WYATT TECHNOLOGY EUROPE

Multi-Angle Light Scattering goes micro

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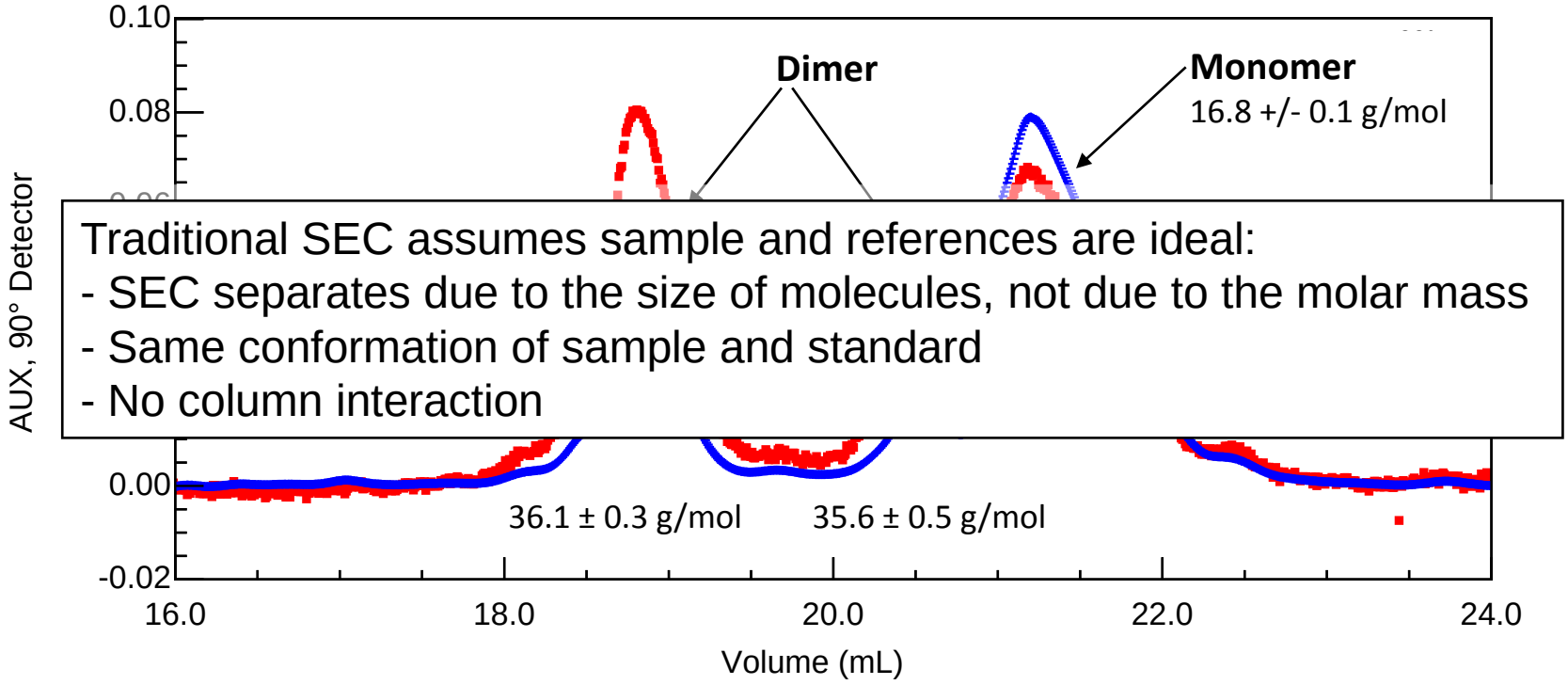
International Symposium on GPC/SEC and Related Techniques
Frankfurt 2014

Overview

- Why use Multi Angle Light Scattering ?
- Challenges coupling UV MALS DRI to UHPLC
- µDAWN and UT-rEX
- First Results
- Summary

SEC-MALS where Column Calibration fails: Fibroblast Growth Factor



Astafieva, A., G. Eberlein, L. Nilsson, D.W. Shortt and P.J. Wyatt, "Multimeric conformation multiangle laser light scattering and reversed-phase high-performance liquid Chromatography," *American Laboratory,* pp. 30, *March 1995.*

Why use Multi Angle Light Scattering ?

- Multi Angle Light Scattering (MALS)
  - Measures molar mass based on the amount of scattered light
  - Absolute method, no assumptions, no standards
  - Independent of size, shape, etc.
  - Destruction free, sample is reusable
  - Measures molecular size based on the angular dependency of the scattered light
  - Measures second virial coefficient A_2 for stability and solubility studies

- Coupled to Size Exclusion Chromatography (SEC / GPC)
  - Adds absolute molar mass detection capability to SEC / GPC
  - Analysis of **polymer conformation and branching**

- MALS in stand alone – batch mode
  - Measures molar mass without separation / column (if columns fail)
  - Measures interaction and solubility

