Small but Bright: The µDAWN Adds Multi-angle Light Scattering to APC
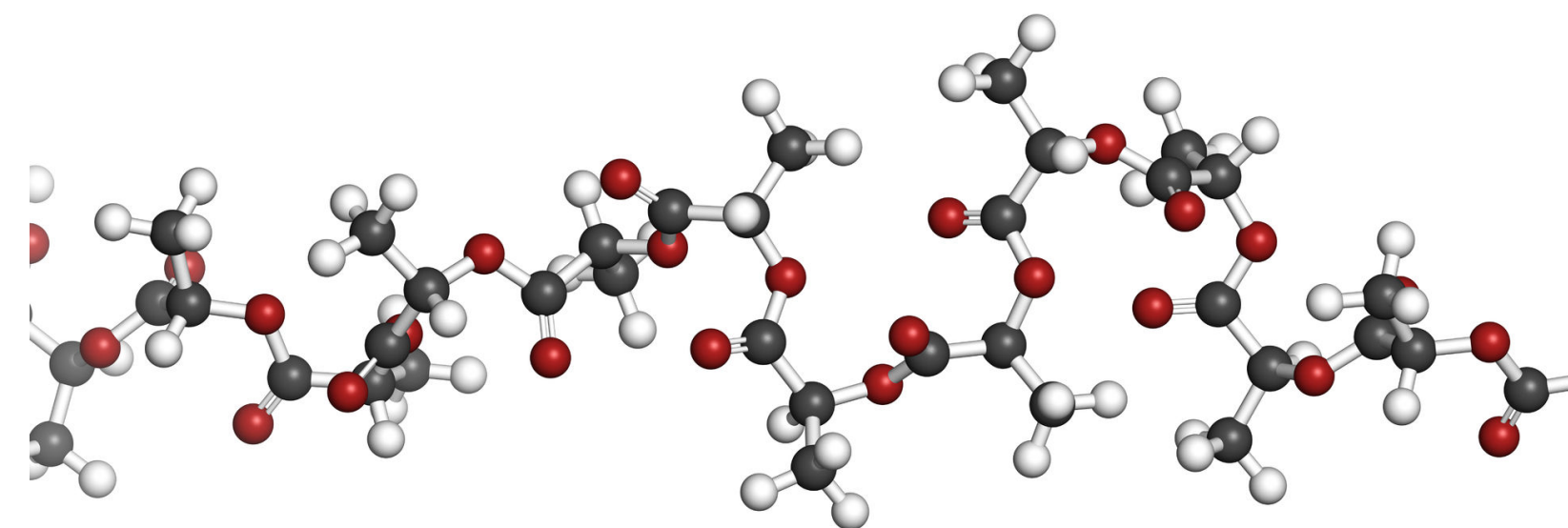
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WYATT TECHNOLOGY

Introduction

Multi-angle light scattering coupled to GPC (SEC-MALS) is well-established as a versatile means of characterizing macromolecules and nanoparticles in solution, independently of retention time and molecular standards, to determine:

- Absolute molar mass from 200 Da up to 1 GDa
- Size (R_g) from 10 nm to 500 nm
- Conformation & Conjugation

Waters APC™, or Advanced Polymer Chromatography, offers several benefits over standard GPC:

a) short run times, b) reduced consumption of sample and mobile phase, and c) improved resolution.

However, MALS instruments designed for GPC are not suitable for low-volume APC.

A new MALS detector, especially designed for UHPLC and APC, fills that need.

1. Why SEC-MALS?

Traditional GPC: Analysis based on column calibration breaks down when samples do not match reference standards in terms of:

- Conformation / shape / density
- Hydrophobicity, charge or other non-ideal column interactions

In addition, column calibration may drift due to aging or pump fluctuations.

