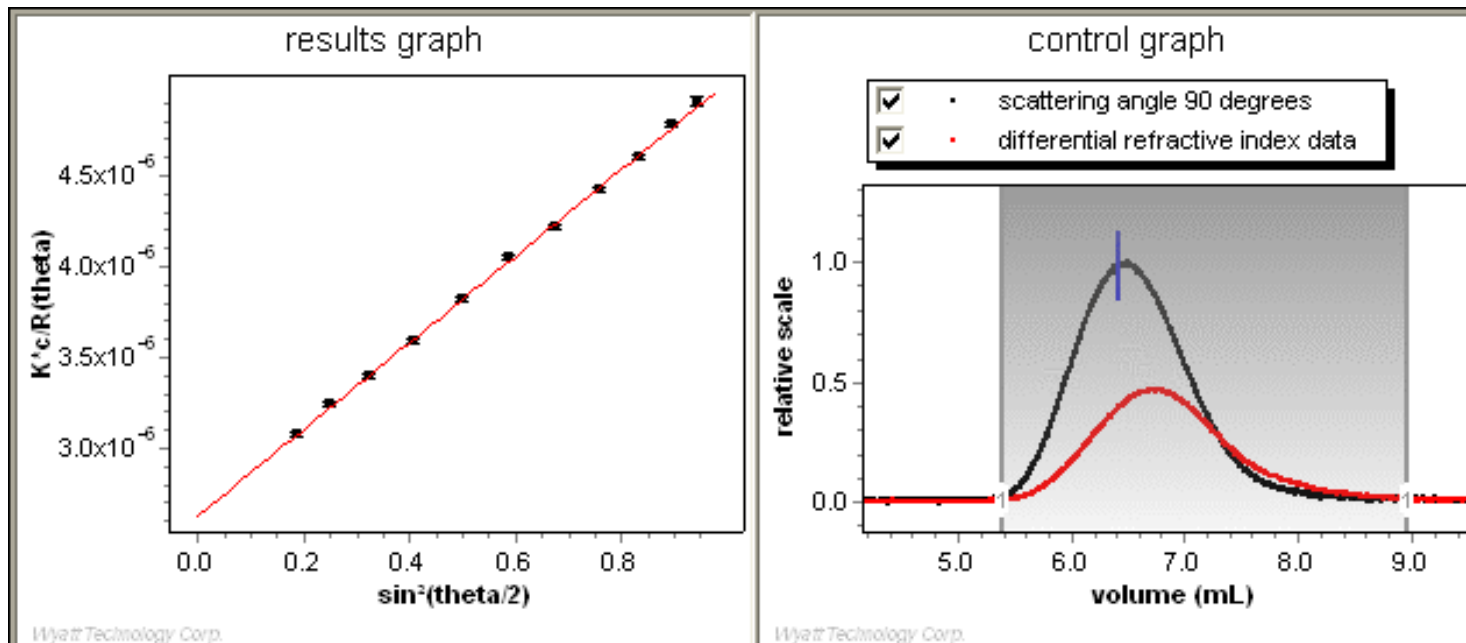
	Value
Molar Mass	(3.798 ± 0.023) e+5 g/mol
rms radius	62.0 ± 0.3 nm

Multi Angle Light Scattering
What do we mean by ABSOLUTE?

NO Reference to standards of mass

ALL parameters measured directly from 1st principles

- Refractive indices
- geometries of cell and detector
- wavelength
- concentrations
- detector response
- temperature
- dn/dc

> Absolute molar mass from light scattering requires calibration with a pure solvent (toluene)

NO assumptions of molecular model/conformation

There are 4 Absolute Methods

- 1) Membrane Osmometry (*Number* Average MM)
- 2) Light Scattering (*Weight* Average MM)
- 3) Sedimentation Equilibrium (Ultracentrifugation) (*z-average* MM)
- 4) Mass spectrometry

Multi Angle Light Scattering
Instrumentation: DAWN HELEOS II
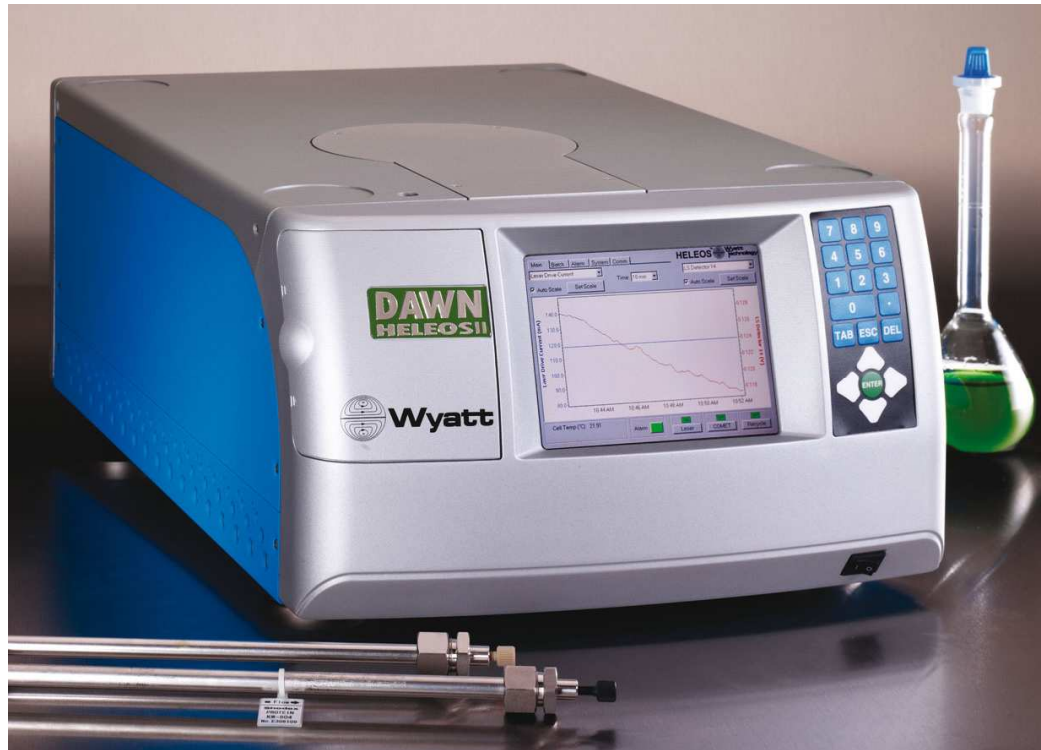
- 18 angular detectors ranging from ~10° to ~160°
- MM's from ~$10E^2$ to ~$10E^9$ g/mol
- Molecular size from ~8 nm to ~1 μm
- Precision of 2-5%
- Reproducibility better than 1%
- 120 mW 658 nm laser
- Ability to measure QELS at 18 different angles

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  - Asymmetrical Flow Field-Flow Fractionation (AF4)