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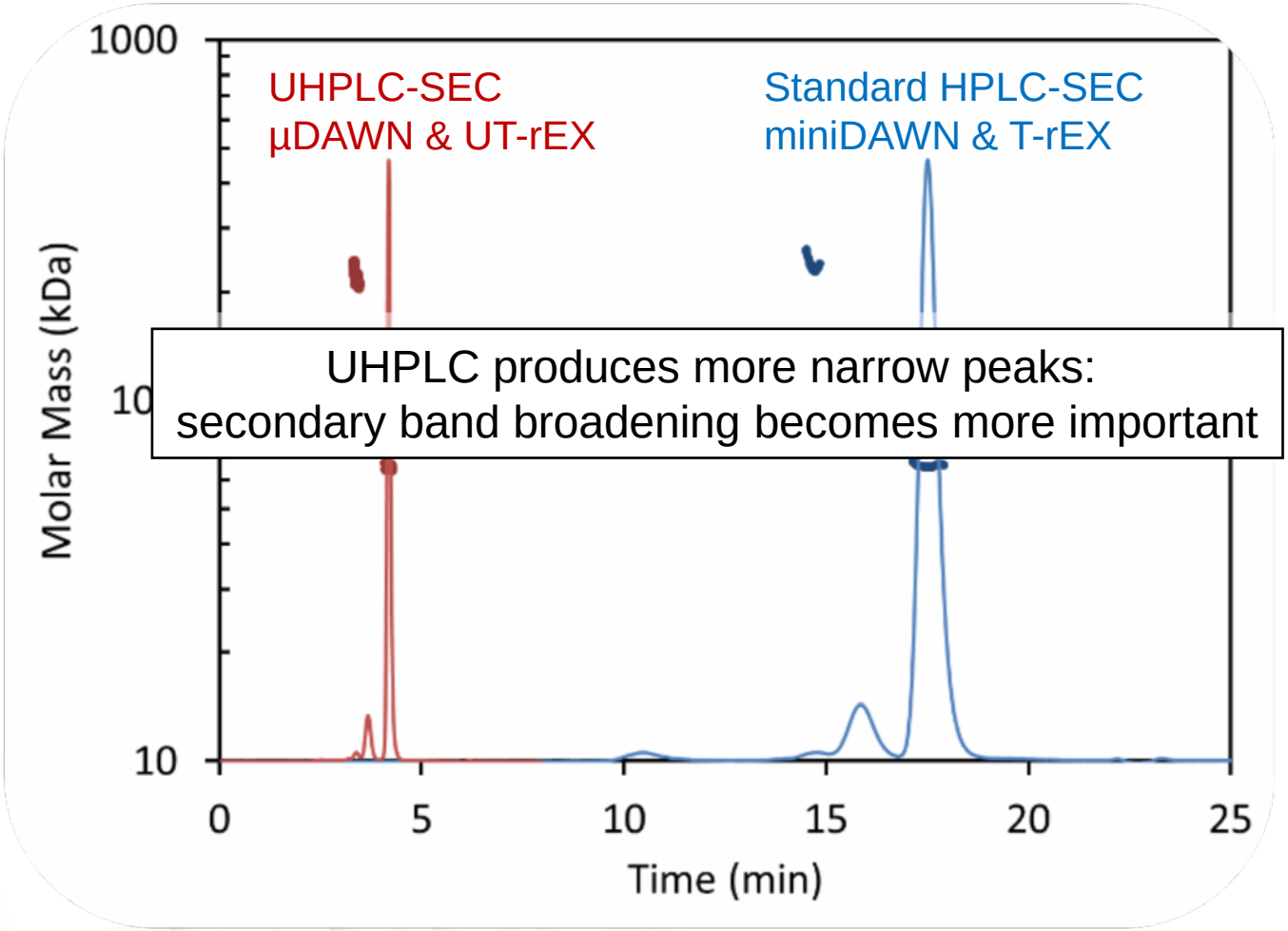
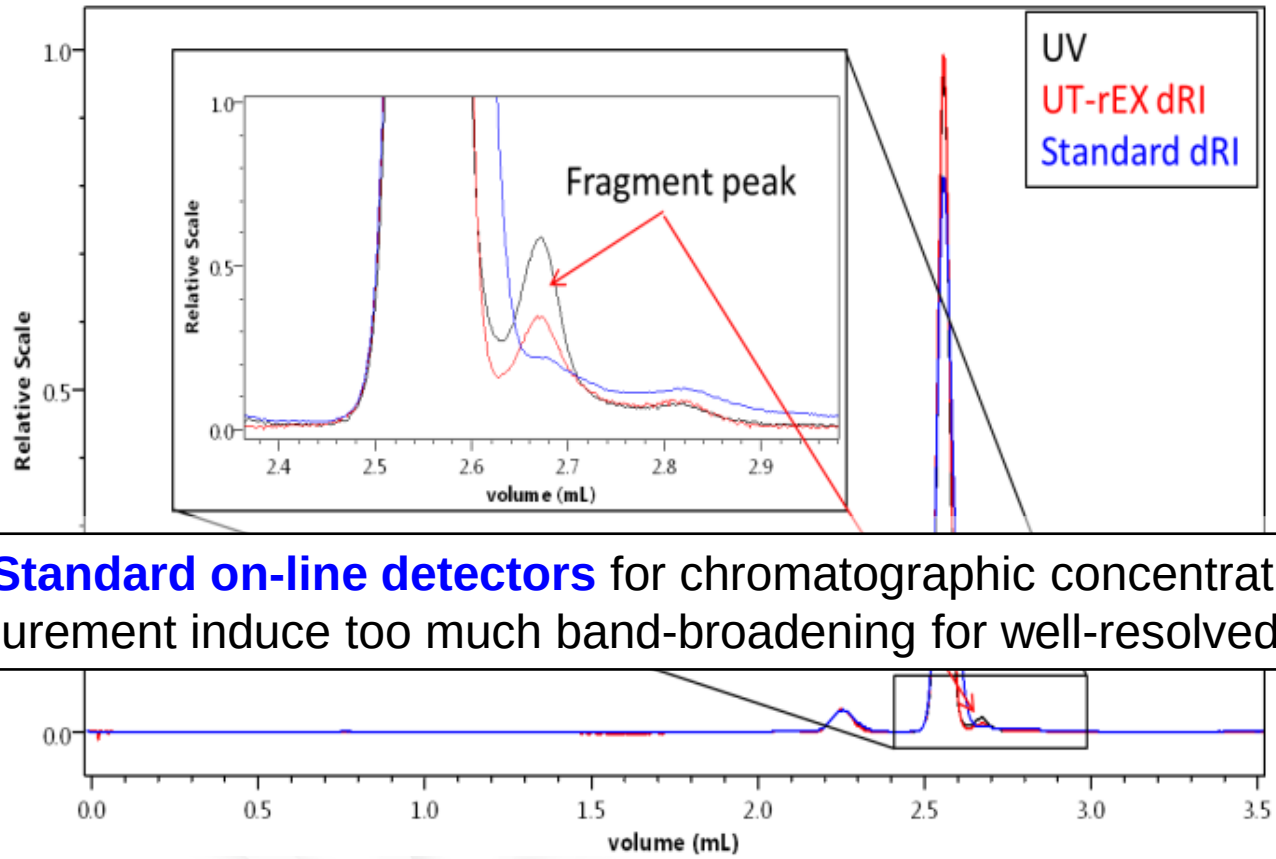
UHPLC SEC-MALS vs. SEC-MALS



- µDAWN + UT-rEX, 150 mm UHPLC column

UHPLC SEC-MALS



Standard on-line detectors for chromatographic concentration measurement induce too much band-broadening for well-resolved UHPLC

- A UHPLC chromatogram overlay comparing standard DRI and UT-rEX DRI signals from bovine serum albumin (BSA). In both cases the DRI detector is downstream of the UHPLC UV detector

