2015 International Symposium on GPC/SEC and Related Techniques

The Sun Rises Over UHP-SEC: µDAWN for Polymer Characterization by MALS

WYATT TECHNOLOGY

Why UHP-SEC?

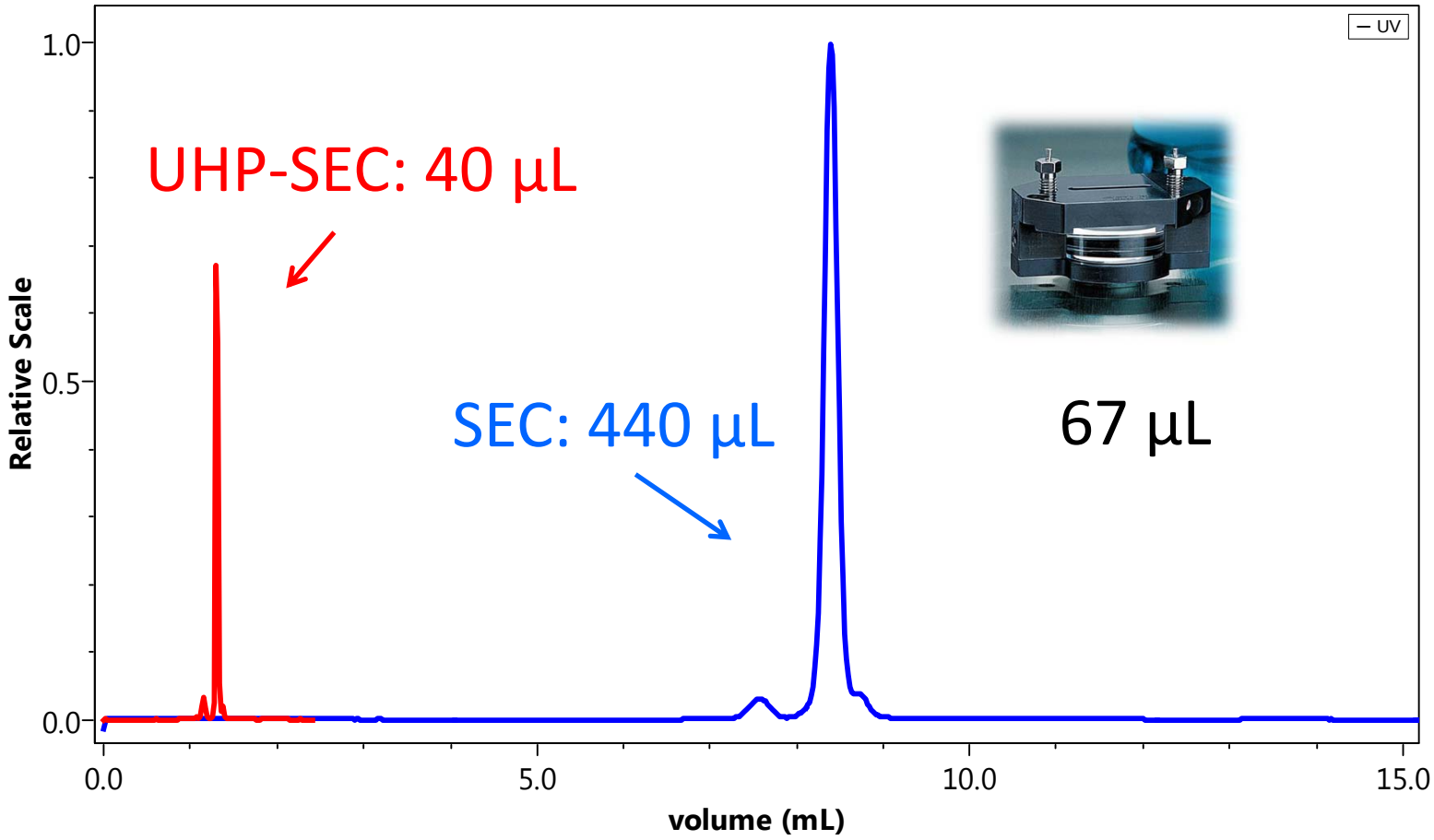
- aka: UHPLC-SEC, SE-UHPLC, APC

  (SEC using 1.7 to 2.5 µm particles for stationary phase)
- Faster results
- Greater resolution
- Higher detection sensitivity
- Smaller sample quantity
- Less solvent usage
- Better productivity

Why MALS & DLS for UHP-SEC?

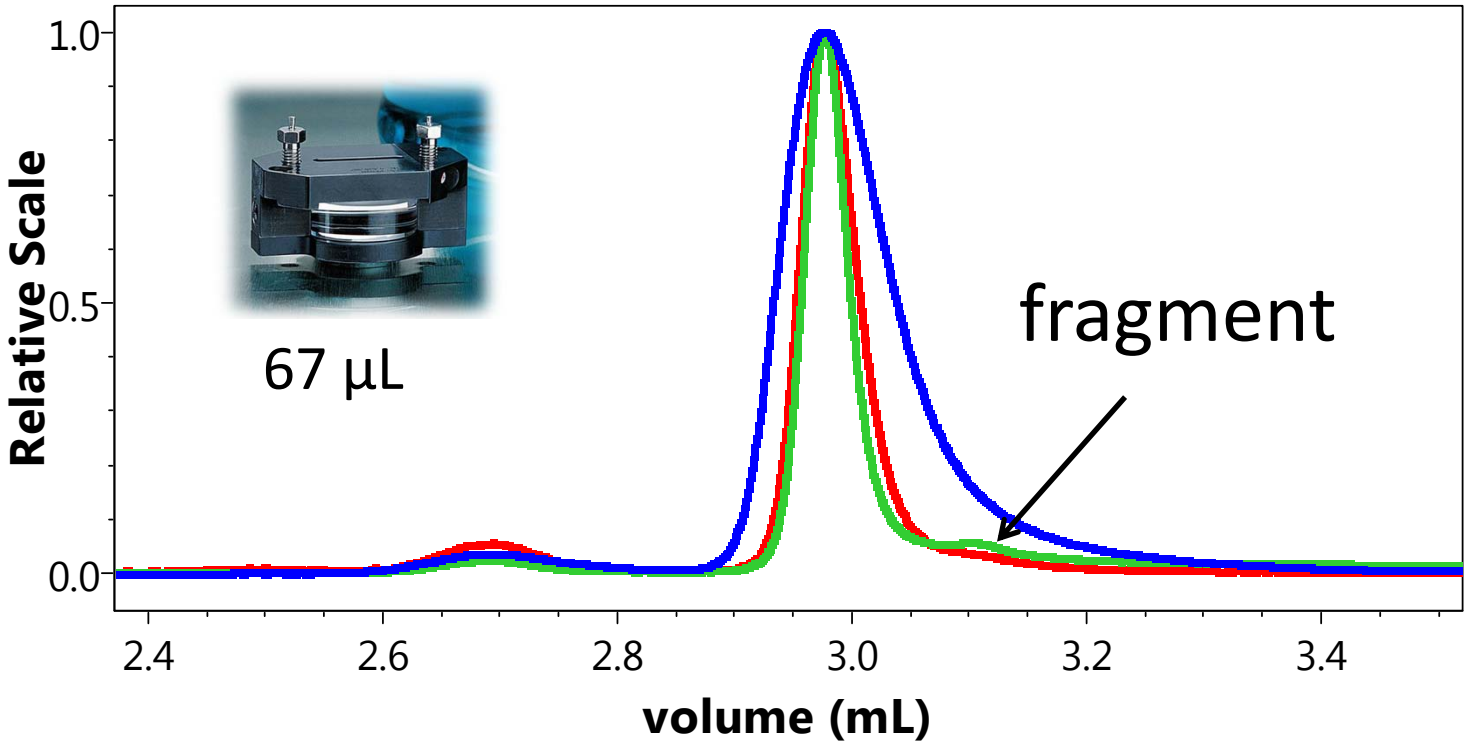
- Measure MW *in solution* without assumptions on polymer shape and chromatography properties
- Reveal conformation by online MALS and DLS
- Detect, characterize, and quantify protein aggregates
- Identify new peaks from UHP-SEC
- Assess heterogeneity of the SEC peak
- Characterize protein conjugates and copolymers