Four Monomeric mAbs

SEC-MALS: Determines molar masses independently of elution time, and does not require calibration against reference molecules. Hence MALS correctly characterizes:
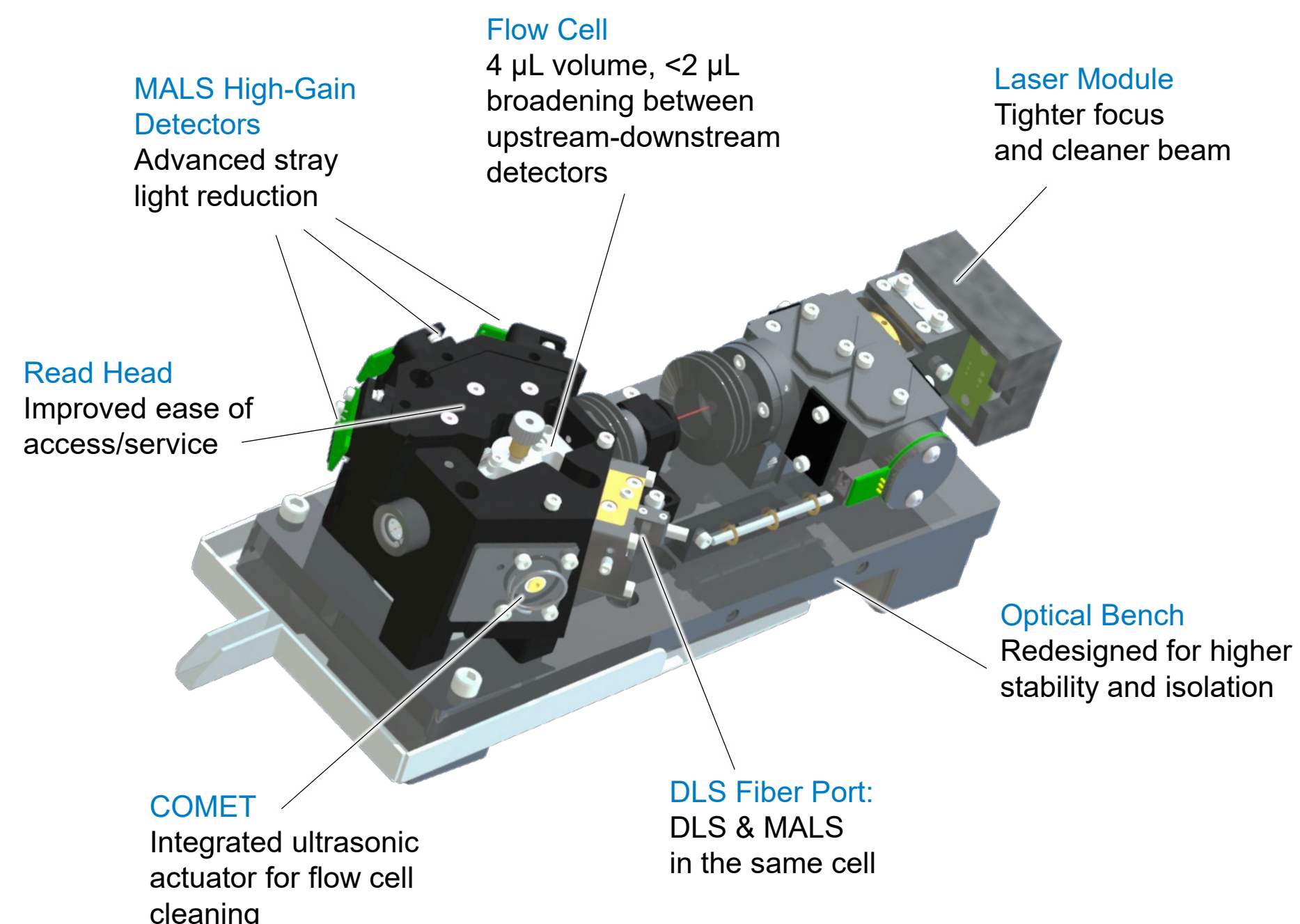
- mAbs with the same molar mass that elute at different times
- Branched as well as linear polymers and copolymers