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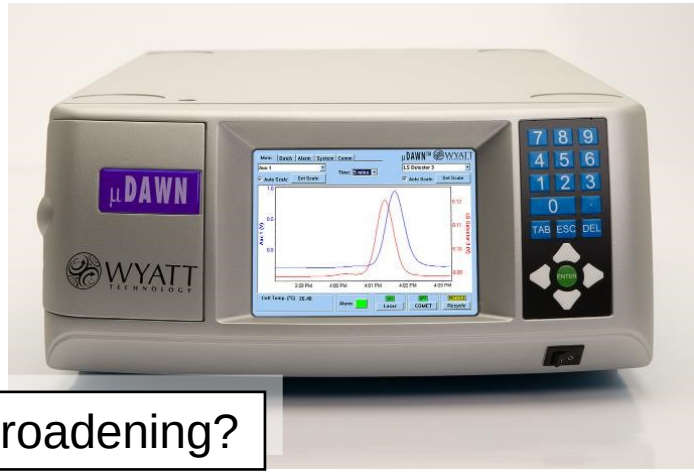
µDAWN and UT-rEX

µDAWN and UT-rEX for UHPLC-SEC:



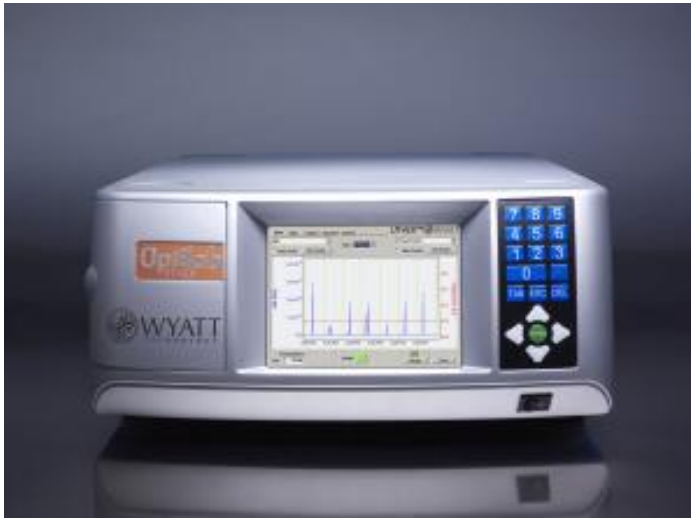
Combining UHPLC performance advantages

- Separation between closely related compounds
- Reduced mobile phase consumption
- Shorter run times

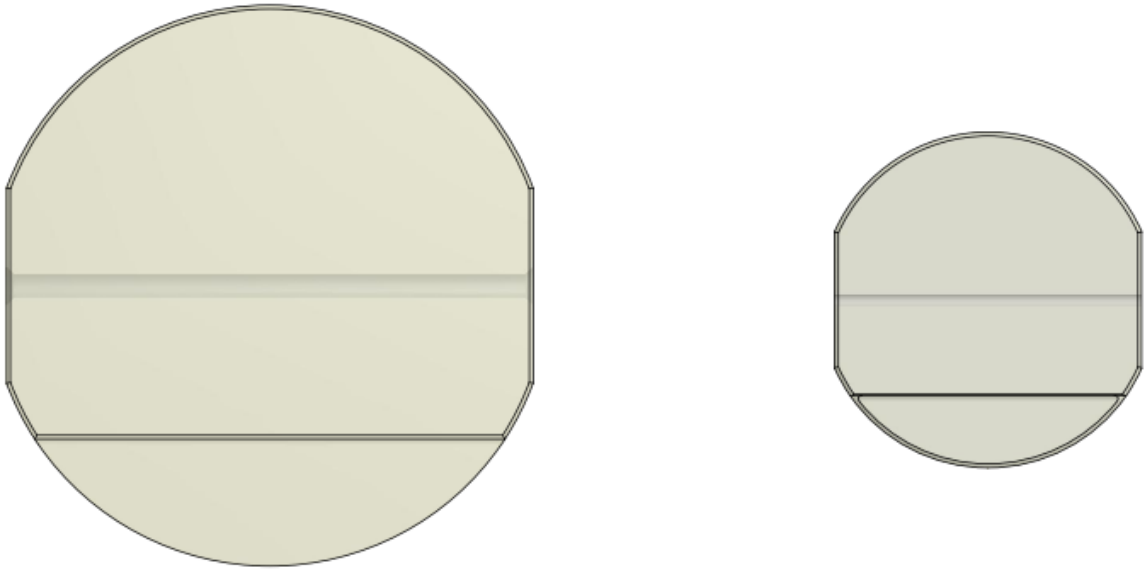
How can we reduce secondary band broadening?

with UV MALS DRI detection capability

- Absolute molar mass and size distributions
  - Based on UV concentration measurement
  - Based on DRI concentration measurement
  - Co-polymer, protein conjugate analysis
- 20 ng BSA typical UHPLC SEC loading (200 ng SEC)
- 200 to 10E7 g/mol