Challenges When Using MALS for UHP-SEC



UHP-SEC *peak volume (5σ)* is small compared to the flow cell volume of a standard MALS detector.

Challenges When Using MALS for UHP-SEC



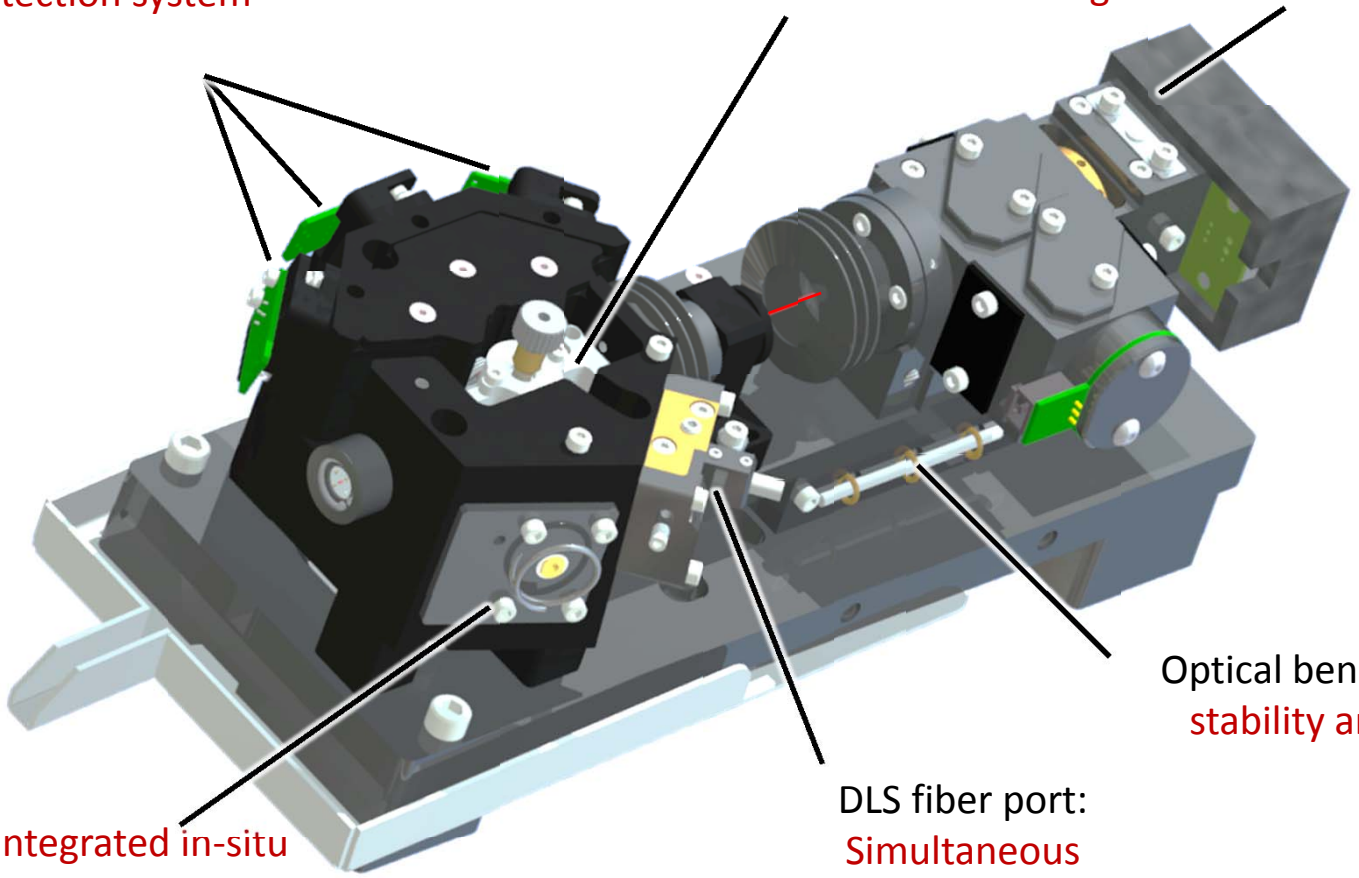
Resolution from UHP-SEC separation was diminished by standard detectors.