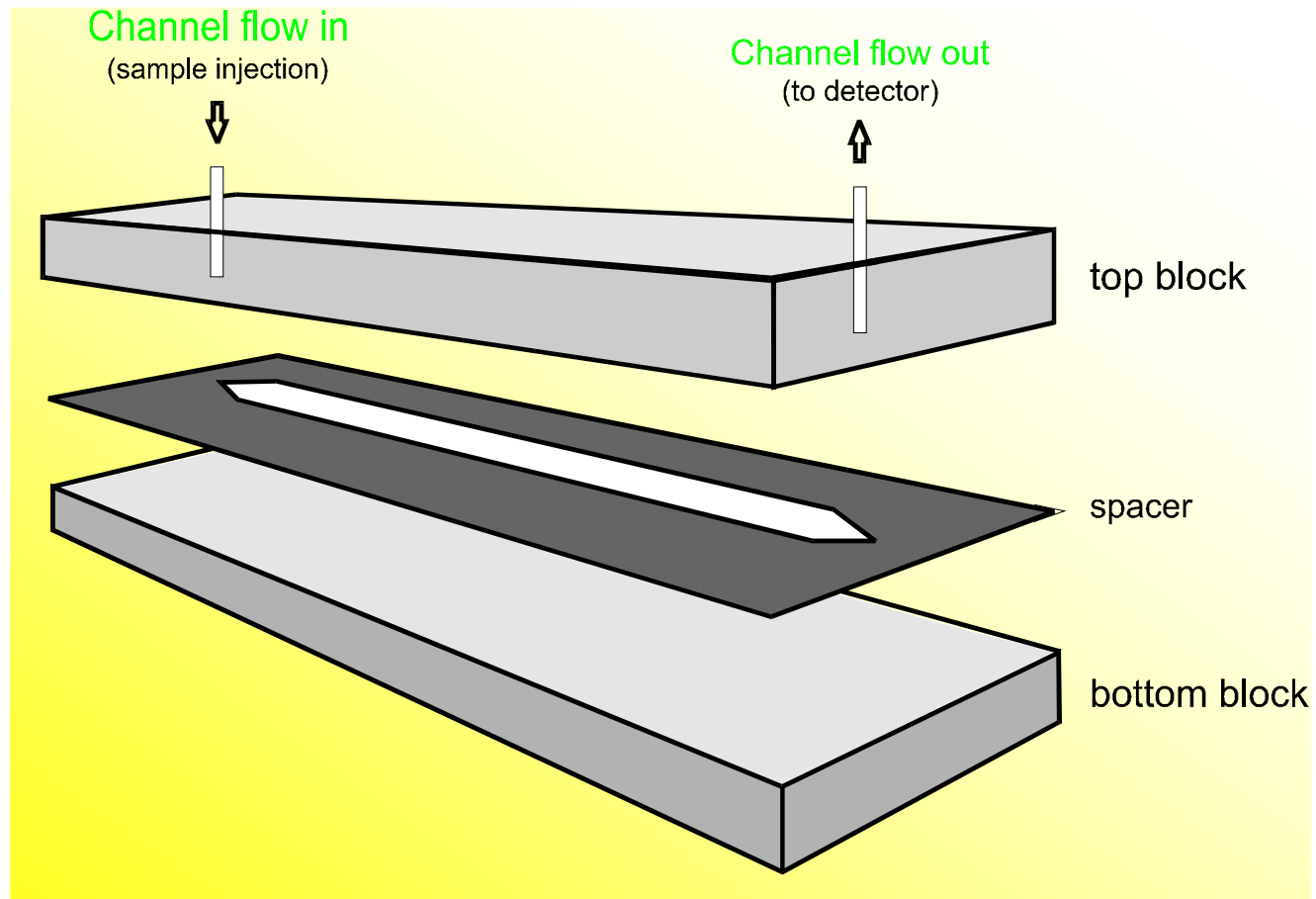- **Comparison SEC versus FFF**

- **Summary**

AF4
Basics of Field-Flow Fractionation (FFF)

- FFF is a well known separation technique with distinct advantages over column separation for many applications

- Asymmetrical Flow FFF (AF4) has originally been developed by Karl-Gustav Wahlund

- AF4 has made a commercial break through in the last few years, mainly in pharmaceutical companies and research institutes
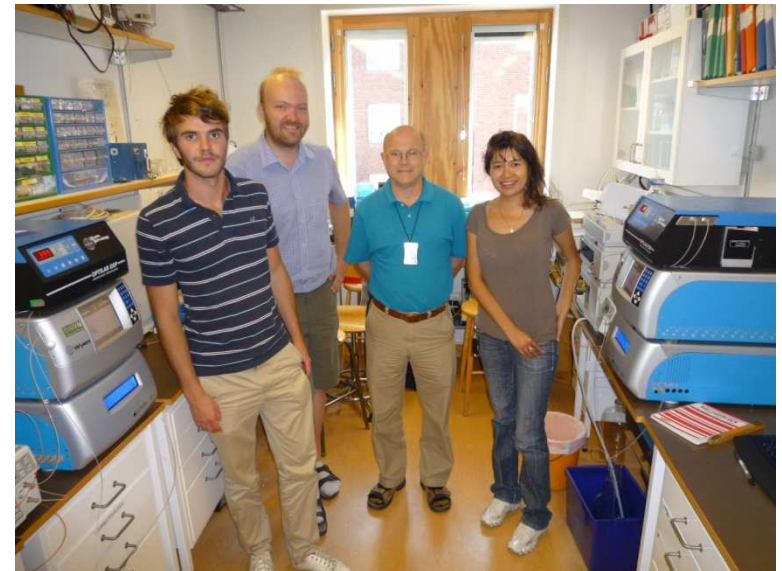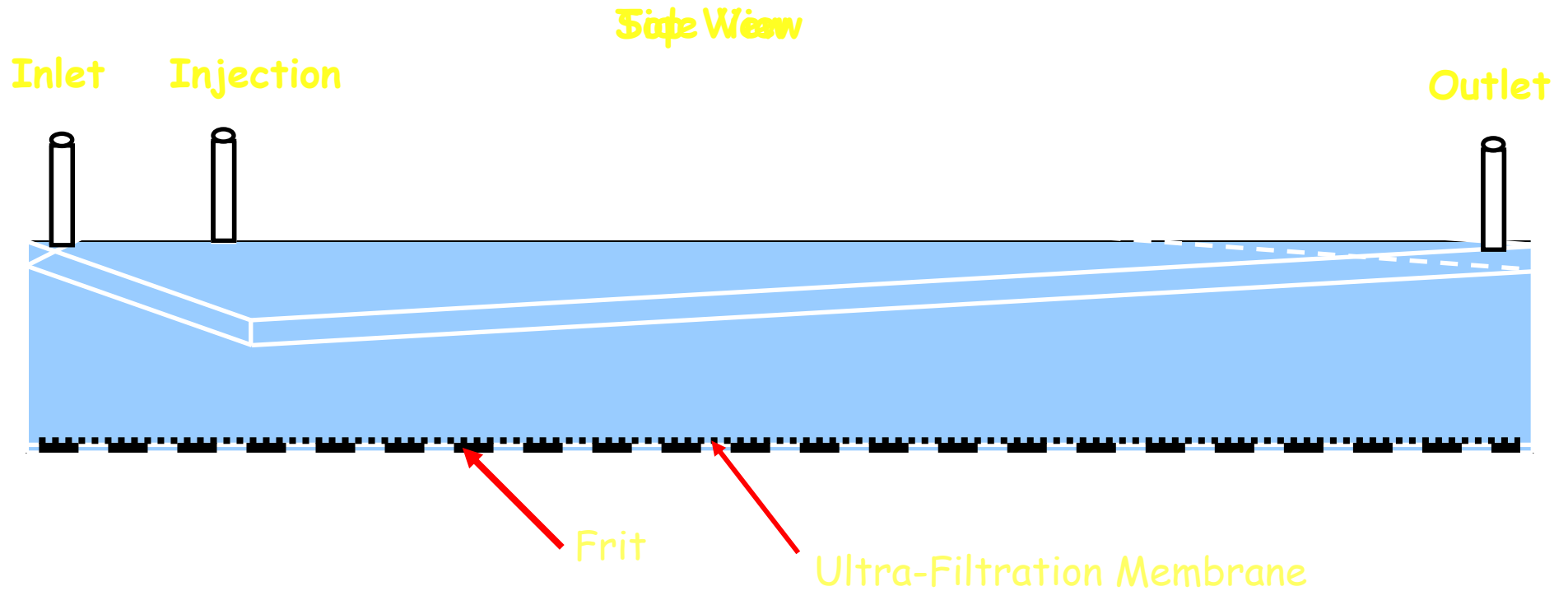
Field-Flow Fractionation Channel