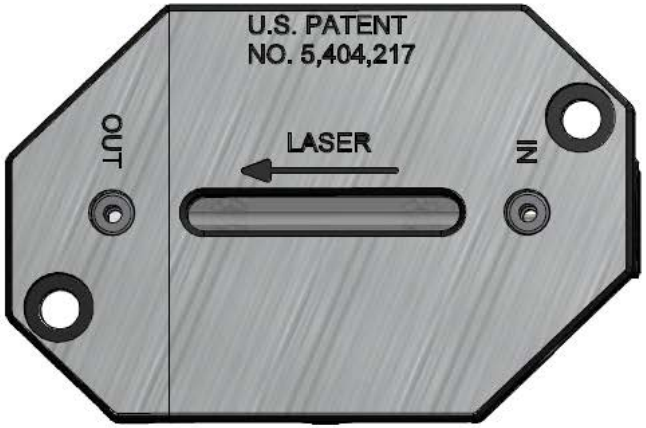


µDAWN and UT-rEX



- Left hand side: DAWN TREOS
- Right hand side: µDAWN

µDAWN and UT-rEX



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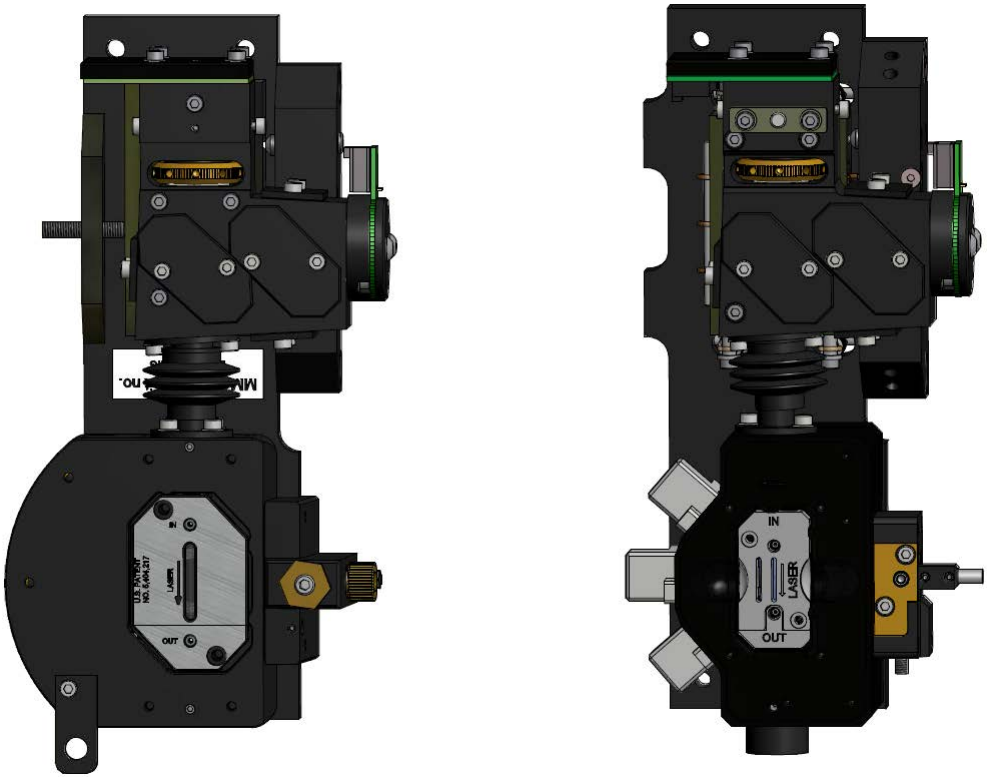
µDAWN and UT-rEX



- Left hand side: DAWN TREOS
- Right hand side: µDAWN

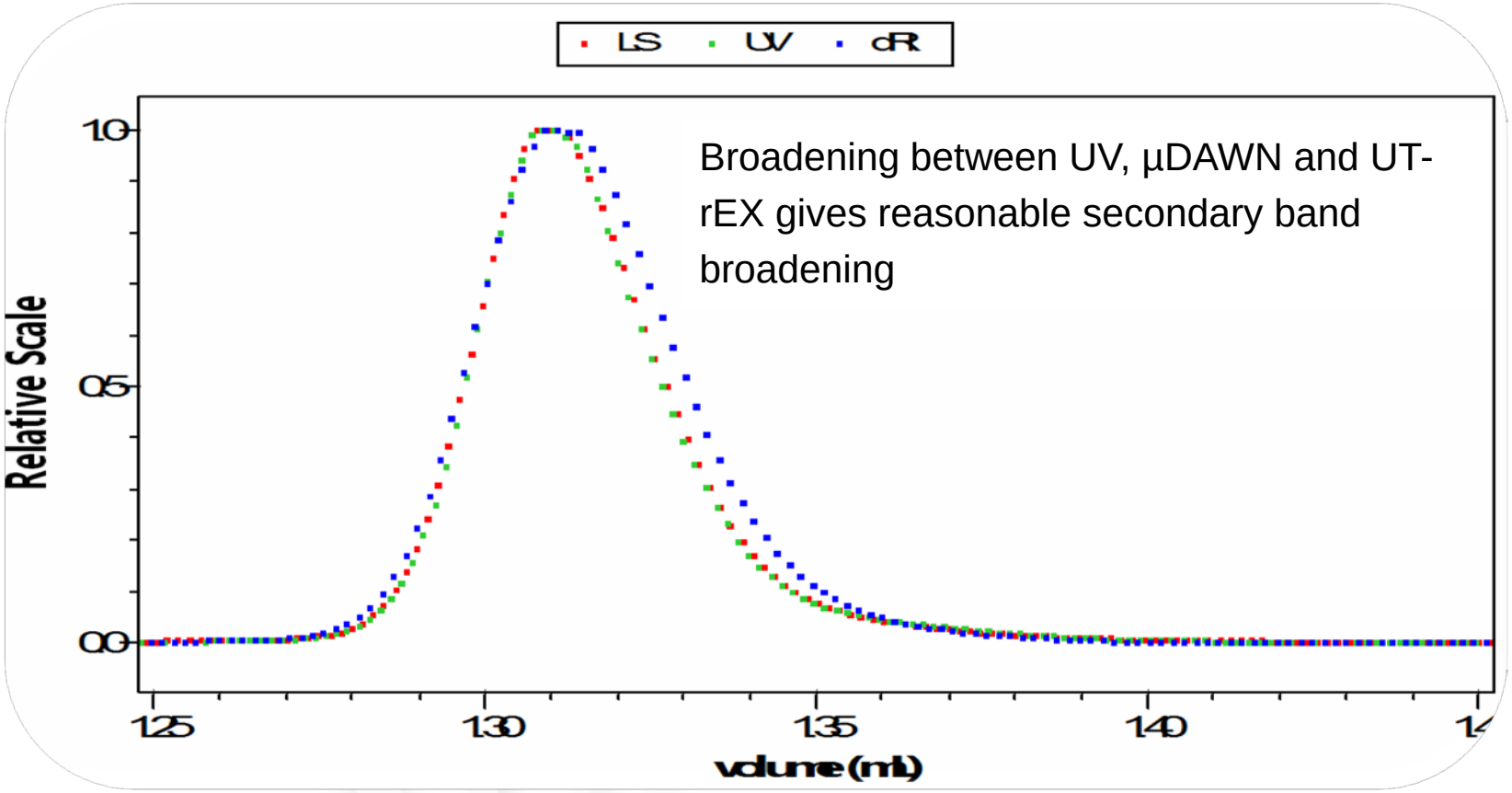
µDAWN and UT-rEX

- Reduced µDAWN flow cell & manifold volume
  - Total cell & manifold volume 63 µL -> 10 µL
  - New read head
  - New tubing i.d. 0.0035” (0.087 mm)
  - New COMET
- Reduced µDAWN flow cell bore diameter
  - New flow cell design inherently removes sources of stray light
  - New detection optics increase signal, reduce noise
  - New laser mount provides more stable laser alignment
- Reduced UT-rEX band broadening
  - Band broadening < 4µL
  - Inlet tubing (0.005” i.d.) 6.5 µL
  - Flow cell 7.4 µL