Copolymer MW and PS Fraction
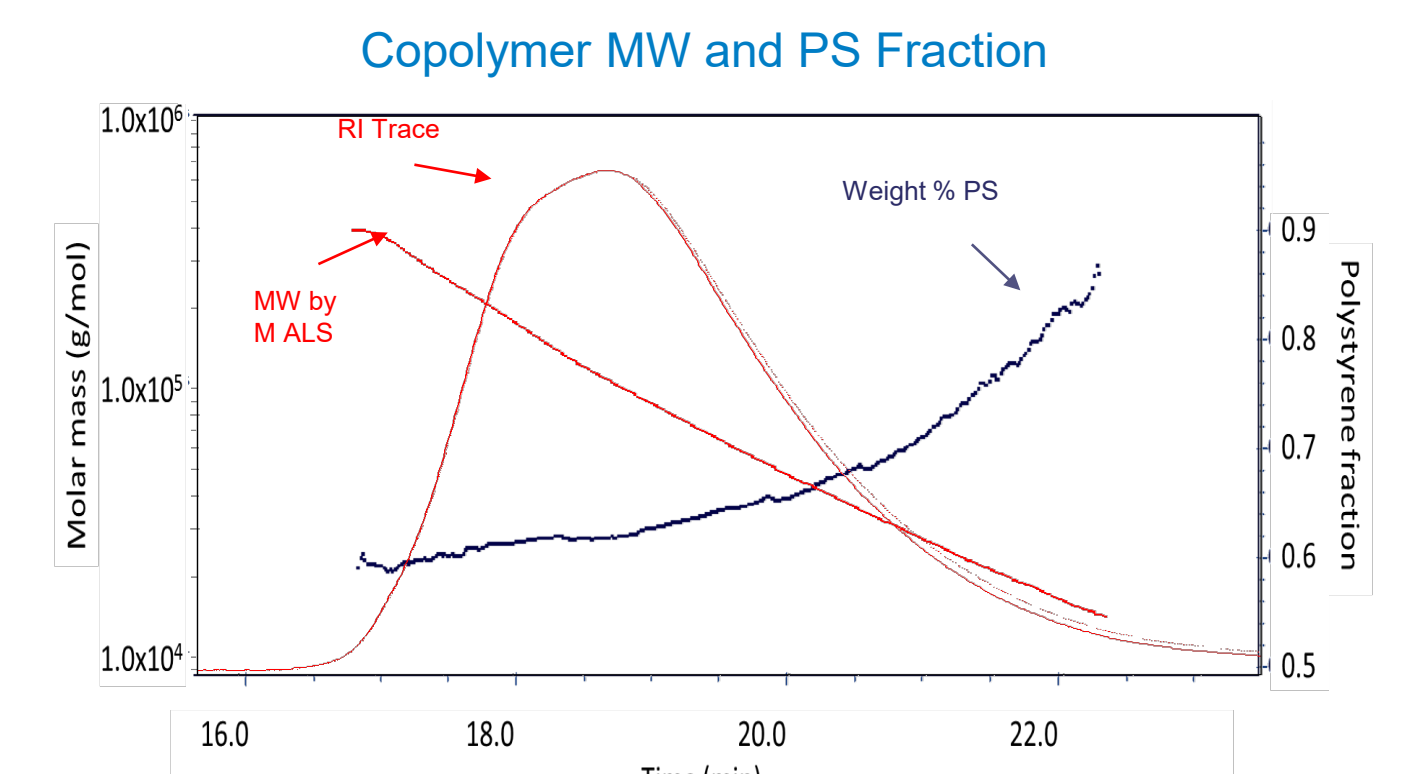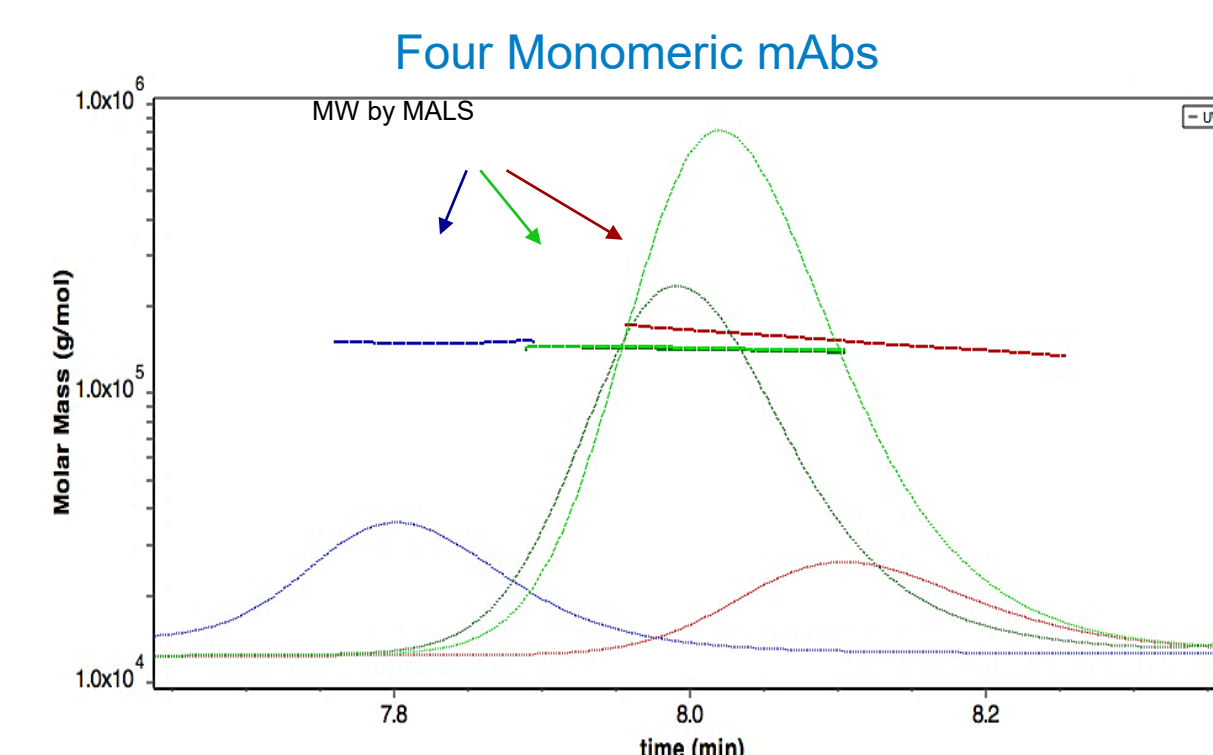
2. A MALS Detector for UHPLC

µDAWN®: A 3-angle MALS detector designed specifically for UHPLC

- Band broadening < 2 µL
- Maintains the high sensitivity and robustness of Wyatt's standard MALS detectors

Molar Mass

Range: 200 to ~10^7 Da
**Sensitivity:** 3.5 ng, 100 kDa
PS in THF by UHPLC

Size

Range: 10 to 50 nm
Accuracy: ± 5%
Precision: 2%

MALS High-Gain Detectors — Advanced stray light reduction

Flow Cell — 4 µL volume, <2 µL broadening between upstream-downstream detectors

Laser Module — Tighter focus and cleaner beam

Read Head — Improved ease of access/service

COMET — Integrated ultrasonic actuator for flow cell cleaning

DLS Fiber Port: DLS & MALS in the same cell

Optical Bench — Redesigned for higher stability and isolation

3. MALS: Comparing APC with GPC

No Difference: µSEC-MALS combining APC with µDAWN and Optilab® UT-rEX achieves similar separation range as GPC, but in much less time and with much less sample and solvent consumption.