AF4
Field-Flow Fractionation Principle

Side View

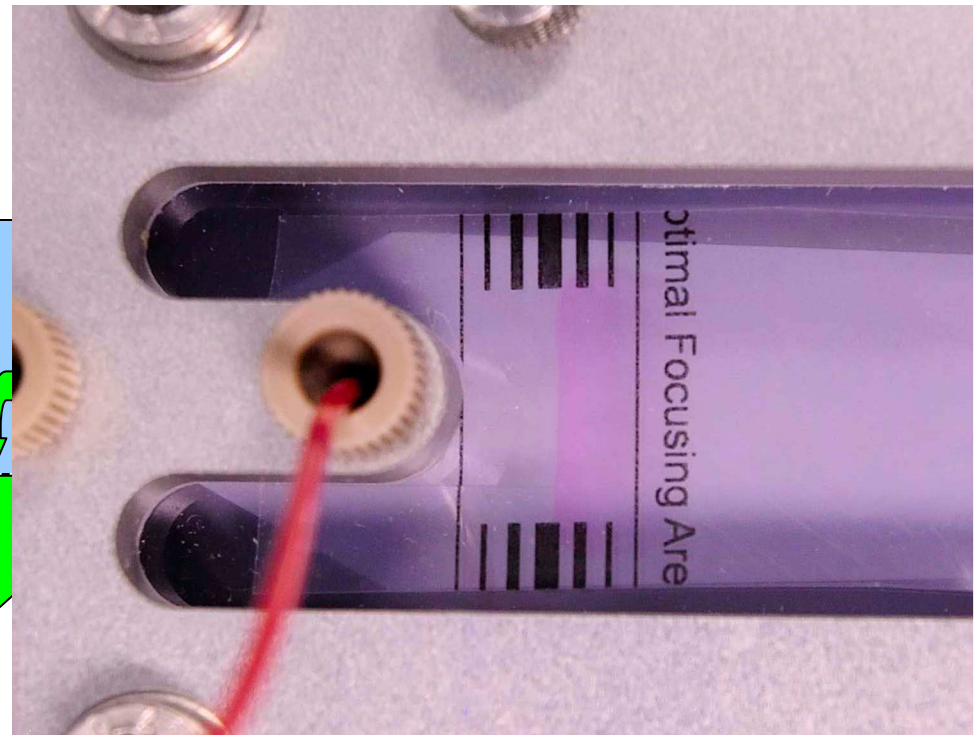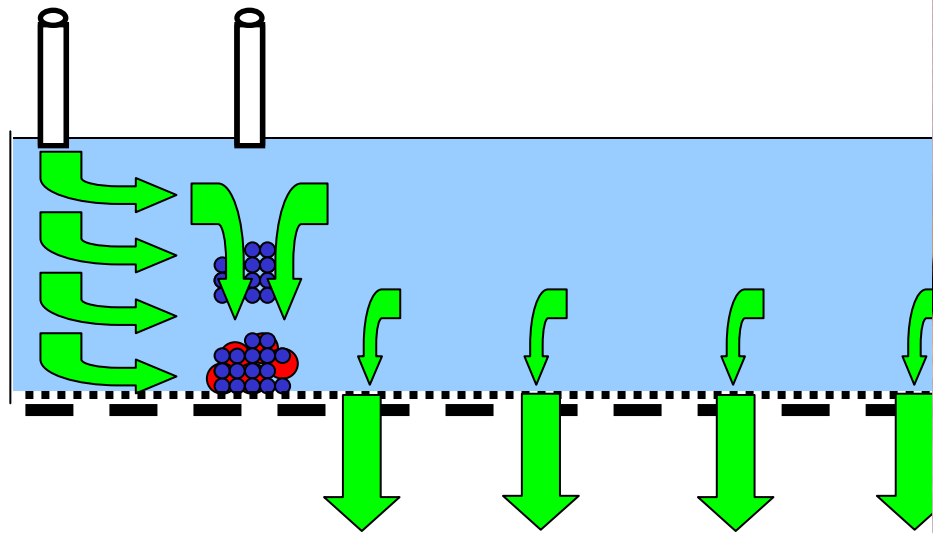Inlet Injection Outlet

Frit

Ultra-Filtration Membrane

AF4
Field-Flow Fractionation Principle

Stage 1: Injection/Relaxation

Inlet   Injection   Exit

AF4
Field-Flow Fractionation Principle
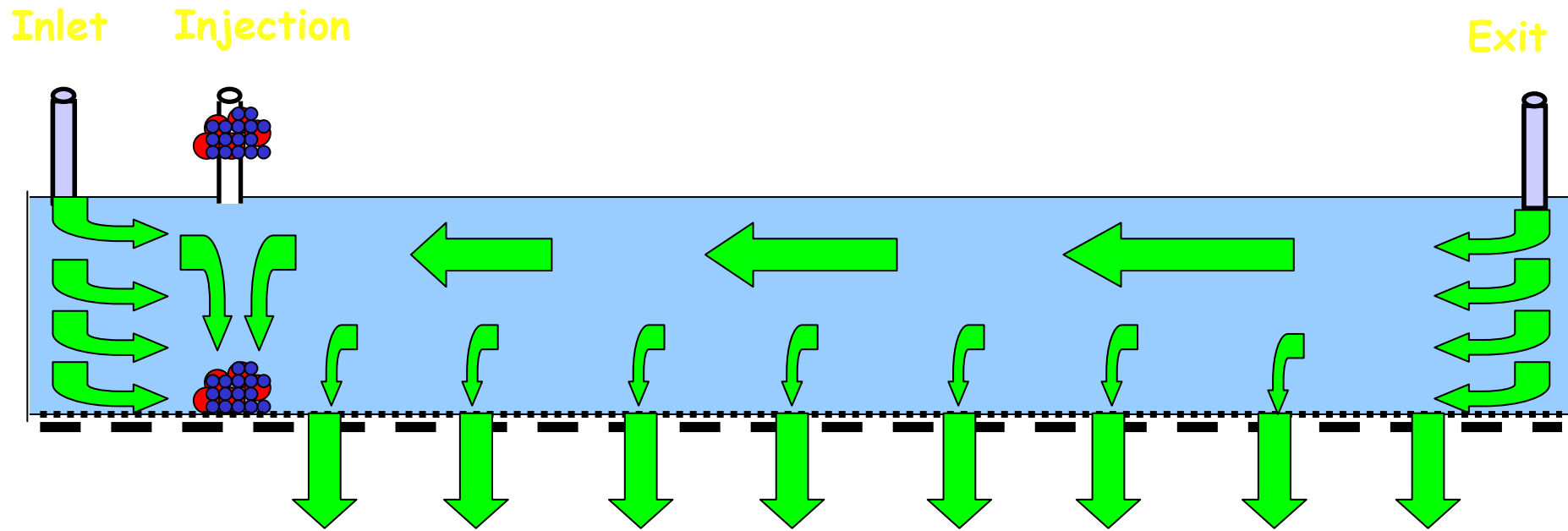
Stage 1: Injection/Relaxation