µDAWN Features

MALS Detectors:
Advanced optical detection system

Flow Cell:
~10x reduction in volume
~20x reduction in broadening

Laser Module: Tighter focus & cleaner beam

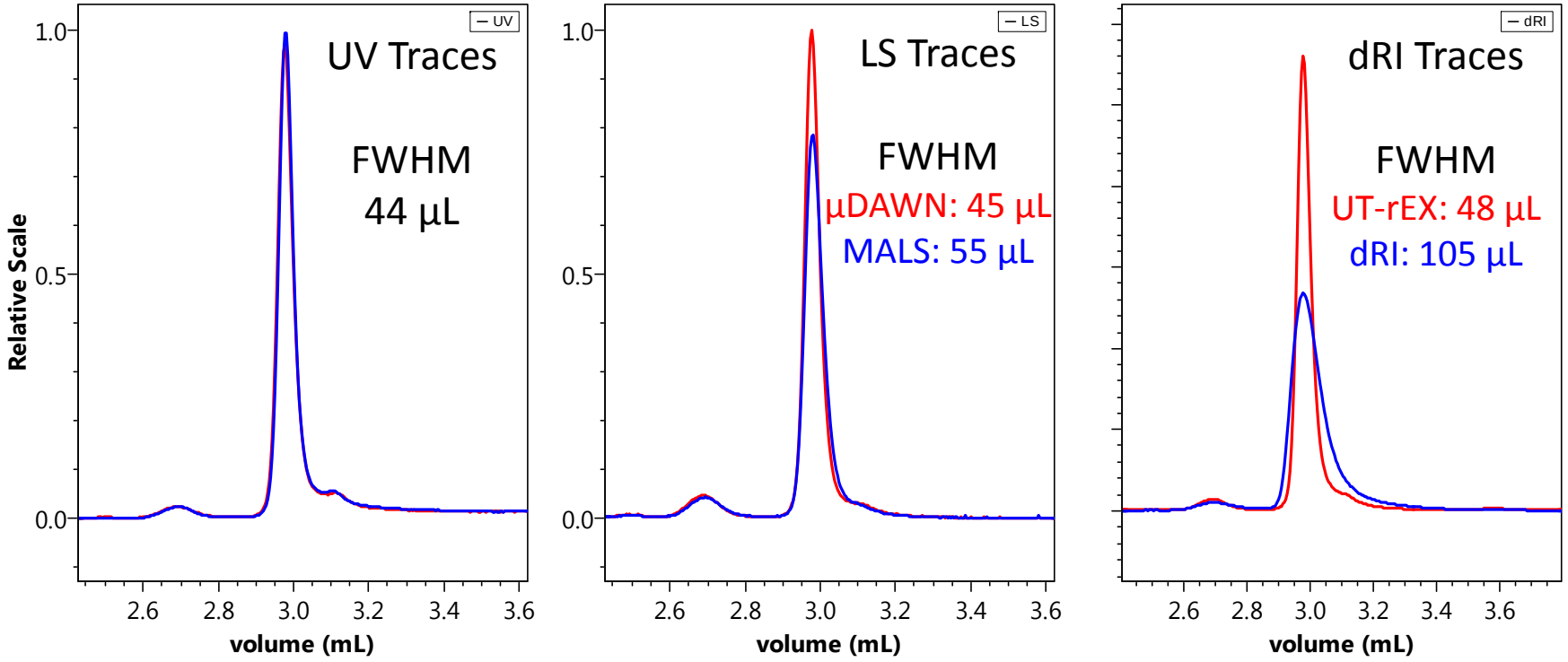


Optical bench: Improved stability and isolation

COMET: Integrated in-situ ultrasonic cell cleaning

DLS fiber port:
Simultaneous DLS capability

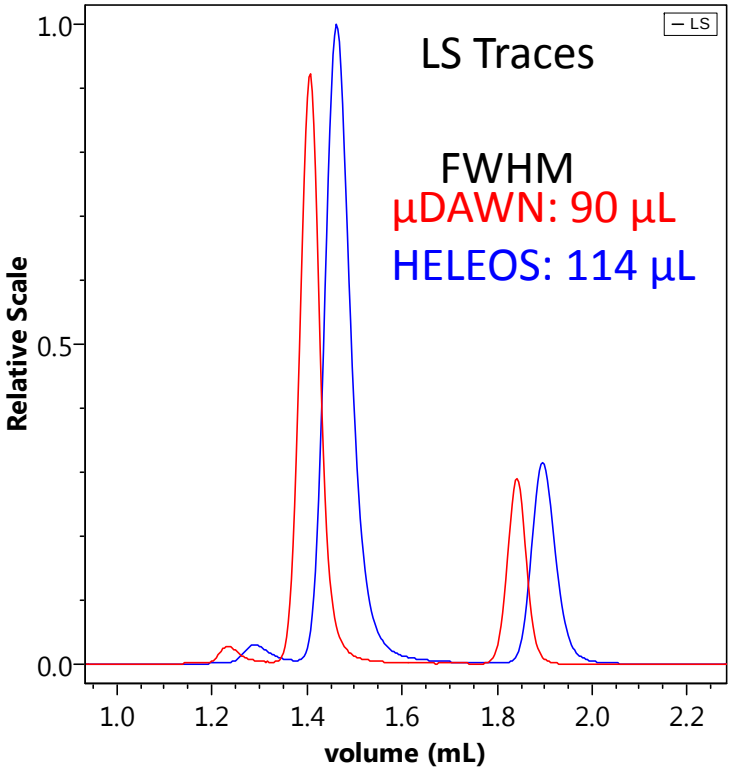
Why µDAWN & UT-rEX: Proteins

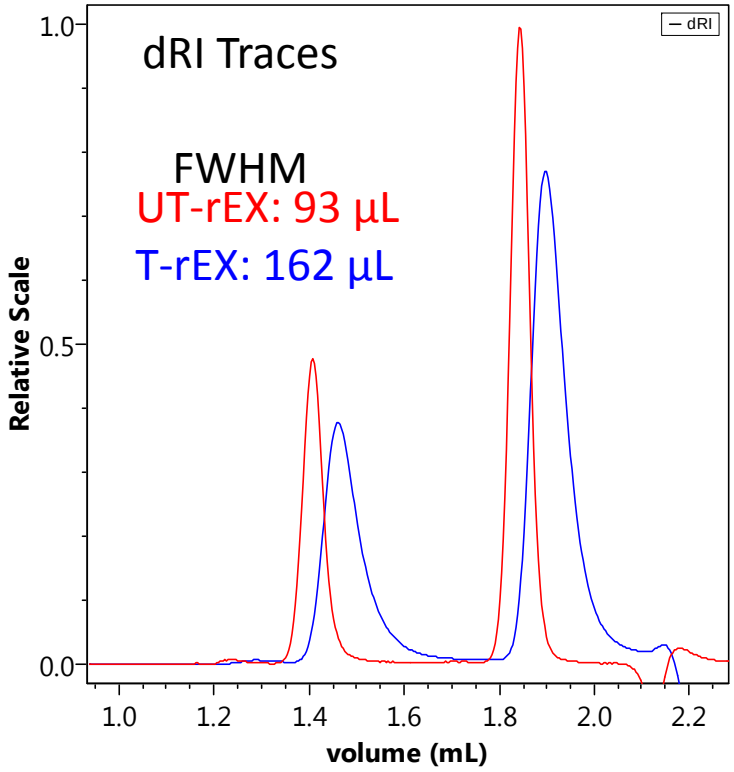


Agilent 1290, Sepax UNIX SEC-300, 4.6 x 300 mm, 0.3 mL/min

µDAWN and UT-rEX dispersion is much less than standard detectors.

Why µDAWN & UT-rEX: Polymers (APC)