Experimental Setup

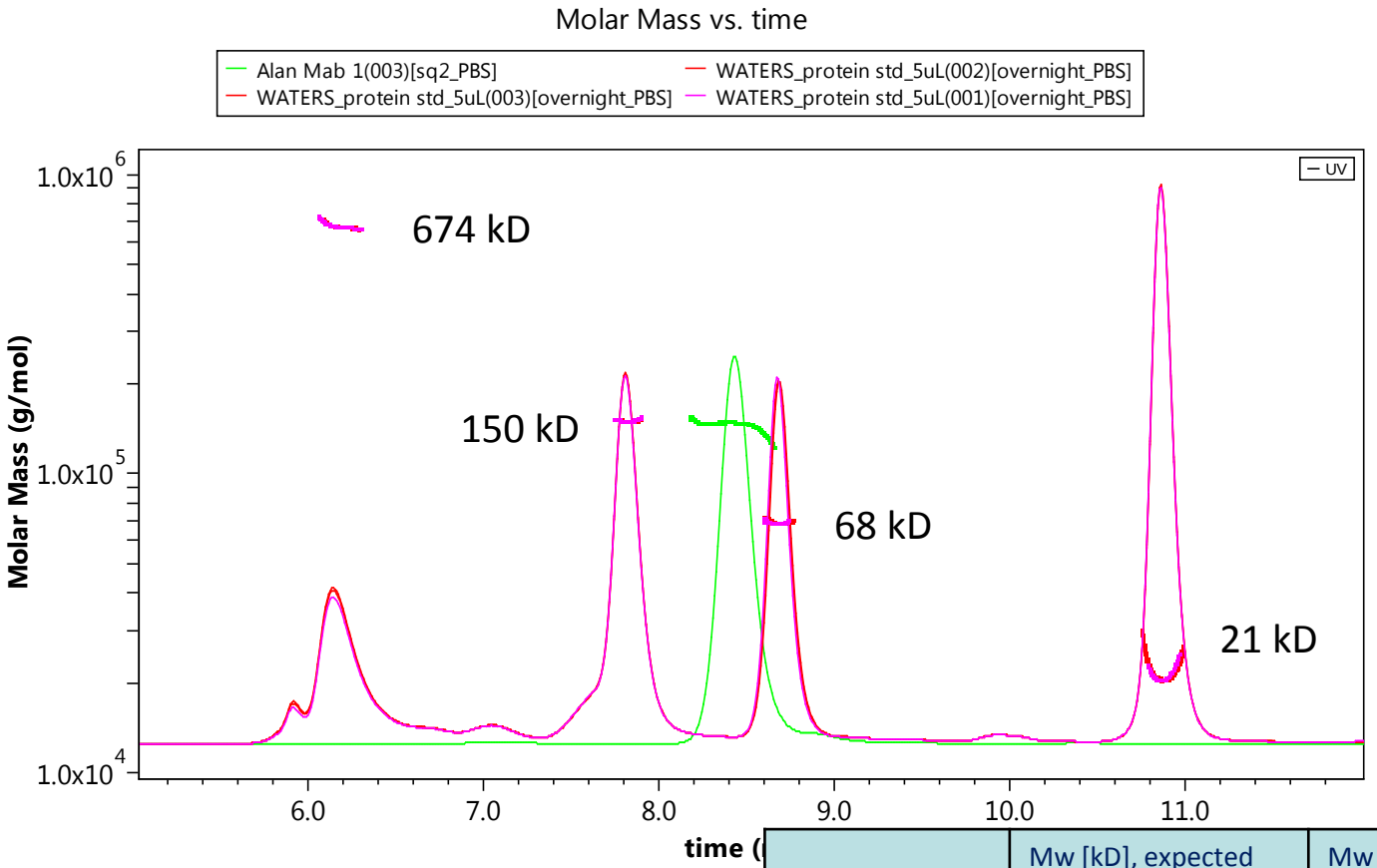
UPLC	Waters Acquity
MALS detector:	Wyatt µDAWN
UV detector:	Waters Ti TUV Detector
dRI detector:	Wyatt UTrEX
Software:	ASTRA 6.1.2
Columns:	Acquity UPLC BEH200, SEC (1.7µm), 4.6 x **150 or 300** mm
Mobile phase:	WTC PBS
Flow rate:	0.3 mL/min
Samples:	Pierce BSA, Waters mAb Standard, Waters Protein Standard Mix, various mAb proteins.
Injected amount:	Varies from 5 to 30 µg

UV, µDAWN and UT-rEX Band Broadening



- µDAWN + UT-rEX, 150 mm UHPLC column

Band Broadening Correction works for UHPLC Peaks

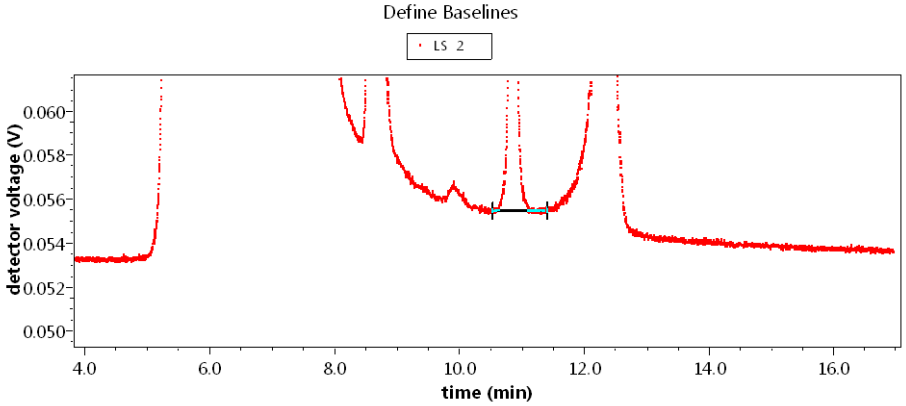


	Mw [kD], expected	Mw [kD] by µDAWN
thyroglobulin	660	674
IgG	150	150
BSA	66.4	68
myoglobin	16.7	21

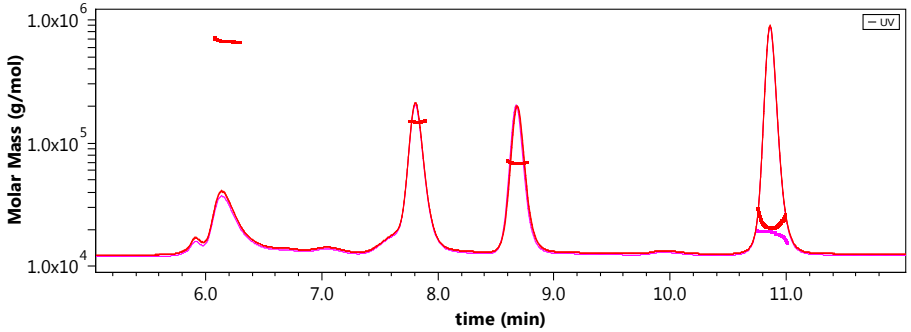
- Band broadening correction parameters w
  applied to the data files of protein mixture