Inlet   **Injection**

AF4
Field-Flow Fractionation Principle

Stage 2: Elution

Inlet   Injection   Outlet
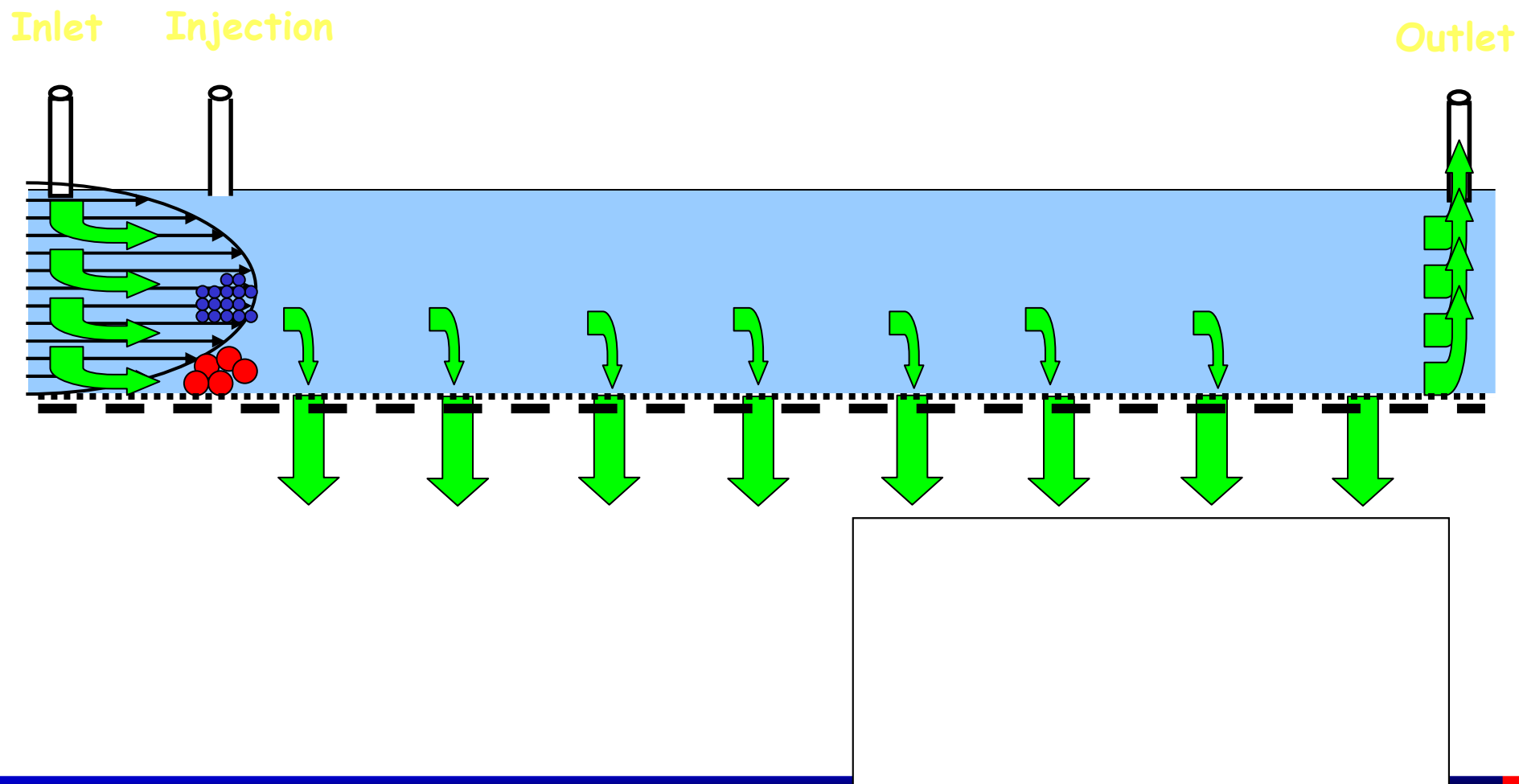
Flow FFF
Retention Function

$$t_r = \frac{\pi \cdot \eta \cdot d \cdot w^2}{2 \cdot kT} \cdot \frac{\dot{V}_{Cross}}{\dot{V}_{Channel}}$$

t_r retention time

w   channel thickness

V   flow rate for channel and cross flow

d   Stokes diameter

h   viscosity of solvent

k   Boltzmann's constant

T   absolute temperature

Use of FFF Theory to Predict a Separation Result

- The very well worked out FFF theory can be used to predict a separation result

- The only parameter which is not known is w, the effective channel height; it is calibrated by running a sample with known R_h

- w is adjusted to reproduce the known size at the measured retention time

AF4
Instrumentation

- AF4 control unit and separation channel

- Regular HPLC components for pump, auto sampler, detectors

- Can be coupled to MALS, ICP, MS

- Fractions can be collected for further off-line analysis

AF4
Different Channel Dimensions to Optimize Separation

- Different channels can be used, e.g.
  115 mm, 152 mm, 240 mm length

- Spacer width
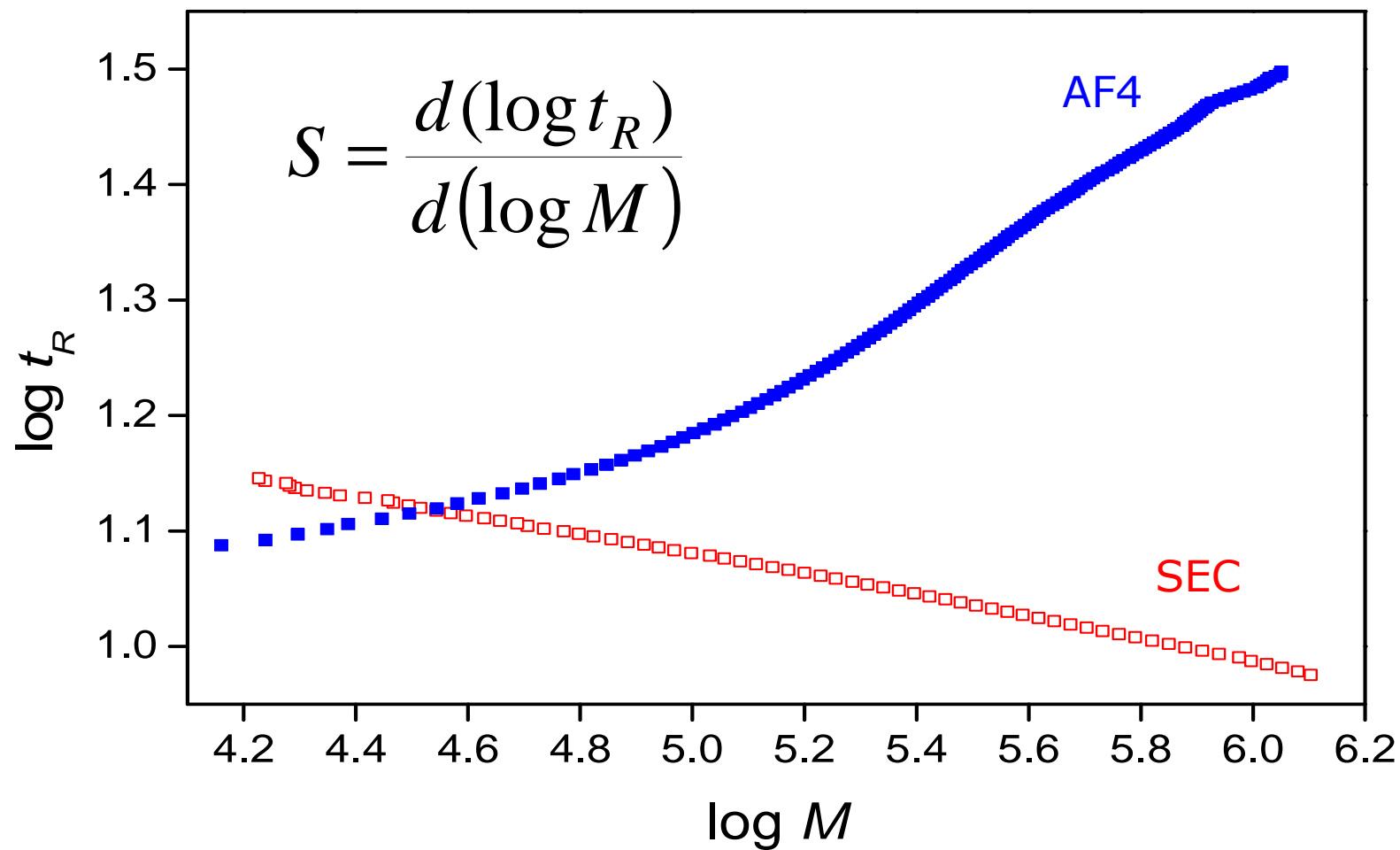 11.5 mm to 44 mm

- Spacer height
  250 µm to 800 µm

Comparison SEC versus FFF

- ■ Selectivity

- ■ Limitations of SEC

- ■ Advantages of FFF

- ■ Limitations of FFF

Selectivity: SEC versus AF4

$$S = \frac{d(\log t_R)}{d(\log M)}$$

AF4

SEC

SEC versus FFF

SEC Limitations

- High-molar-mass limit $\approx 10^7$ g/mol

- Possibility of shearing degradation of high-molar-mass fractions

- Large particles can be retained by SEC columns
  Information loss and reduced column life time

- Interactions of polymer molecules with column packing:
  e.g., amine containing polymers have strong tendency to interactions

- Limited injection volume: maximum 200 µL per 300 x 8 mm column

- Change of resolution by change of column: porosity, length, particle size

- Change of solvent requires time to flush and stabilize columns or use of column sets designated for different solvents