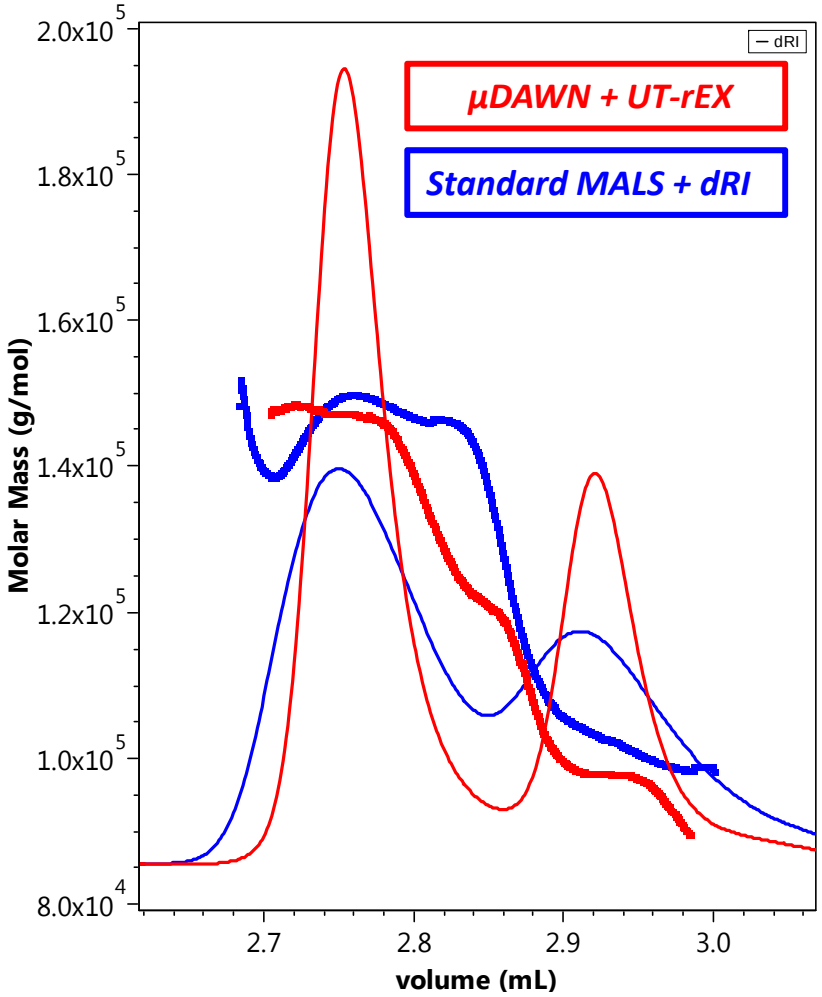




APC also benefits from low dispersion of µDAWN and UT-rEX.

Why are µDAWN & UT-rEX Needed?

Example: IgG with fragment



- Maintain resolution from UHP-SEC
- Provide accurate MW and size information
- Proper band-broadening correction by ASTRA software
- Enable all key MALS applications on the UHP-SEC platform