Band Broadening Correction works for UHPLC Peaks

- Baseline correction to subtract the LS signals from co-eluted species



- Forward laser monitor correction for absorption



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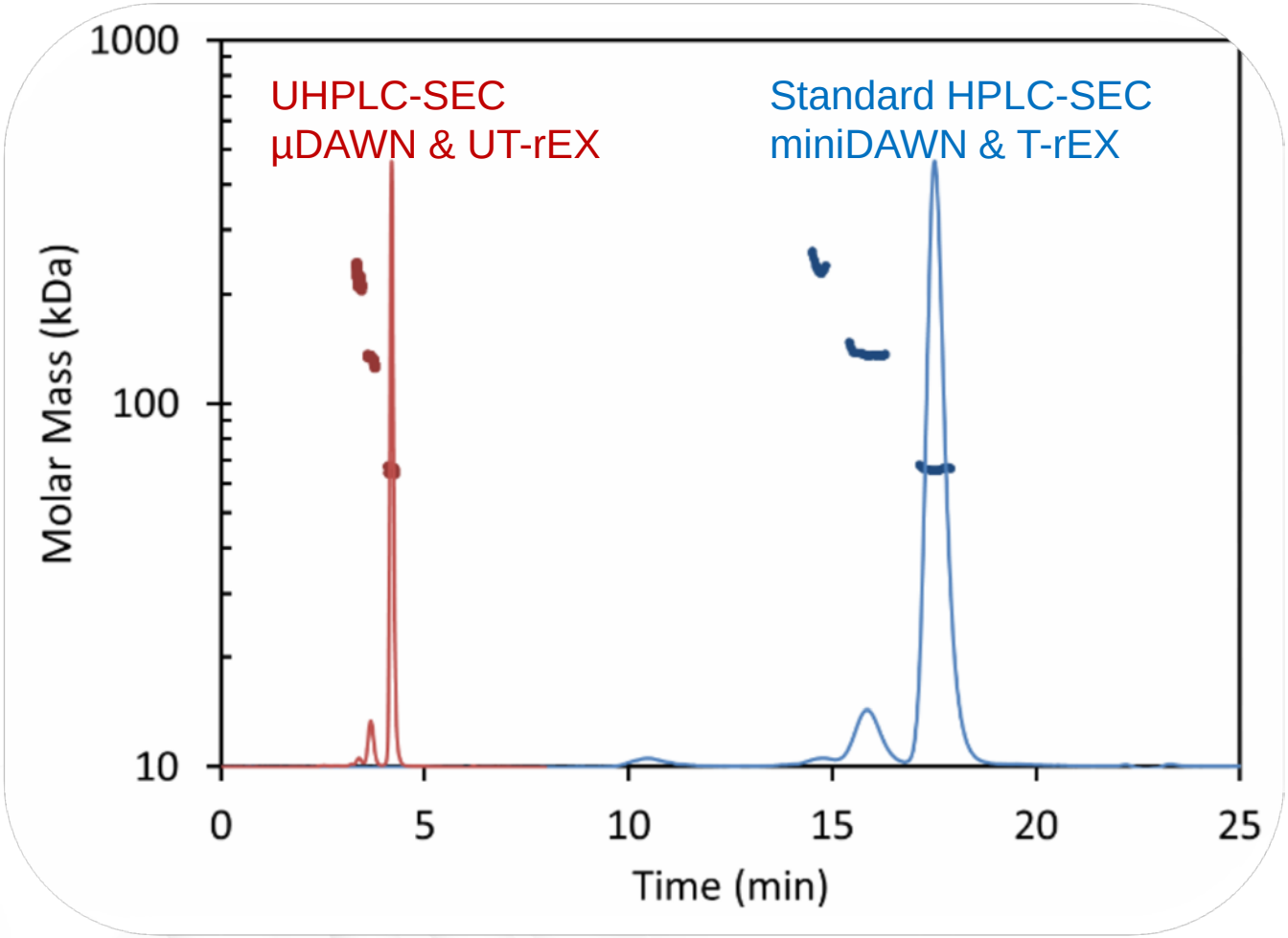

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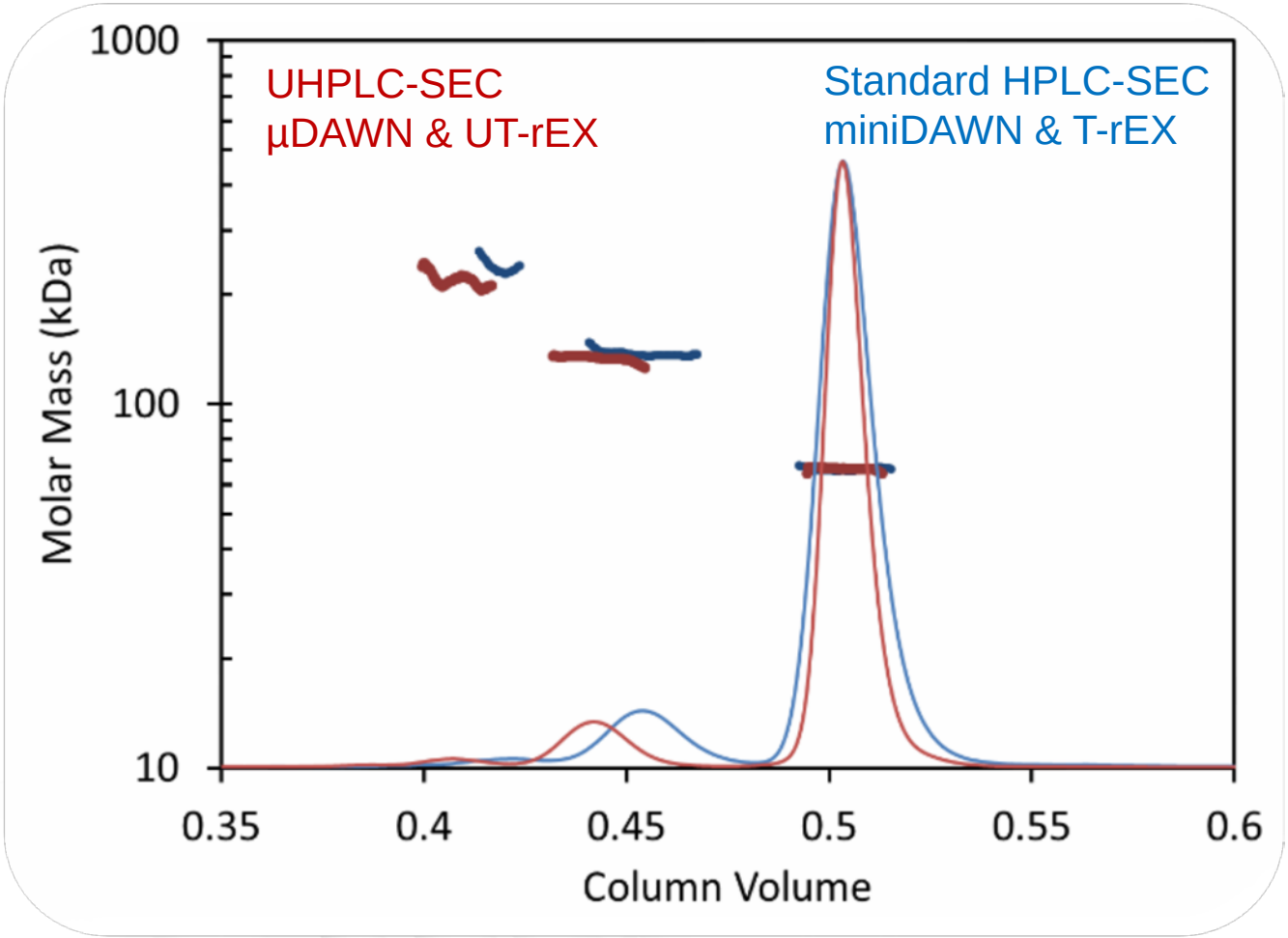