- Bleeding of particles disturbing light scattering signal

SEC versus FFF

FFF Advantages

- Broad separation range (molar mass $\approx$ 5000 – 10^9 g/mol)

- Characterization of particles up to μm scale

- Detection of aggregates and large species (microgels, …)

- Reduced possibility of shearing degradation

- No interactions with column packing

- Possibility to inject large injection volumes (very low concentration of solutions when needed)

- Change of resolution by change of separation conditions

- Easy and fast change of solvents with almost no time for system stabilization

- No bleeding of particles

- Low cost of membranes

SEC versus FFF

FFF Limitations

- Permeation of oligomeric fractions through membrane (overestimation of M_n)
- Sample is concentrated during focusing, this may lead to aggregation or entanglement

Three Key Application Areas of Flow-FFF

1. Branched polymers

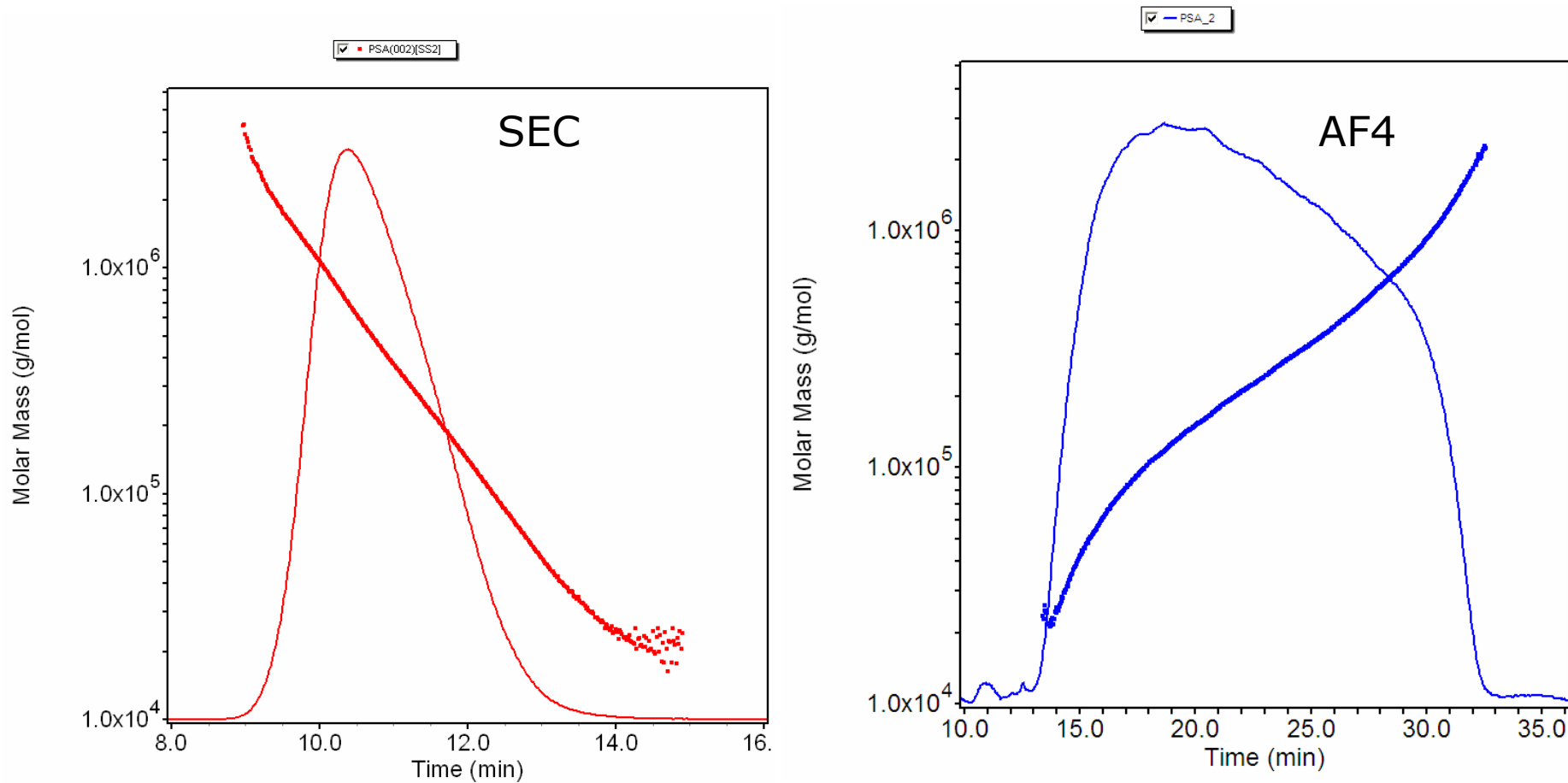   Superior conformation plots compared to SEC

2. Polymers containing ultra-high-molar-mass species

   Reduced shearing degradation

3. Polymers interacting with SEC packing material

   Total surface area in SEC is significantly larger than in AF4
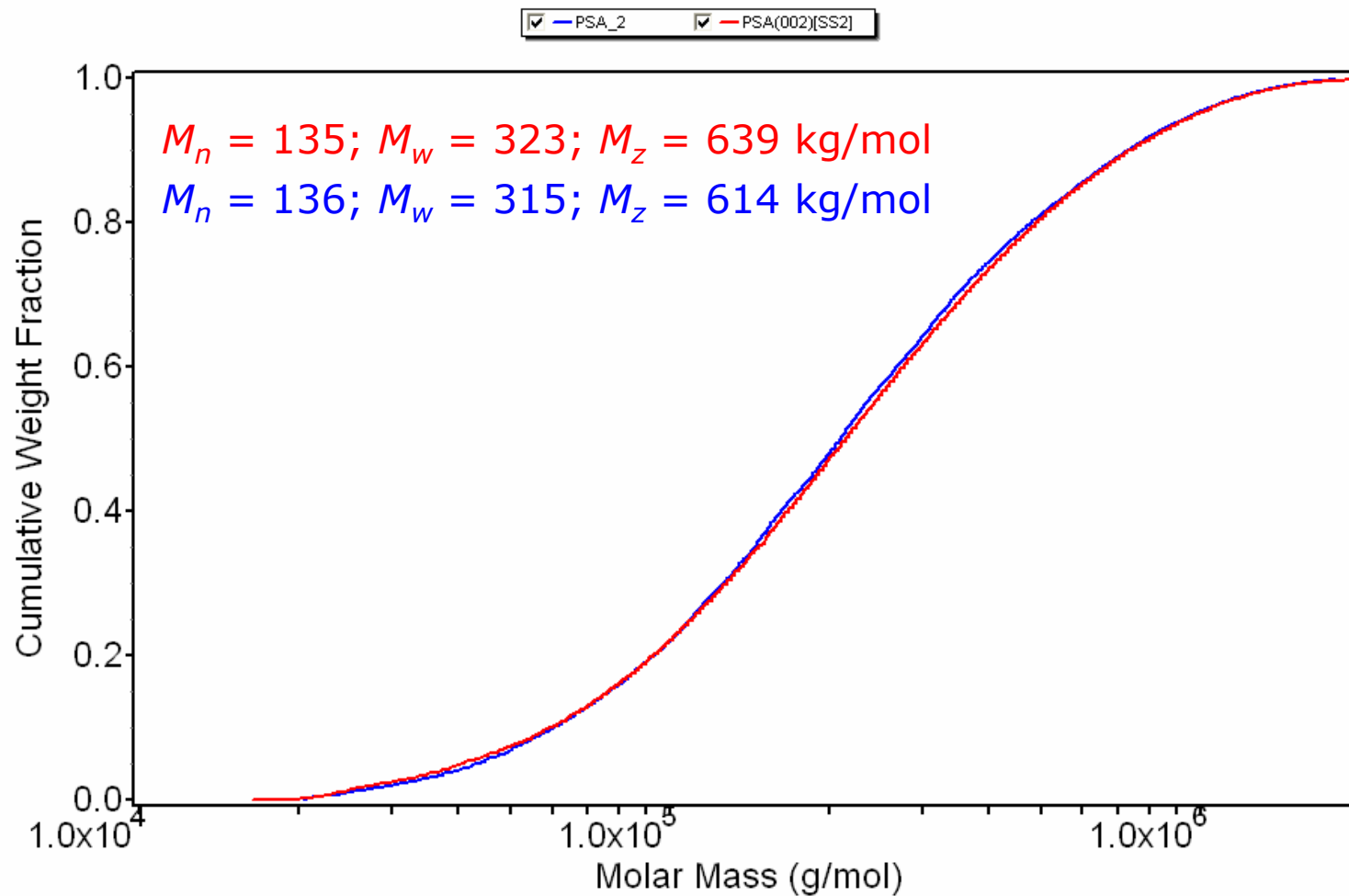
SEC vs. FFF: Polydisperse Linear Polymer