Experimental Conditions

UHP-SEC Waters Acquity

Columns Proteins – BEH200

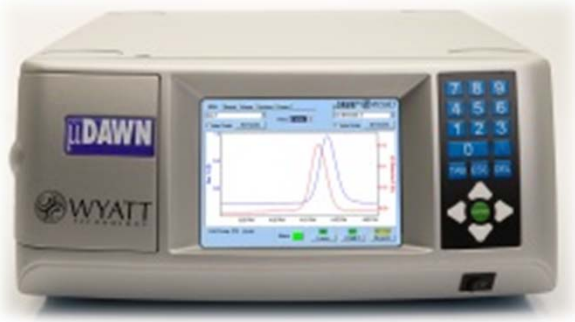
Polymers – APC XT

Detectors

UPLC TUV from Waters

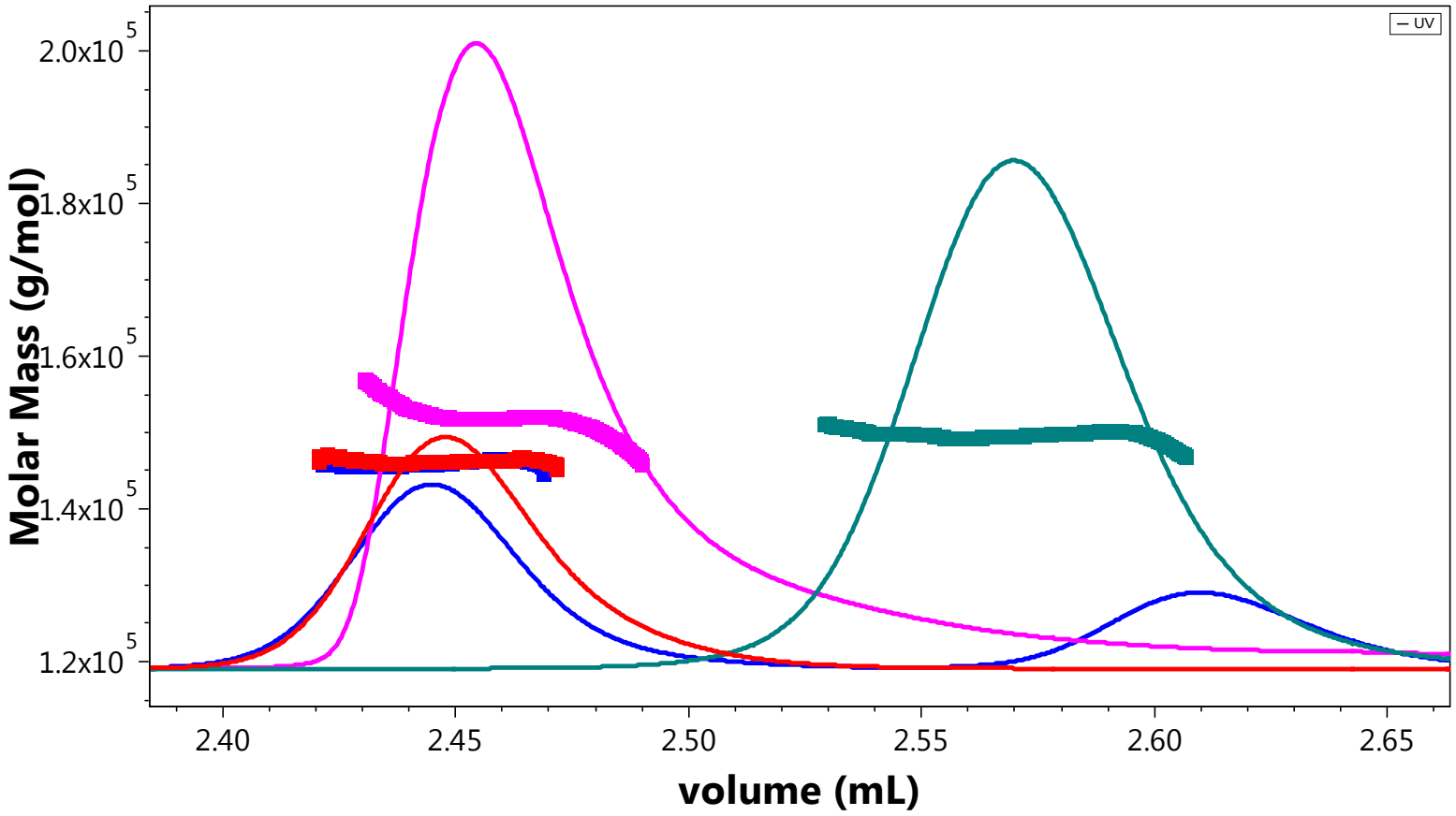
µDAWN (MALS) from Wyatt

Optilab UT-rEX (dRI) from Wyatt



For Proteins and Biotherapeutics

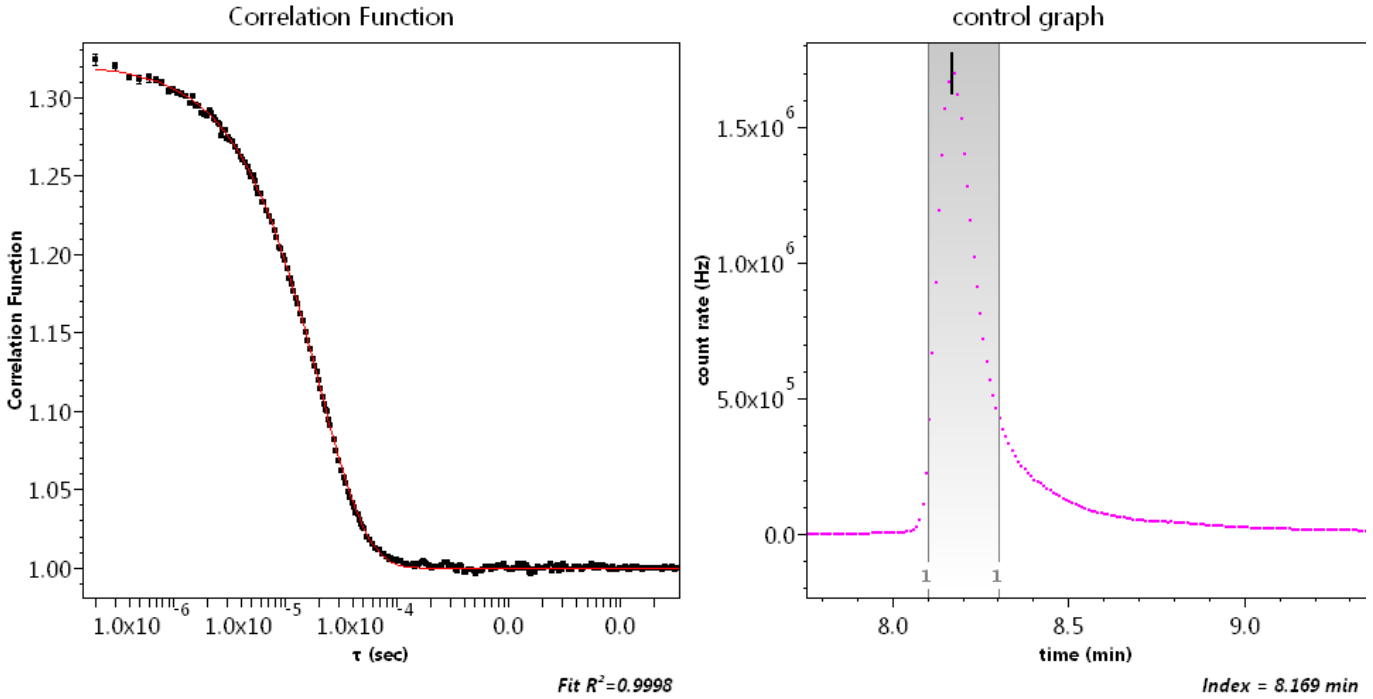
Are These Proteins Really mAb Monomers?



µDAWN revealed all four mAbs are indeed monomeric.

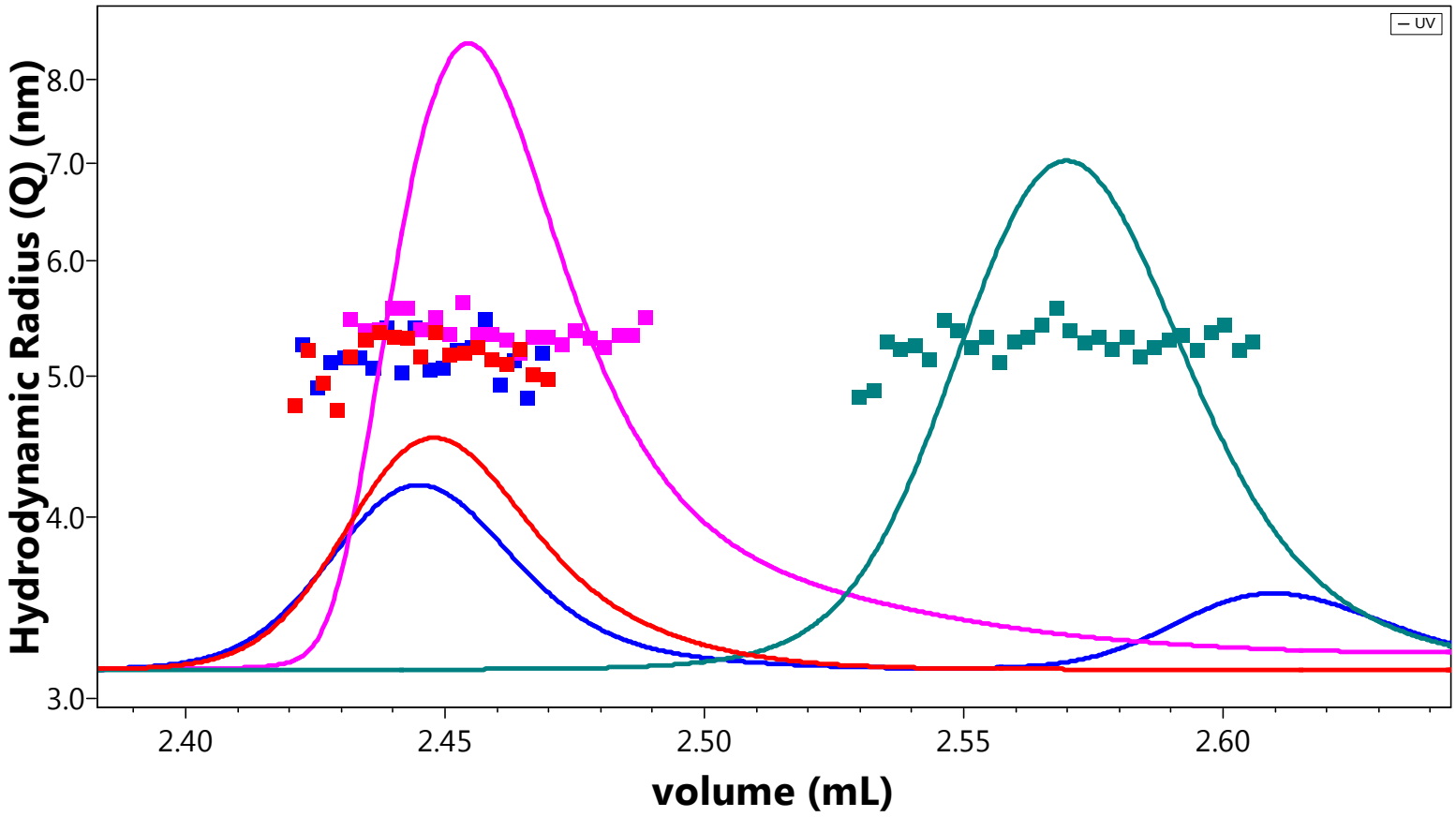
Why Did These mAbs Elute Differently?

Could be due to different shapes. Check with online DLS.