|	$M_w \times 10^{-6}$ [g/mol]	R_z [nm]
GPC: DAWN + T-rEX	1.56 ± 0.01	63.0 ± 0.05
APC: µDAWN + UT-rEX	1.56 ± 0.01	61.5 ± 0.05
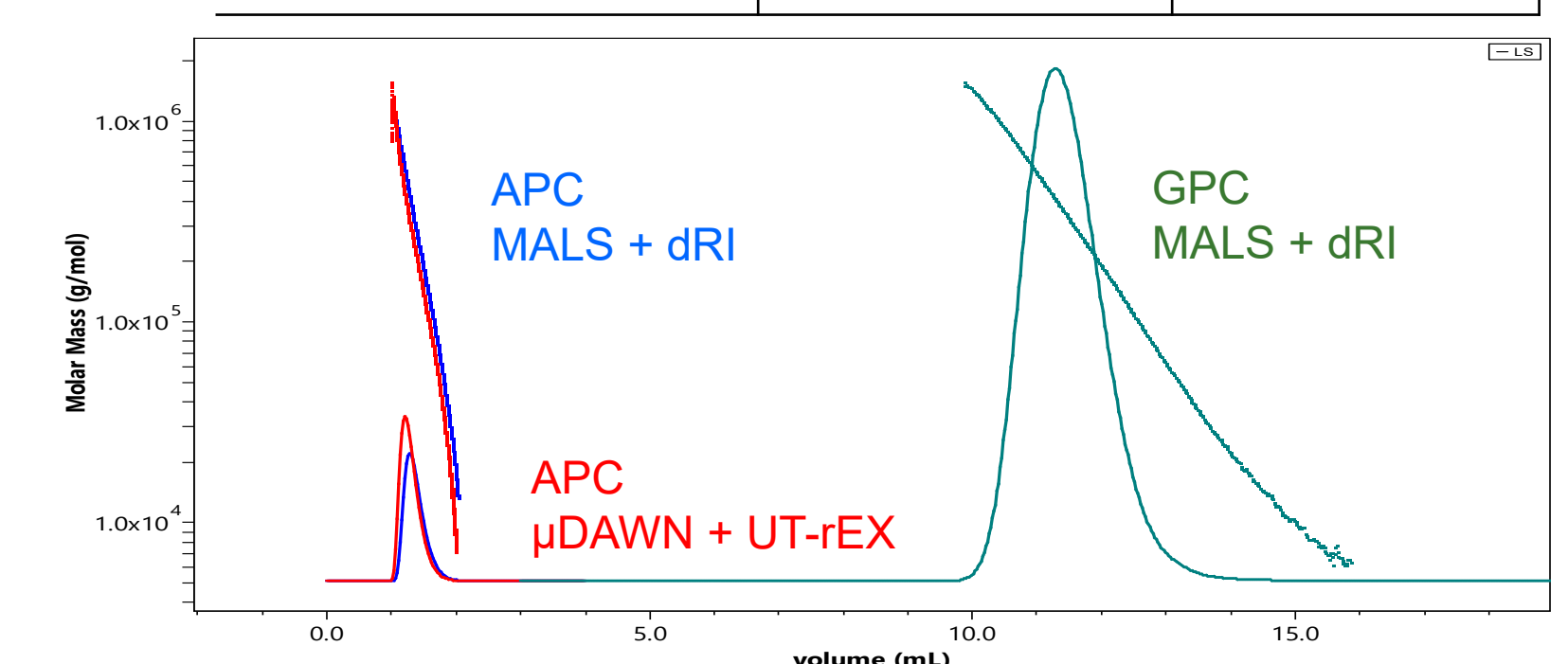
No Shear: Absolute MW and rms radius values measured in SEC-MALS and µSEC-MALS proves that this polymer did not experience shear degradation in the APC column at 0.5 mL/min.
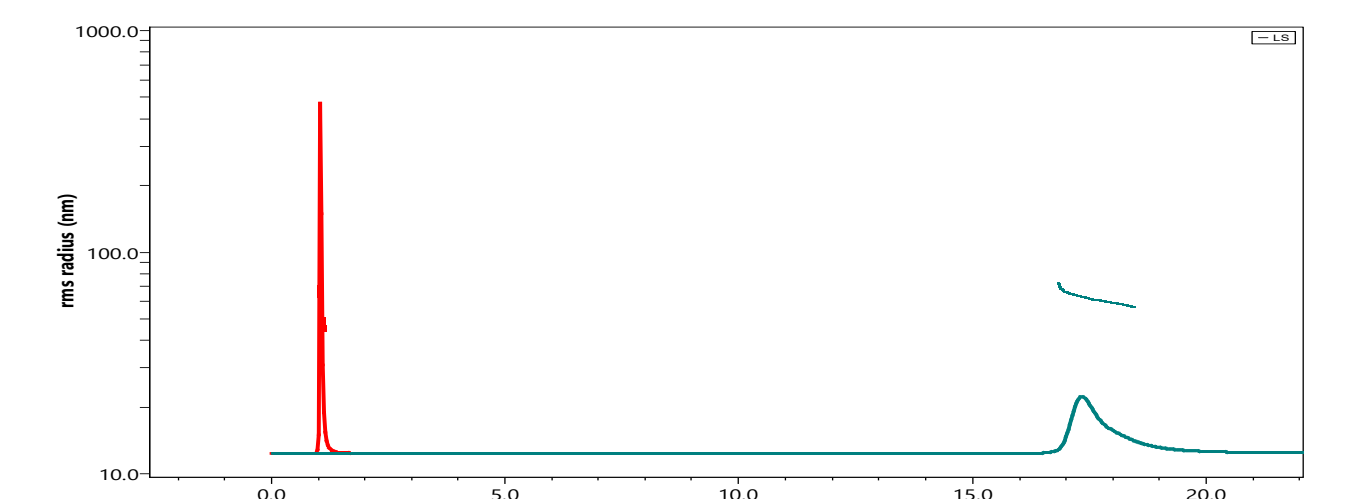
4. Polystyrene: Calibration and Injection Volume Bias