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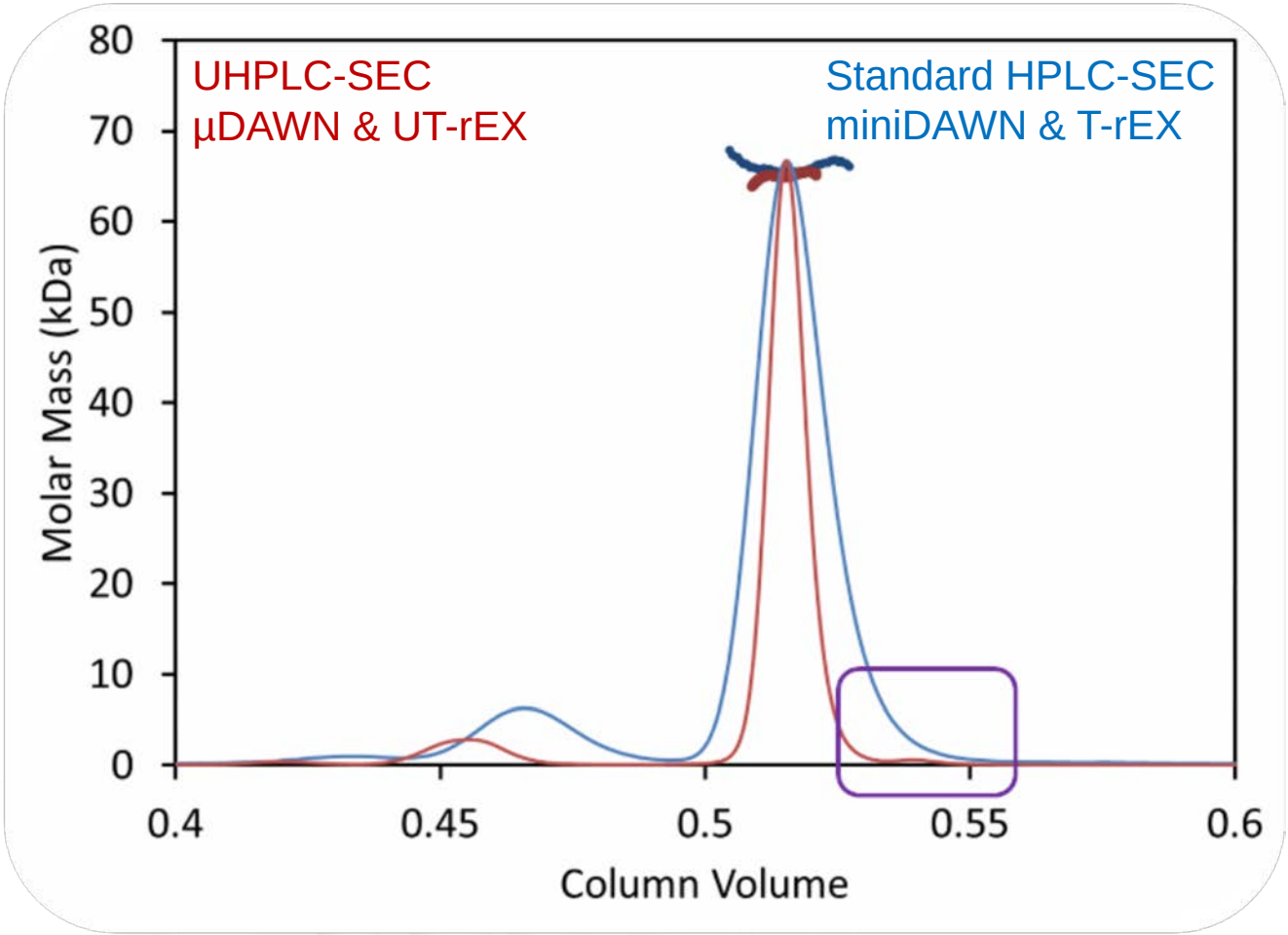
BSA Fragment on 300 mm BEH Column