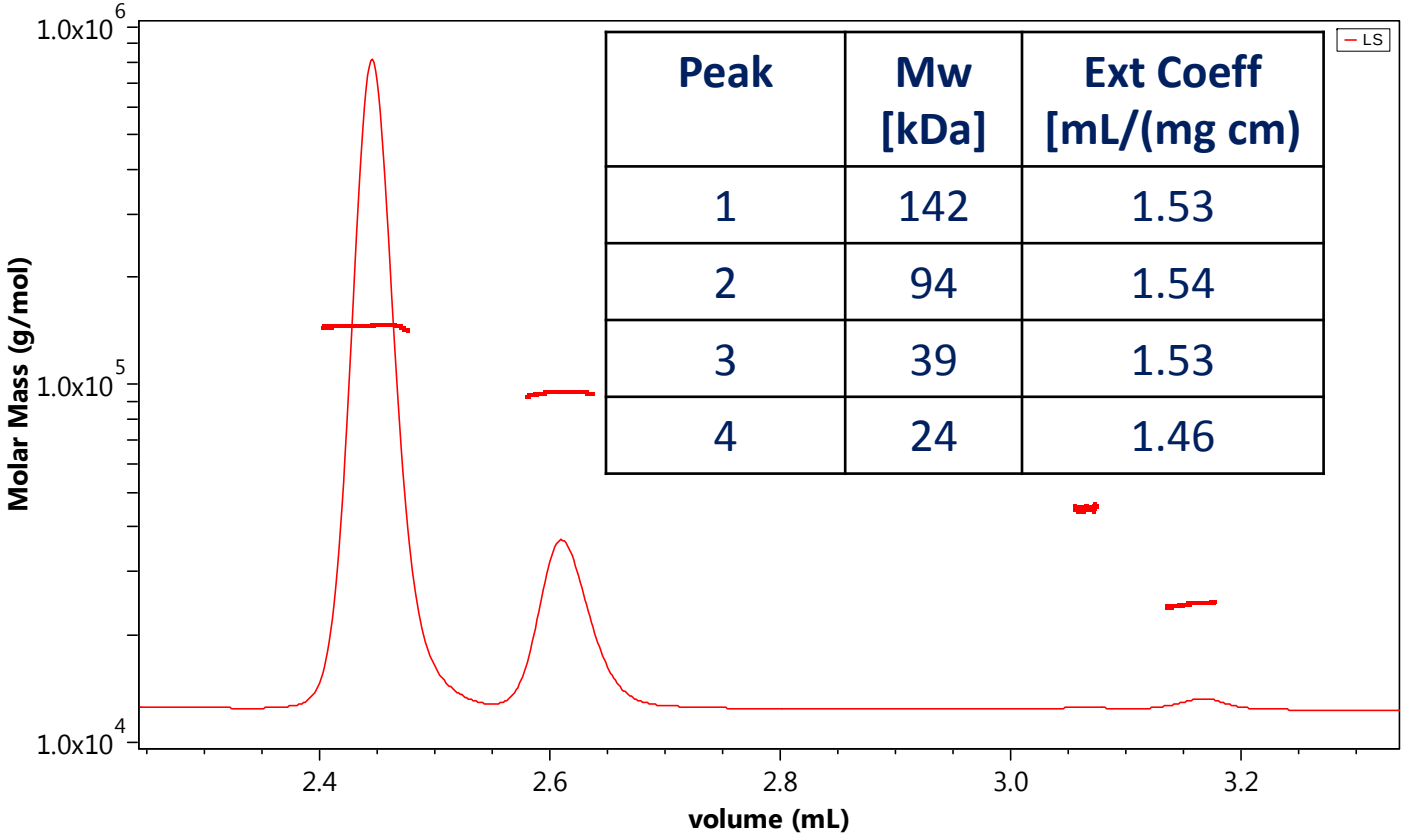
Online DLS measures R_h of mAb down to 20 µg/mL.

Why Did These mAbs Elute Differently?



Different elution times + similar R_h values → column interaction

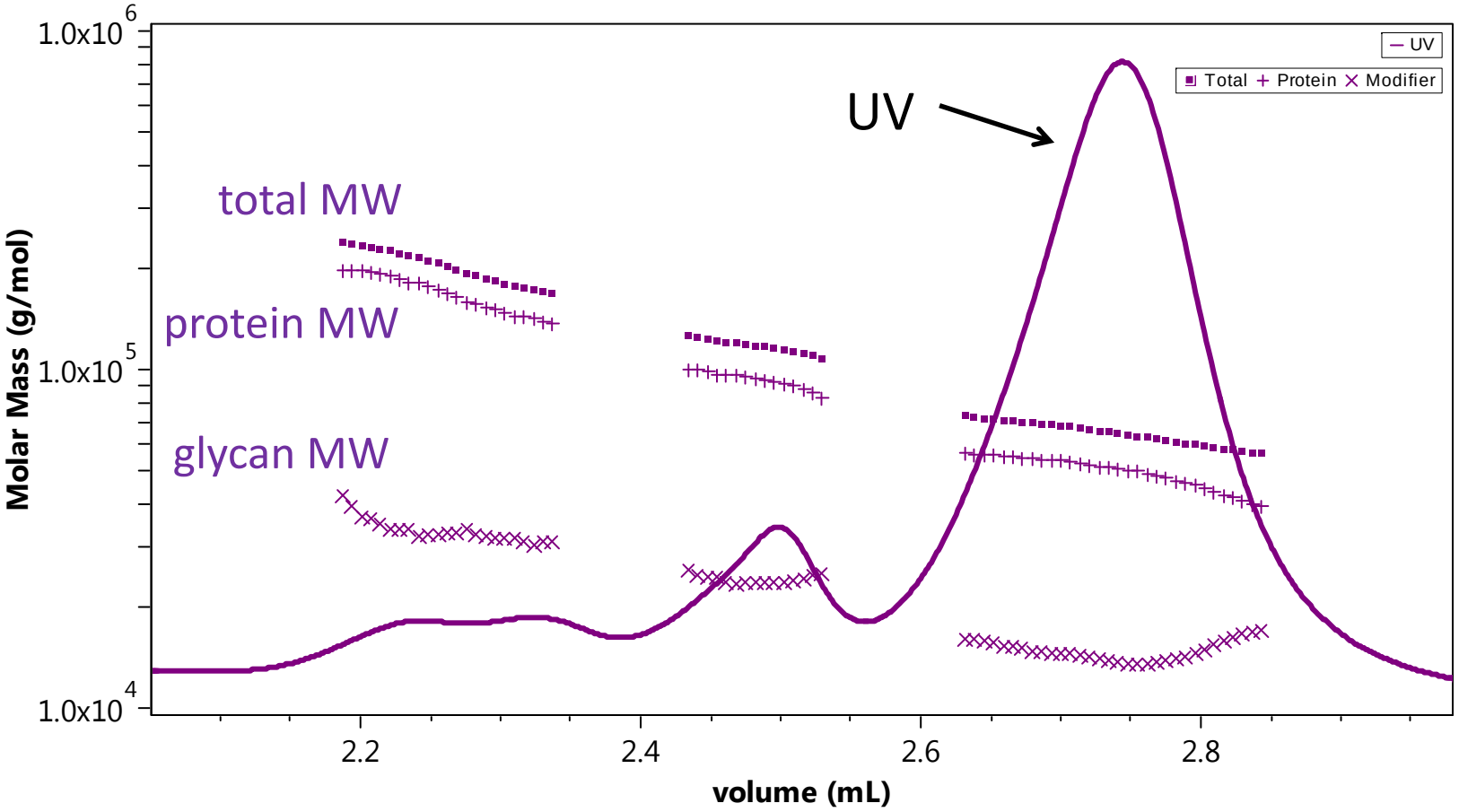
IgG Fragments Identified



Peak	Mw [kDa]	Ext Coeff [mL/(mg cm)]
1	142	1.53
2	94	1.54
3	39	1.53
4	24	1.46

IgG and fragments were identified by MW and confirmed by UV extinction coefficient, both calculated by ASTRA software.