A Broad PS Standard is Narrow

Column calibration dictates a finite, relatively large polydispersity for every peak. MALS shows that the polystyrene standard is actually quite homogeneous.
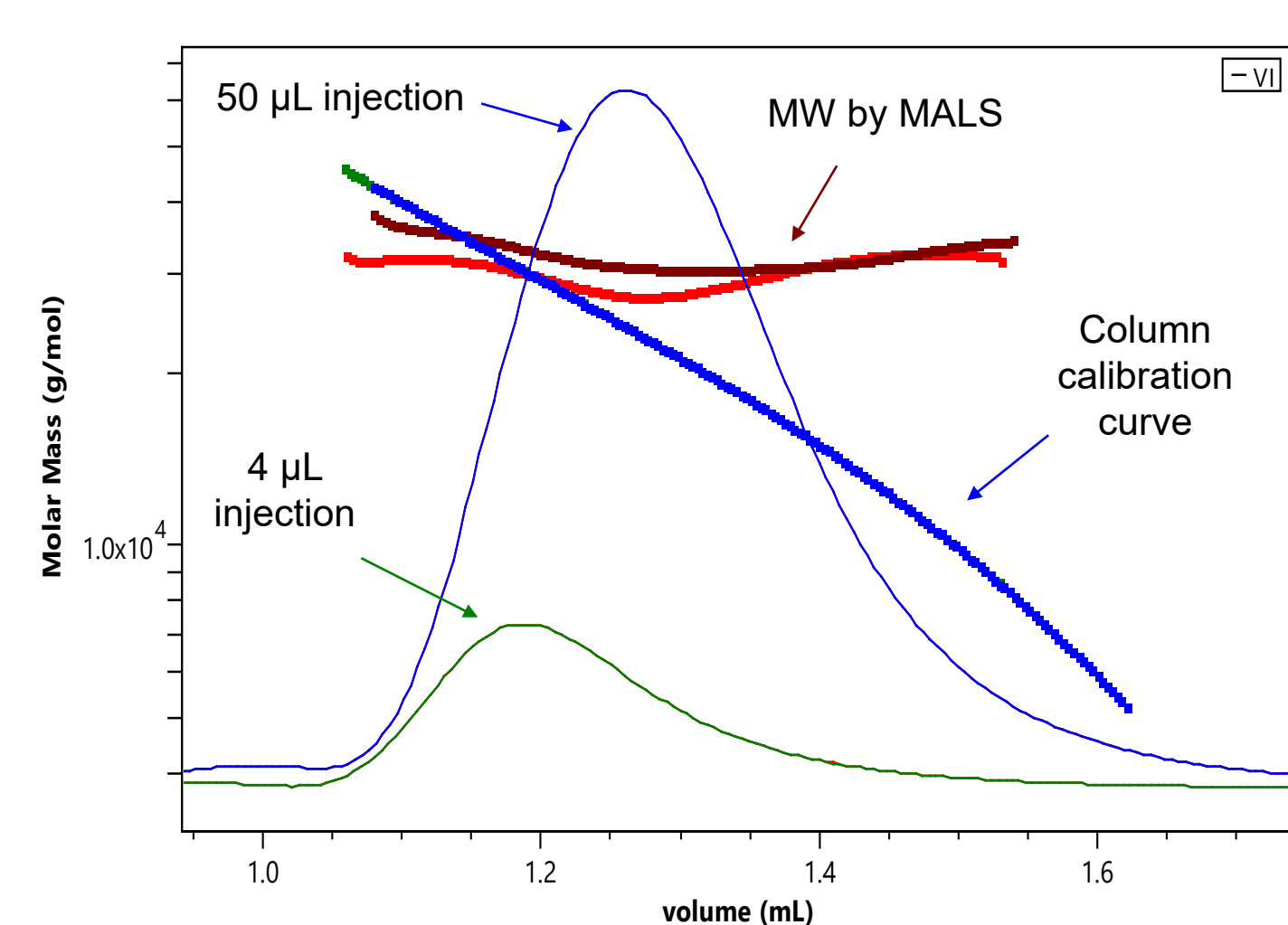
Which Injection Volume is Correct?

Injections of a 30 kDa polystyrene standard on APC with two different volumes, 4 µL and 50 µL, exhibit different retentions. MW by MALS indicates the same molar mass for both injections while column calibration shows different apparent molar masses.