RI chromatograms and molar mass versus elution time plots of broad polystyrene obtained by **SEC-MALS** and **AF4-MALS**.
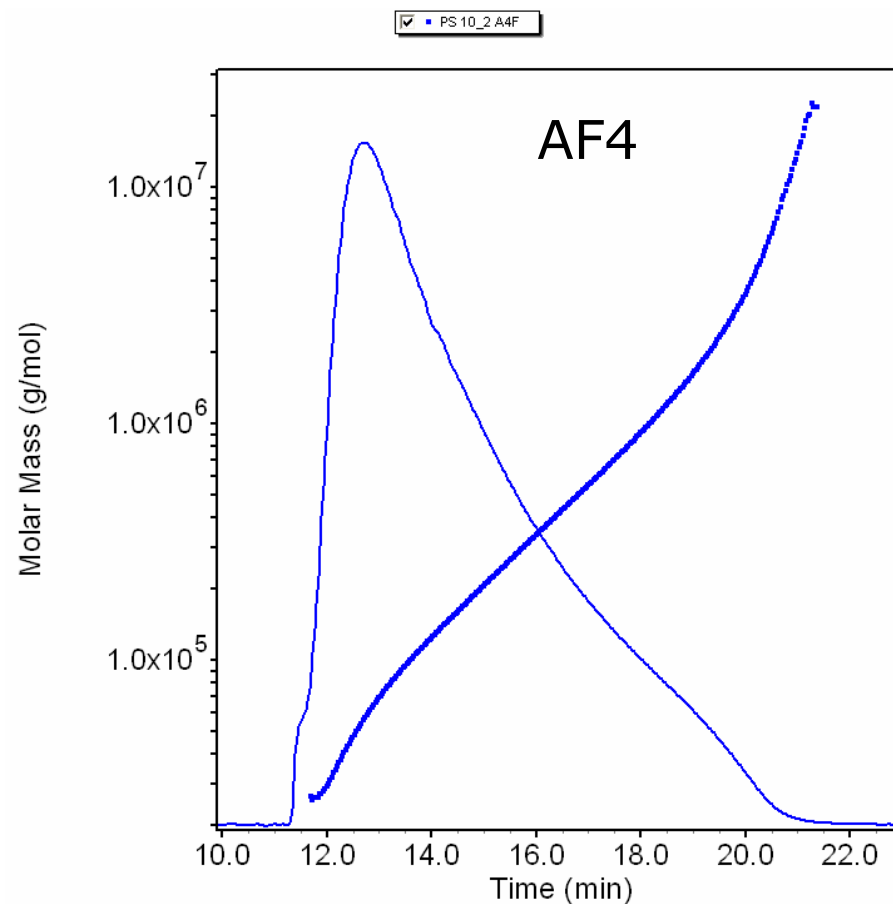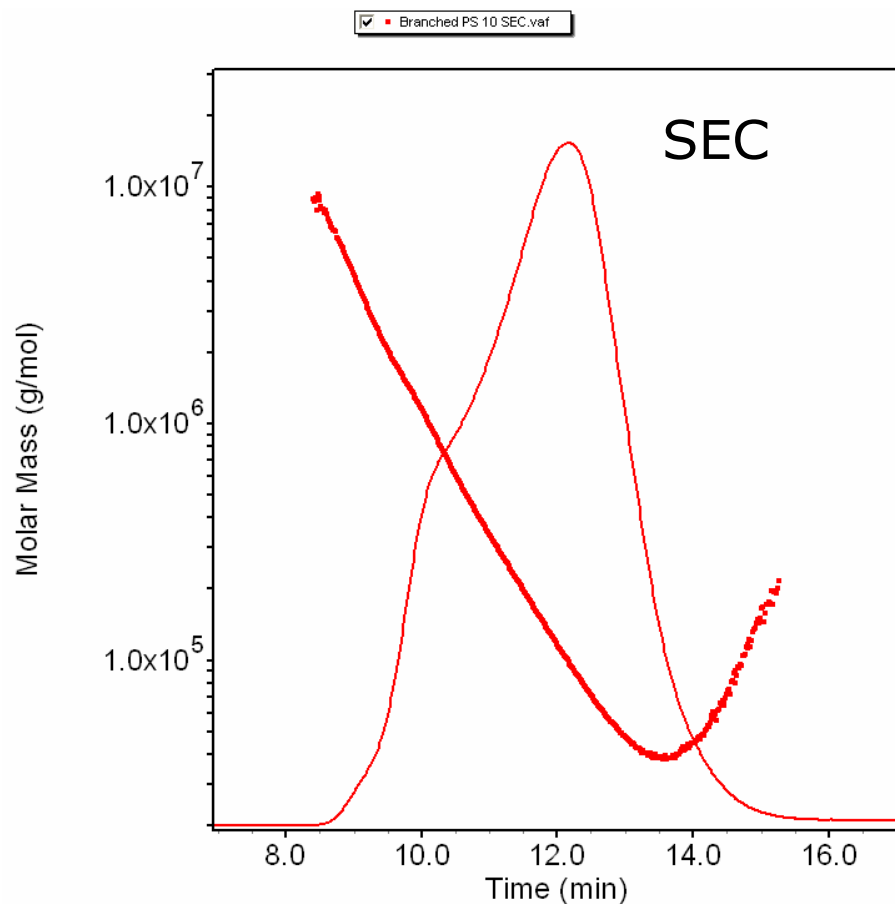
SEC vs. FFF: Polydisperse Linear Polymer