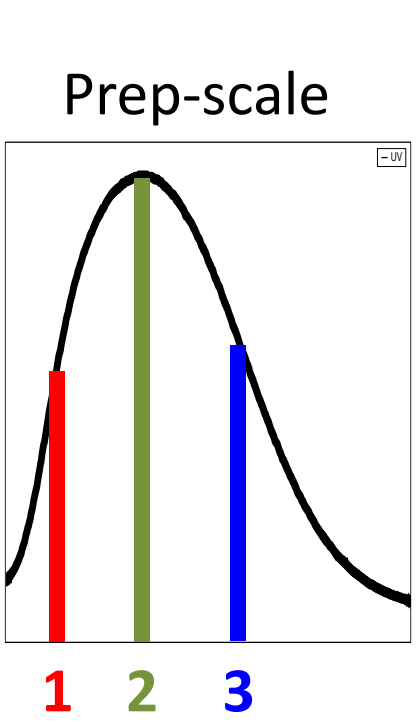
Protein Conjugate Analysis
Glycoprotein by µDAWN-UV-RI

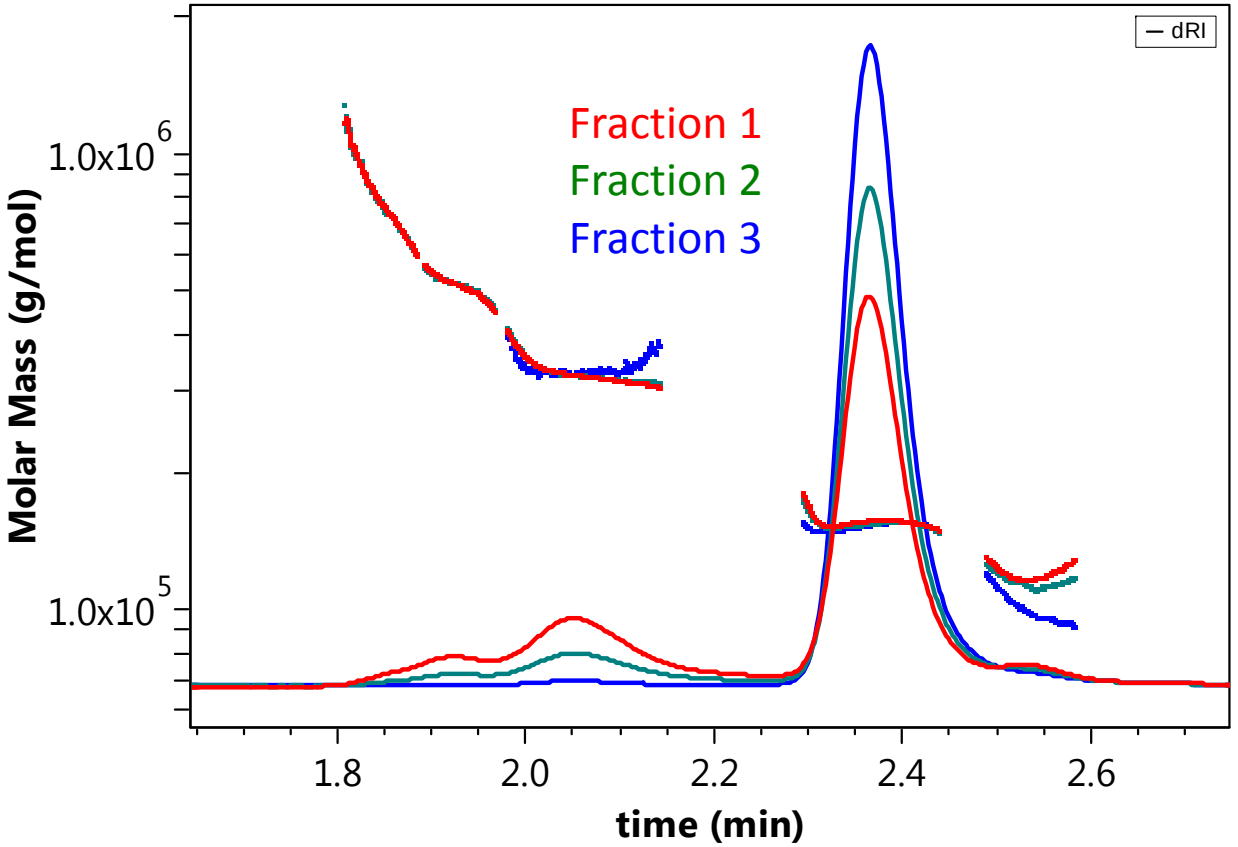


All key MALS applications are readily transferrable to UHP-SEC.

Characterizing Fractions from Preparative HPLC

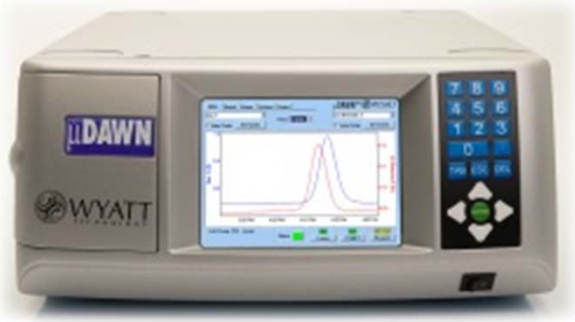
UHP-SEC separation shows potential for process monitoring





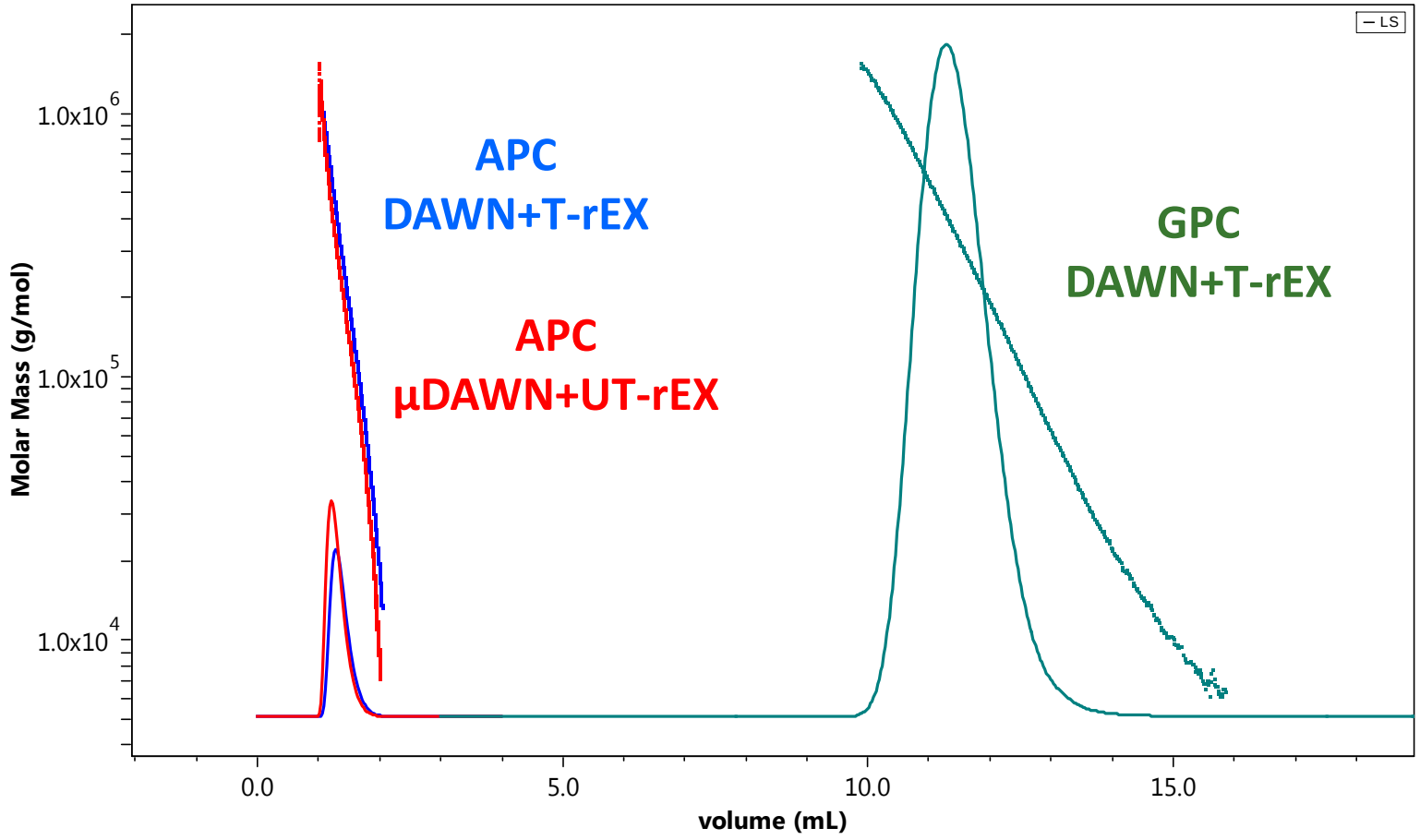
µDAWN identifies aggregates and fragments.