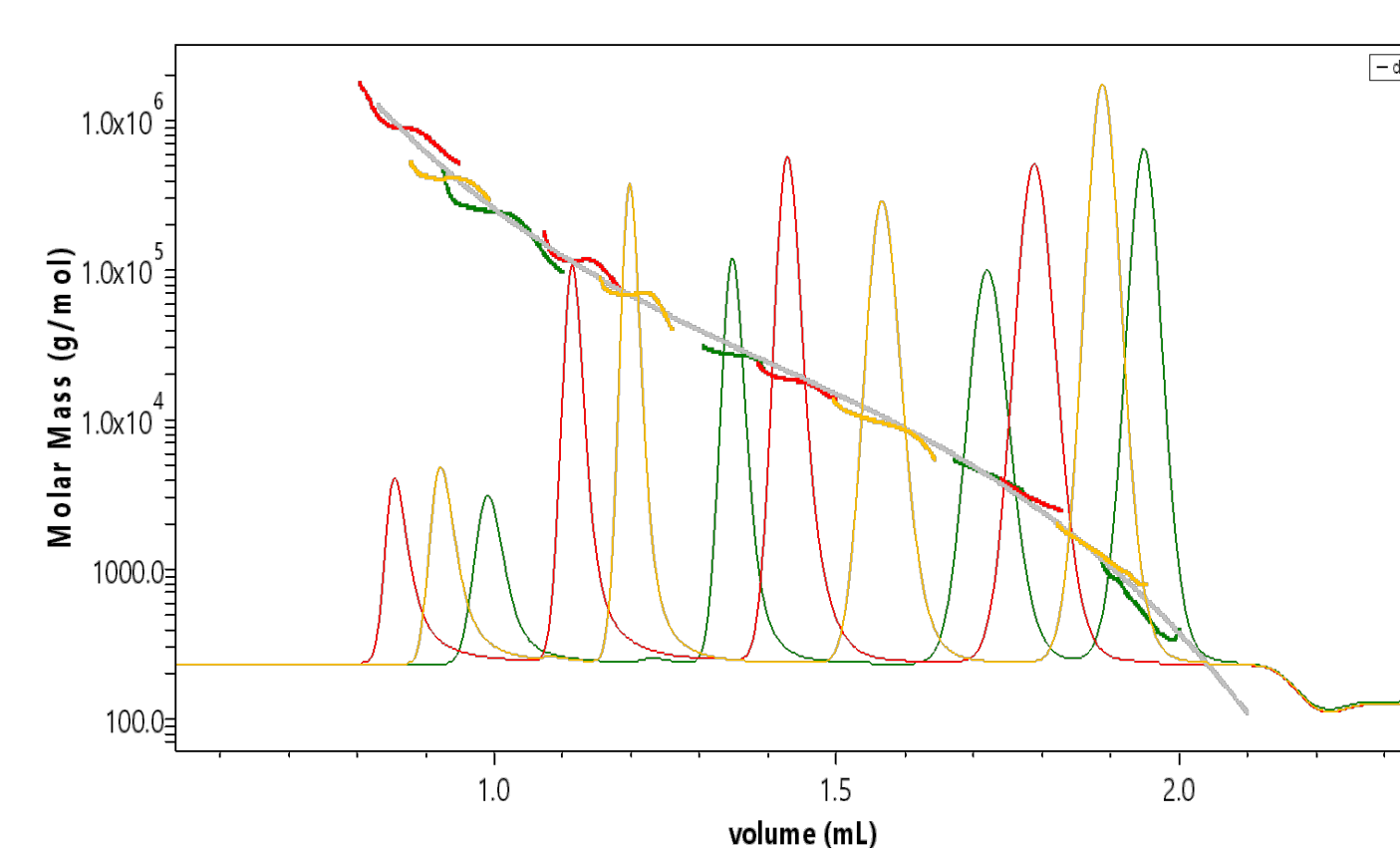|	M_n (kDa)	M_w (kDa)	PD (M_w/M_n)
By Column Calibration	26.1	28.0	1.073
By MALS	29.3	29.4	1.003

5. Standards and Unknowns

Polystyrene Standards:
- Agilent PS (red)
- Agilent PS (green)
- Agilent PS (yellow in DMC)

The separation was performed with two 4.6 x 75 mm Waters APC columns 125 and 450.

MW by MALS precisely matches the column calibration curve obtained with PS standards.

'Unknown' Samples:
- Agilent PMMA (red)
- Agilent PMMA (green)
- Agilent PMMA (yellow in THF)