Cumulative distribution curves of broad polystyrene obtained by **SEC-MALS** and **AF4-MALS**.
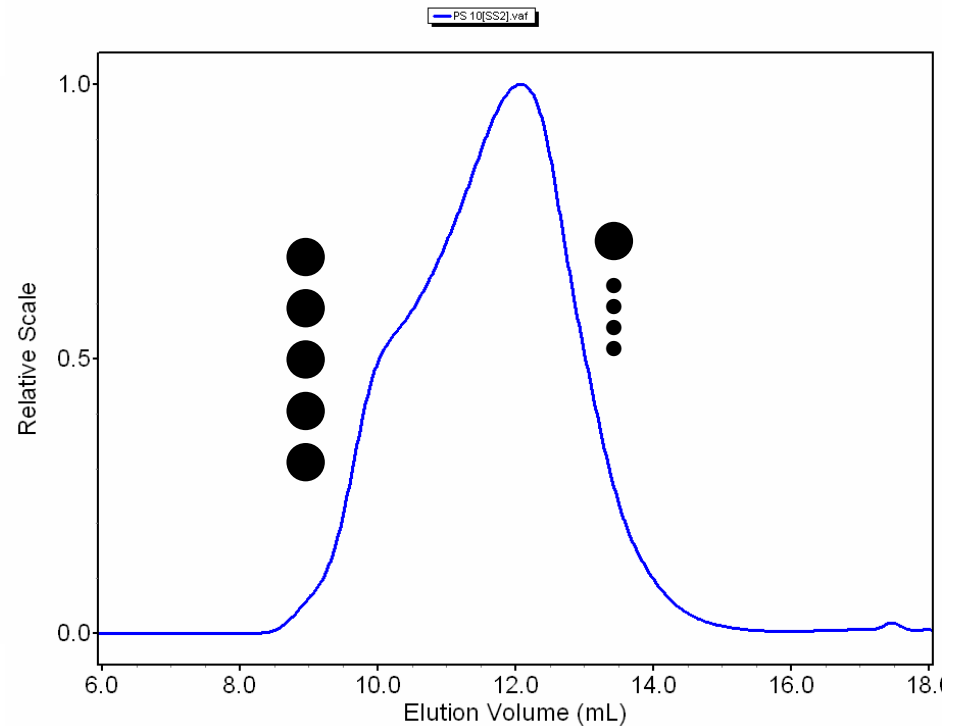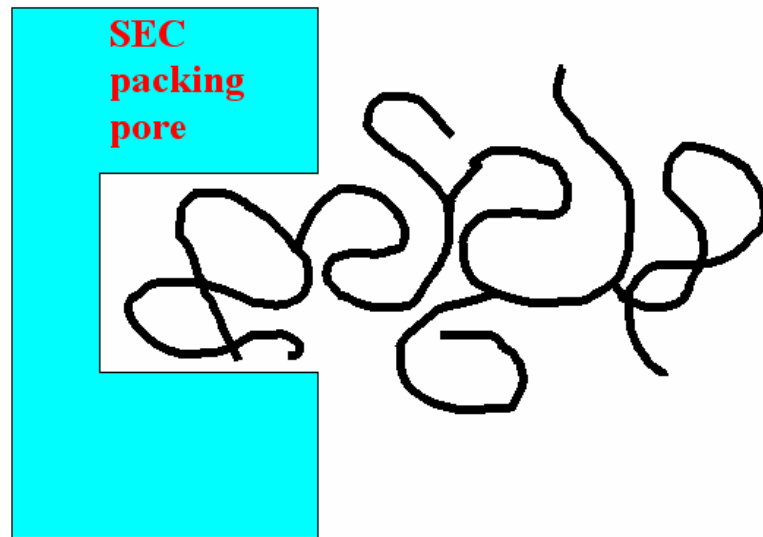
SEC vs. FFF: Branched Polymer

RI chromatograms and molar mass versus elution time plots of highly branched polystyrene obtained by **SEC-MALS** and **FFF-MALS**.

The upswing in SEC plot is due to the anchoring of highly branched molecules in pores of column packing and increased polydispersity of elution volume slices.

SEC Elution Behavior of Branched Polymers

- Large branched molecules are anchored in packing pores and elute later than it would correspond to their hydrodynamic size
- Slices at high elution volumes are contaminated by delayed large molecules and MALS detector measures M_w and R_z
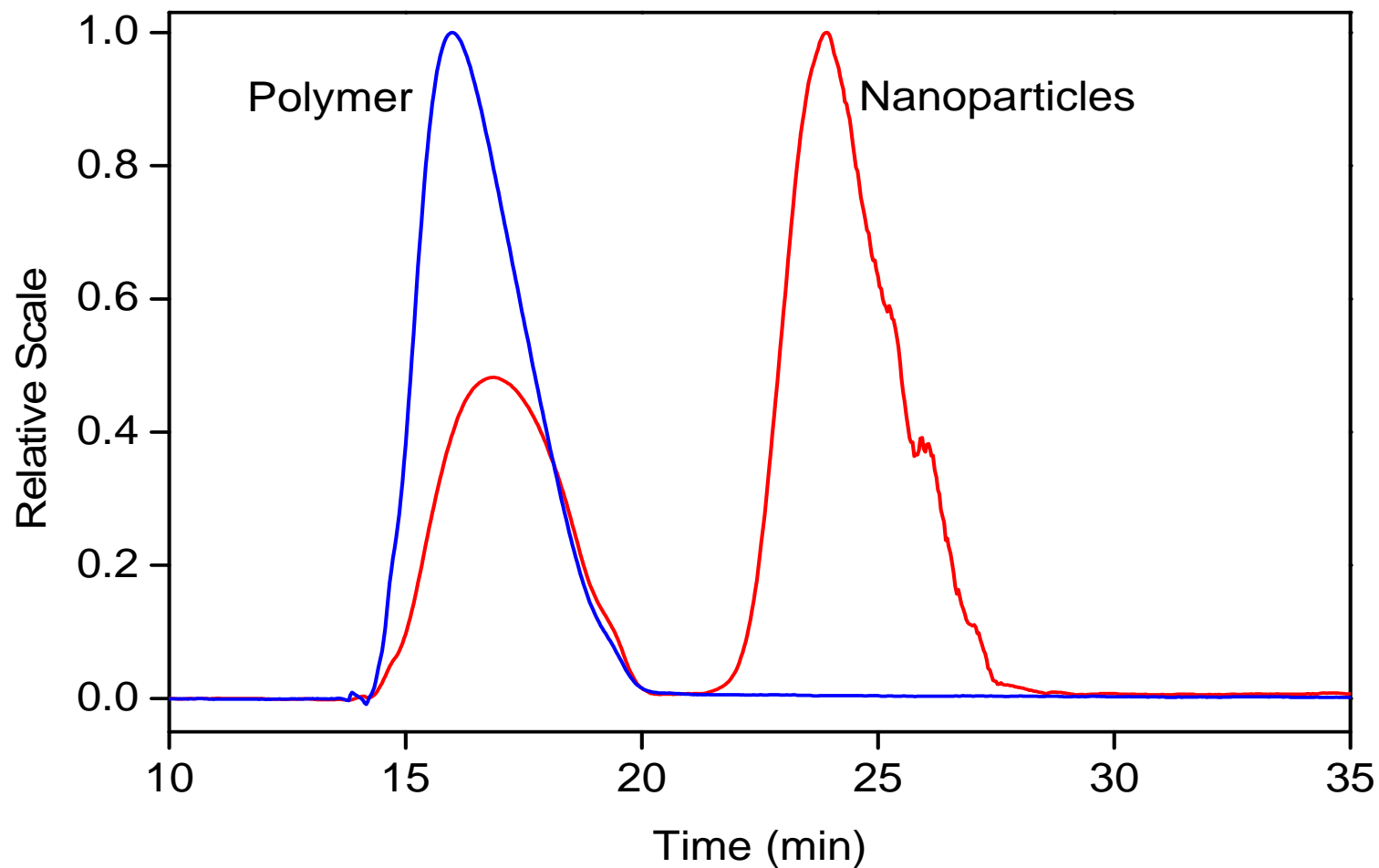- Z-average is more sensitive to small amounts of big molecules than the weight-average