WYATT TECHNOLOGY



APC (Advanced Polymer Chromatography) for Synthetic Polymers

APC vs. GPC for a Polydisperse Polystyrene



Comparable MW results, but much faster with APC

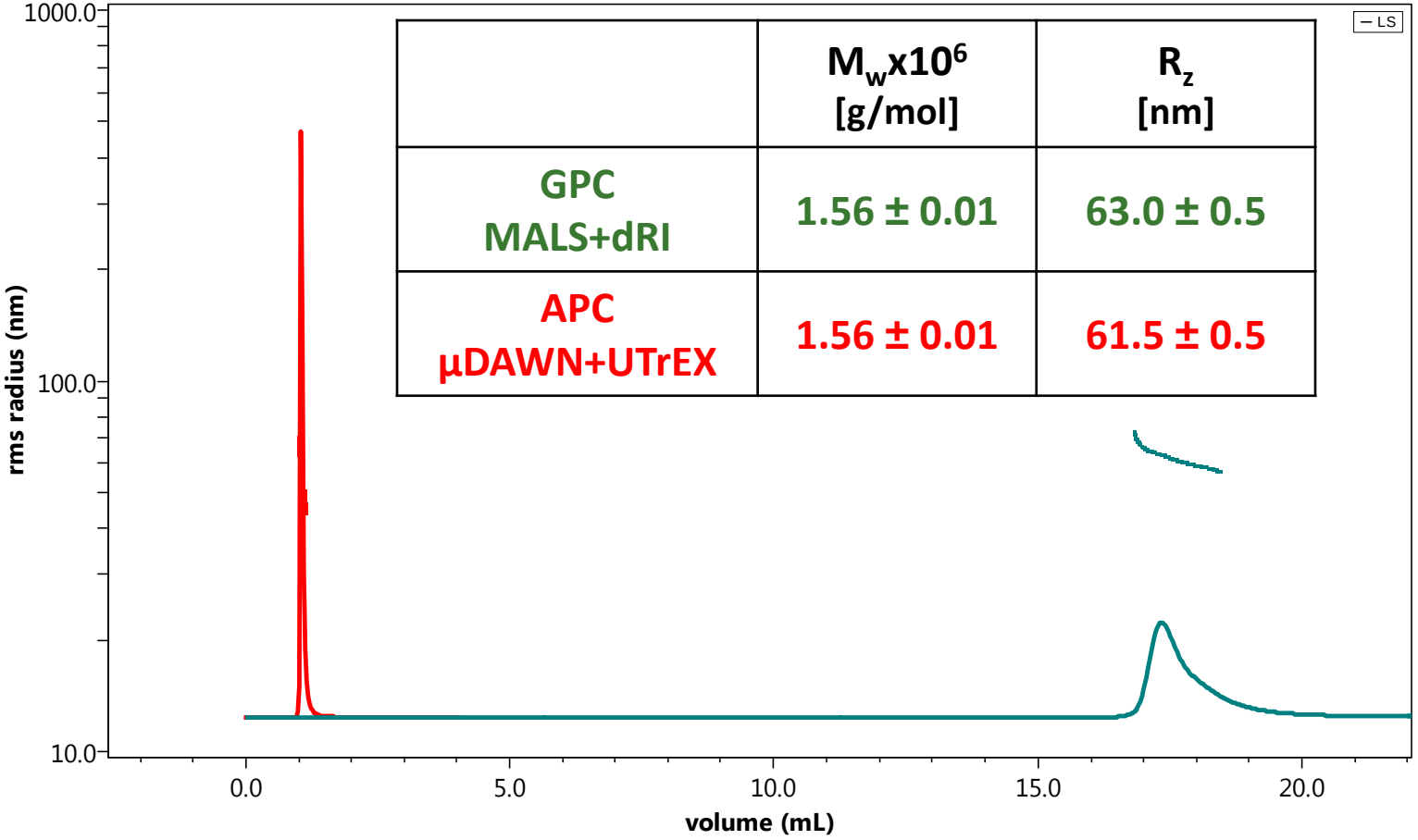
APC vs. GPC for a Polydisperse Polystyrene

	M_n (x10^5) [g/mol]	M_w (x10^5) [g/mol]	PDI	R_z [nm]
GPC MALS+dRI	1.11 ± 2	2.72 ± 1	2.45 ± 0.03	26.9 ± 0.2
APC MALS+dRI	1.29 ± 2	2.68 ± 1	2.07 ± 0.03	26.9 ± 0.2
APC µDAWN+UT-rEX	1.17 ± 2	2.69 ± 1	2.29 ± 0.03	26.7 ± 0.1

Difference between GPC and APC is mainly due to MW ranges covered by respective columns.

Instrument dispersion likely causes small (but noticeable) difference in polydispersity between µDAWN/UT-rEX and standard MALS/dRI.

Does APC Cause Shearing Degradation?



	$M_w x10^6$ [g/mol]	R_z [nm]
GPC MALS+dRI	1.56 ± 0.01	63.0 ± 0.5
APC µDAWN+UTrEX	1.56 ± 0.01	61.5 ± 0.5

MALS proves no appreciable degradation at 0.5 mL/min.

µDAWN & UT-rEX for UHP-SEC

- µDAWN and Optilab UT-rEX preserve speed and resolution from UHP-SEC.
- MALS and DLS measurements determine MW, R_g , R_h, extinction coefficient, and conformation.
- All key online MALS applications are readily transferrable to UHP-SEC.
- High speed and rich information open new applications in process monitoring and other areas.

µSEC-MALS™