The separation was performed with four 4.6 x 30 mm Waters APC columns: 45, 125, 200 and 450.
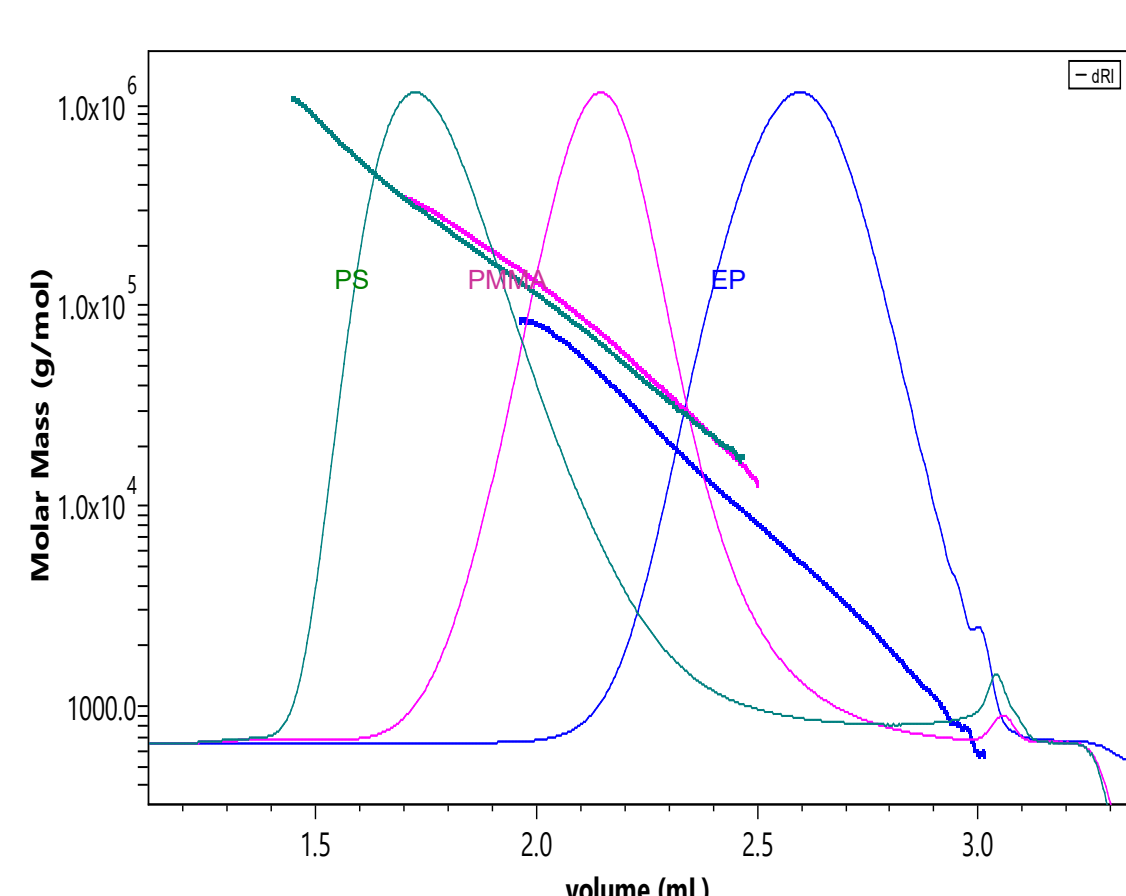
Different branching ratios cause deviation from ideal calibration curve. Absolute MW values are determined by MALS from high MW and down to the smallest molecules separated by the columns.
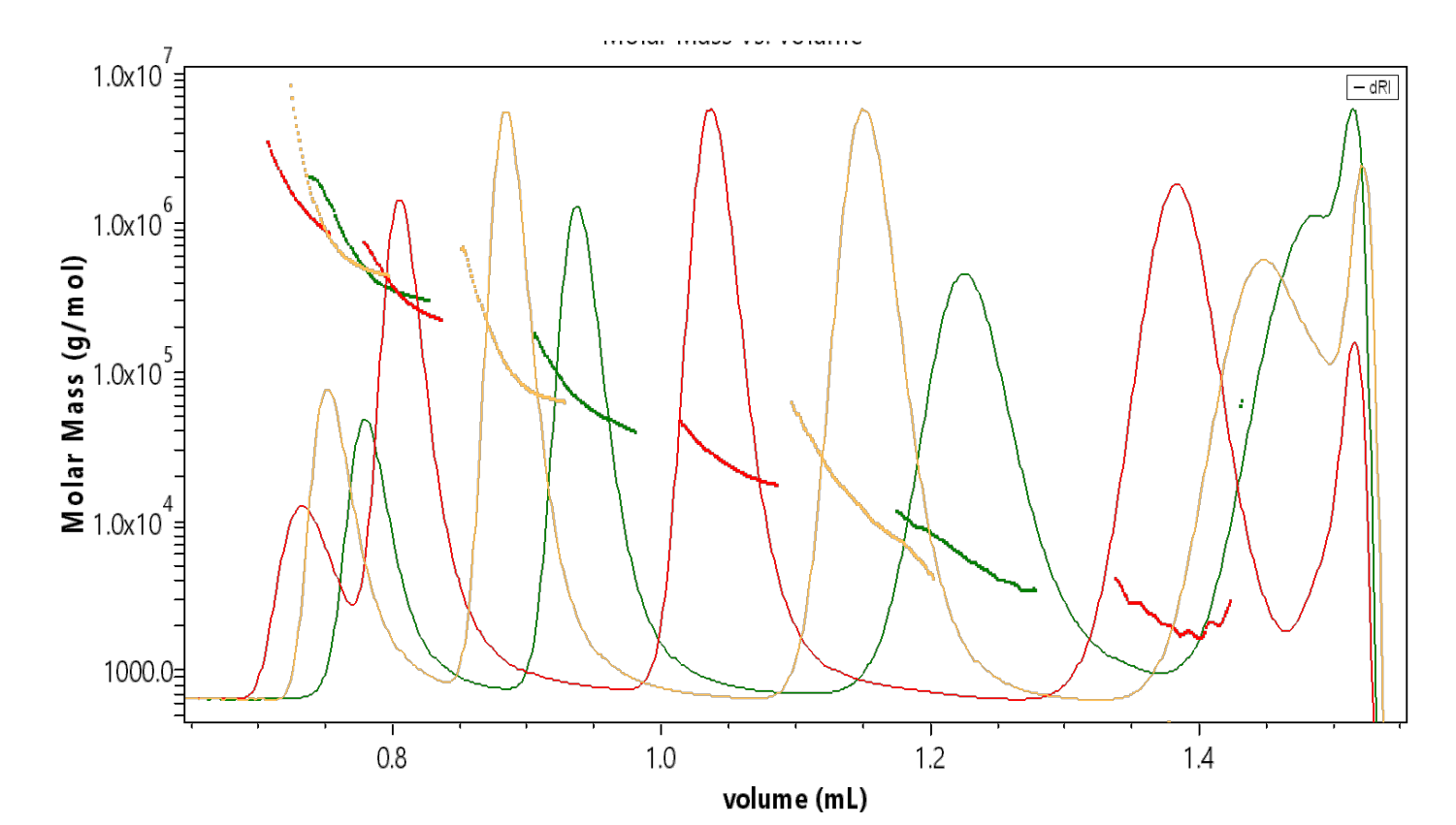
6. Epoxy Resins: High Precision, Low MW