SEC vs. AF4: Branched Polymer
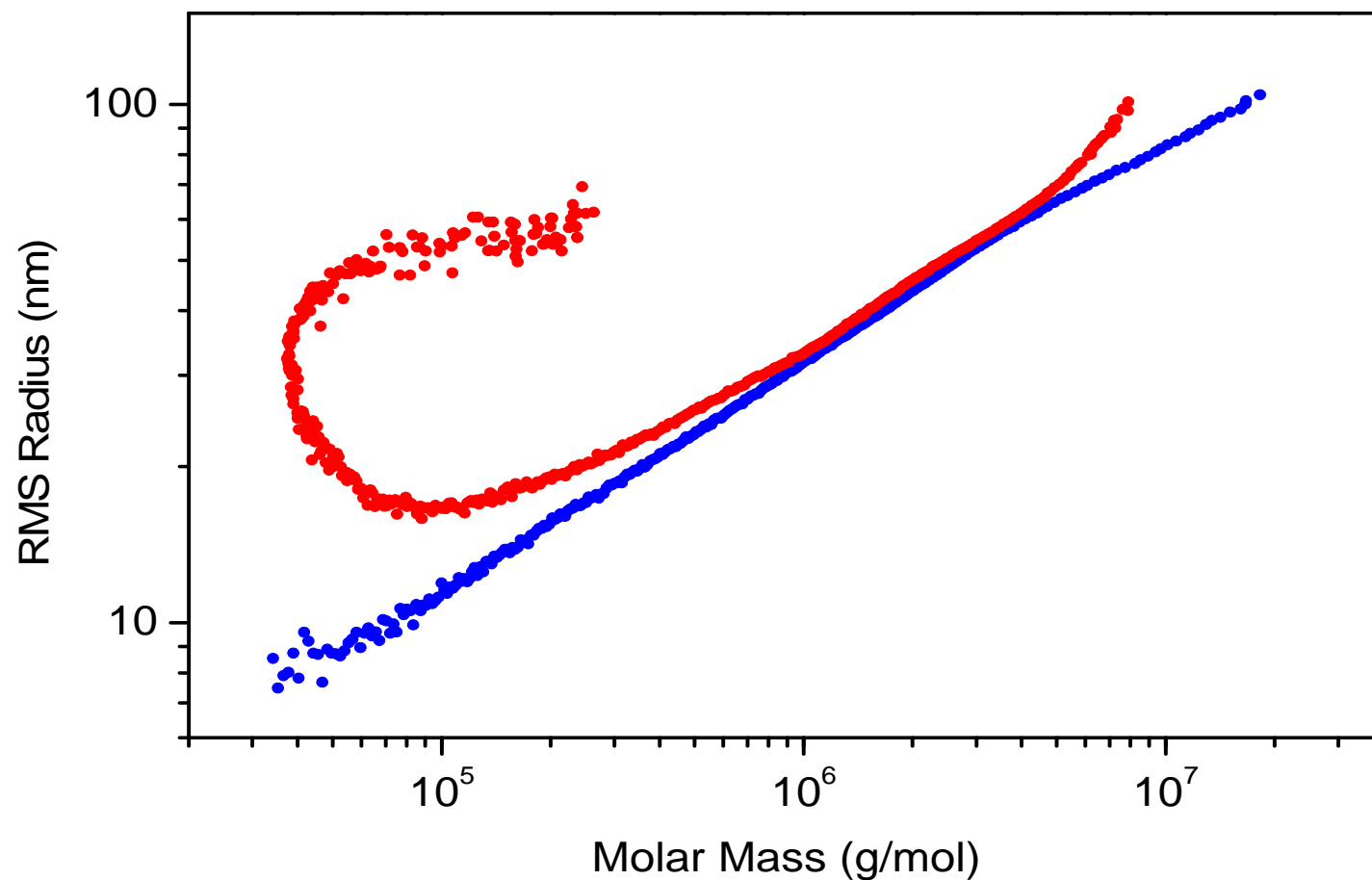
RMS radius versus molar mass plots of branched polystyrene obtained by **SEC-MALS** and **AF4-MALS**. No anchoring in empty FFF channel.
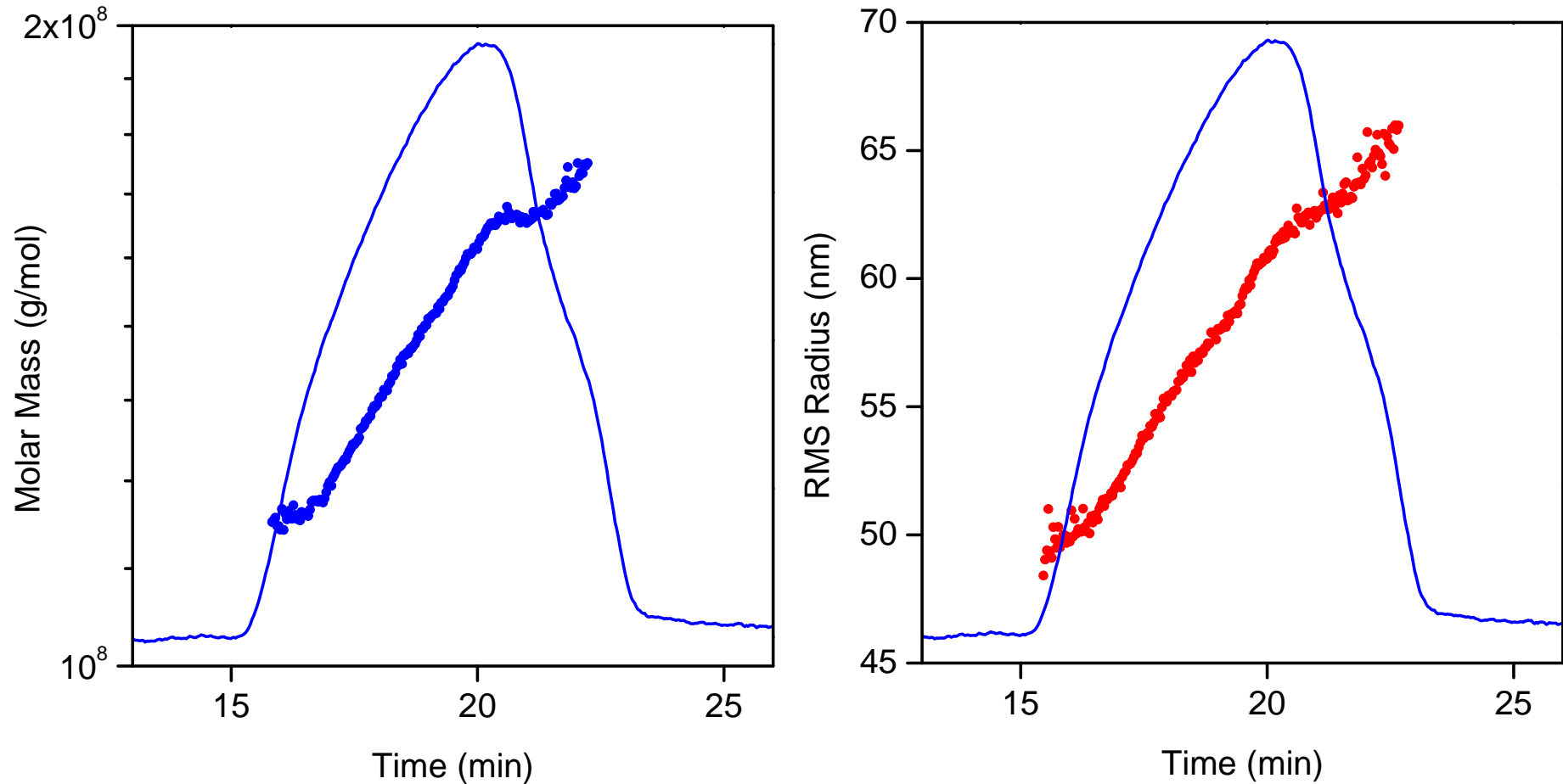
Polycarbonate with Nanoparticles

RI and **MALS** fractograms of polycarbonate containing nanoparticles.

Organic Nanoparticles in Epoxy Resin

RI fractogram and molar mass vs. retention time and RMS radius vs. retention time plots for organic nanoparticles added to epoxy resin.

Summary

- Static light scattering is used to calculate molar mass and RMS radius

- Flow-FFF is a versatile separation method specifically for large molecules and colloidal particles and it can be coupled to MALS to produce high resolution molar mass and size distributions

- Flow-FFF is also useful to prepare fractions for subsequent off-line analysis

- Flow-FFF can be superior to SEC specifically for branched polymers and composite materials containing both soluble macromolecules and colloidal material

Literature

- J. C. Giddings. *Field-Flow Fractionation: Analysis of Macromolecular, Colloidal, and Particulate Materials* - In Science, Vol. 260 pages 1456 1993.

- K.-G. Wahlund,J. Calvin Giddings. *Properties of an Asymmetrical Flow Field-Flow Fractionation Channel Having One Permeable Wall* - In Anal. Chem., Vol. 59 pages 1332-1339 1987.

- S. Podzimek T. Vlcek Christoph Johann. *Characterization of Branched Polymers by Size Exclusion Chromatography Coupled with Multiangle Light Scattering Detector. I. Size Exclusion Chromatography Elution Behavior of Branched Polymers* - In J. Appl. Polym. Sci., Vol. 81 pages 1588-1594 2001.

- S. Podzimek, T. Vlcek. *Characterization of Branched Polymers by SEC Coupled with a Multiangle Light Scattering Detector. II. Data Processing and Interpretation* - In J. Appl. Polym. Sci., Vol. 82 pages 454 -460 2001.