MW Regardless of Conformation

Polystyrene is a random coil, PMMA slightly branched, and epoxy resin stiff and elongated. Hence each elutes at a different volume relative to their respective molar masses. µSEC-MALS provides accurate molar masses, regardless of conformation.
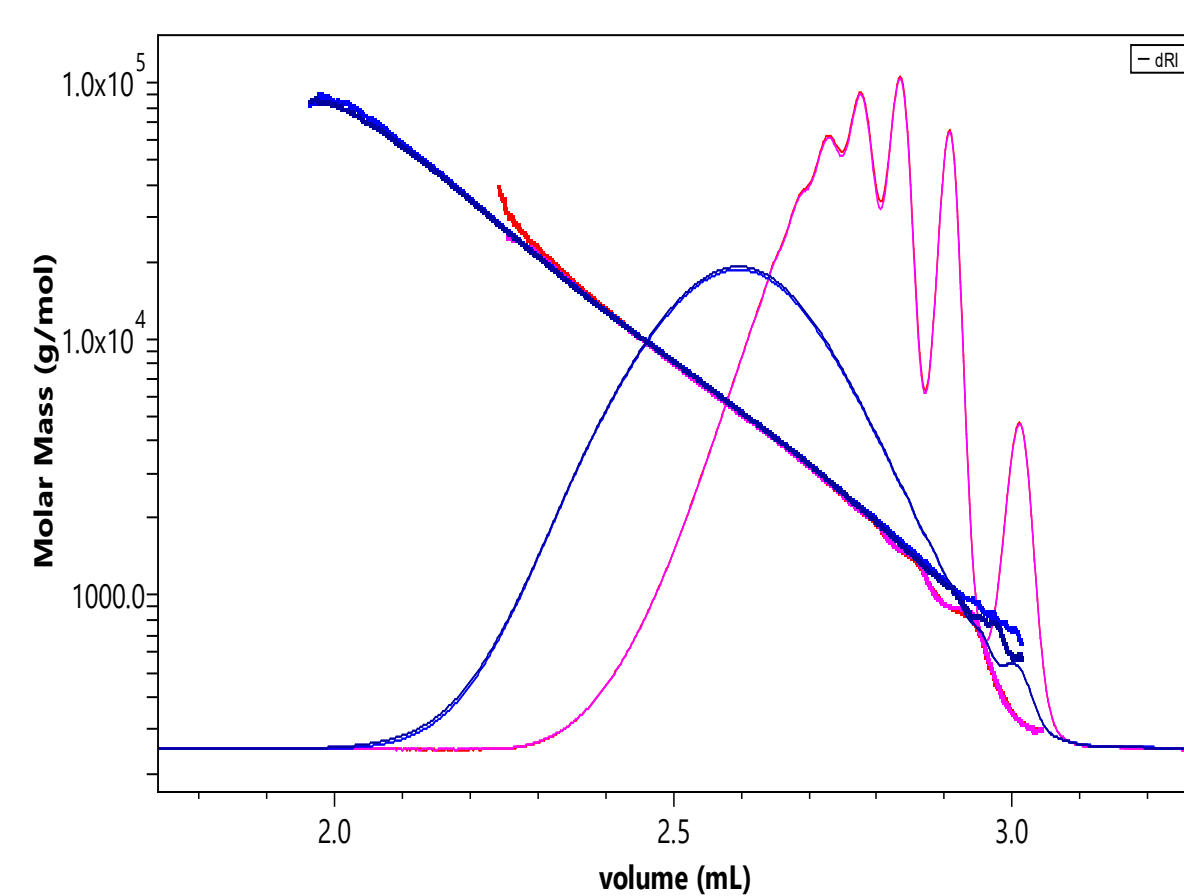
µSEC-MALS Resolves LMW Species

EP1 and EP2 are two epoxy resins with similar conformations but different overall distributions.

µSEC-MALS with APC resolves oligomers and quantifies MW from millions down to hundreds of g/mol.

	M_n (kDa)	M_w (kDa)	PD (M_w/M_n)
EP1(001)	1.55 ± 0.02	3.26 ± 0.01	2.10 ± 0.03
EP1(002)	1.55 ± 0.02	3.25 ± 0.01	2.10 ± 0.03
EP2(001)	3.58 ± 0.07	7.90 ± 0.02	2.21 ± 0.04
EP2(002)	3.47 ± 0.06	7.96 ± 0.02	2.30 ± 0.04

Conclusions

We present data from the first complete APC+MALS system that robustly analyzes polymers, with unprecedented resolution compared to standard SEC-MALS.

- *Polymers with different conformations* are accurately characterized.
- *Small oligomers* of an epoxy resin are clearly separated and quantified.
- *Errors* due to column loading bias and conformation differences are overcome.
- *Column shear* is proven to have negligible effect on polymer MW.
- *These results are achieved in 1.5 to 5 minutes* with just micrograms of material.