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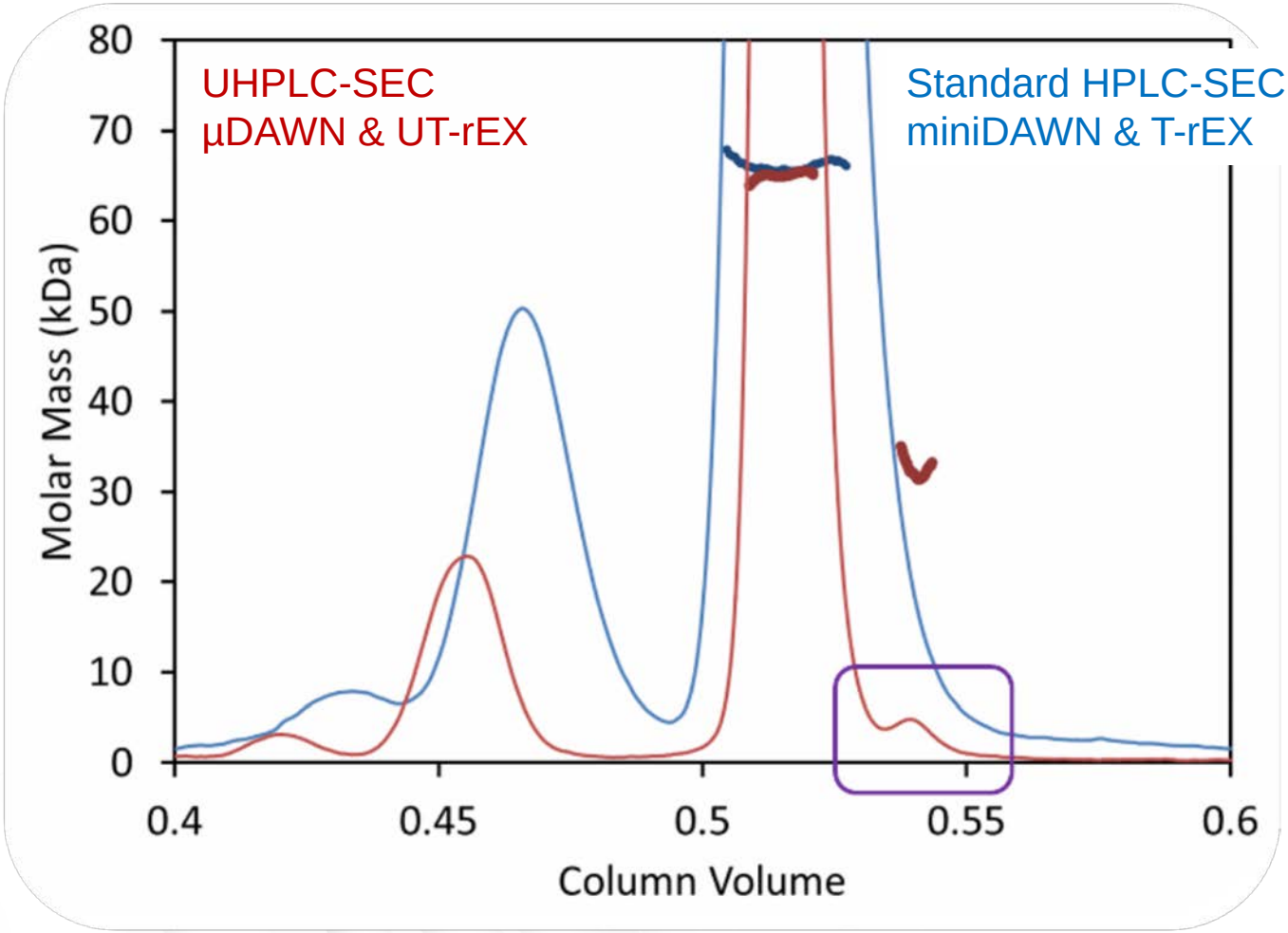
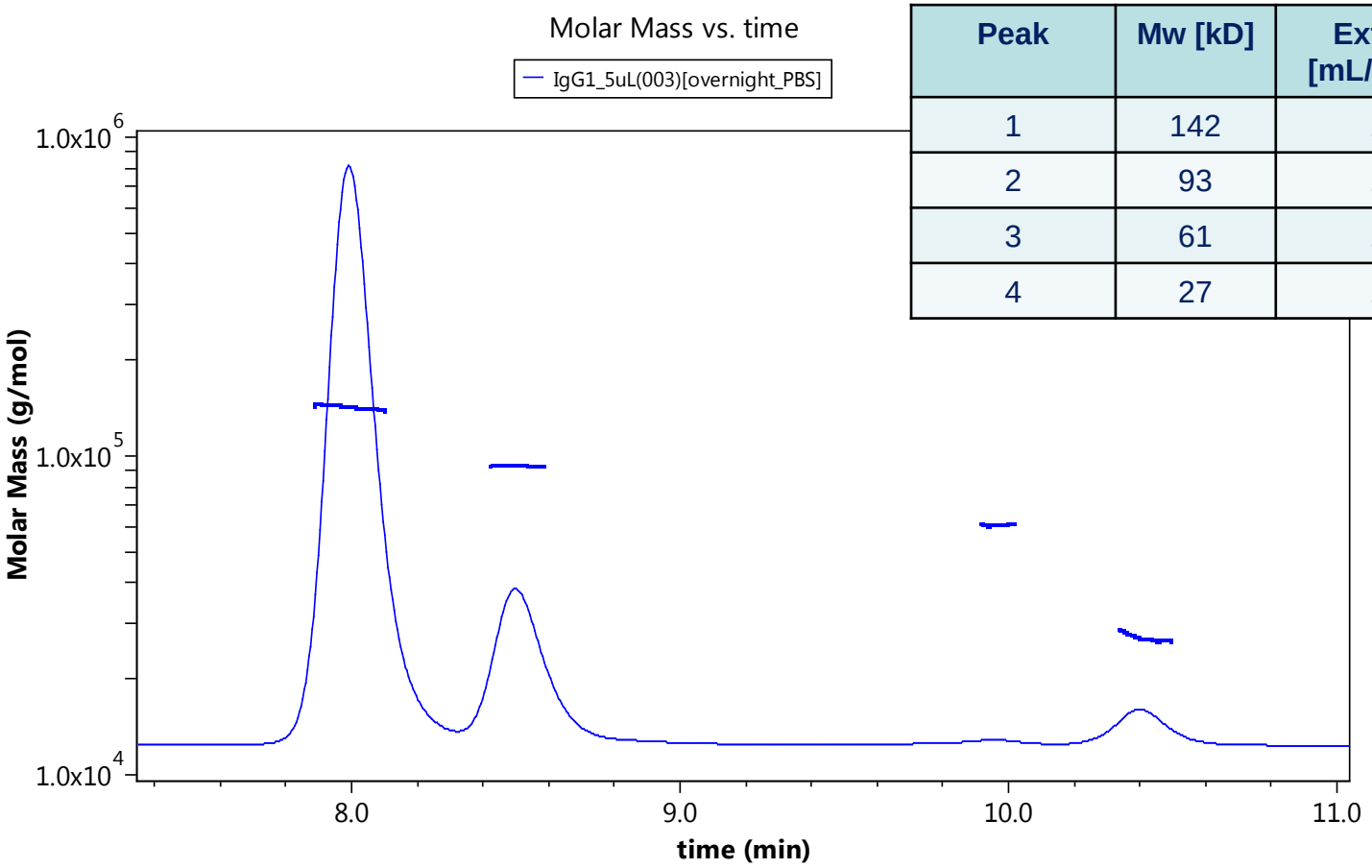


- µDAWN + UT-rEX, 300 mm UHPLC column

IgG Fragments identified



Peak	Mw [kD]	Ext Coeff [mL/(mg cm)]
1	142	1.53
2	93	1.54
3	61	1.53
4	27	1.46

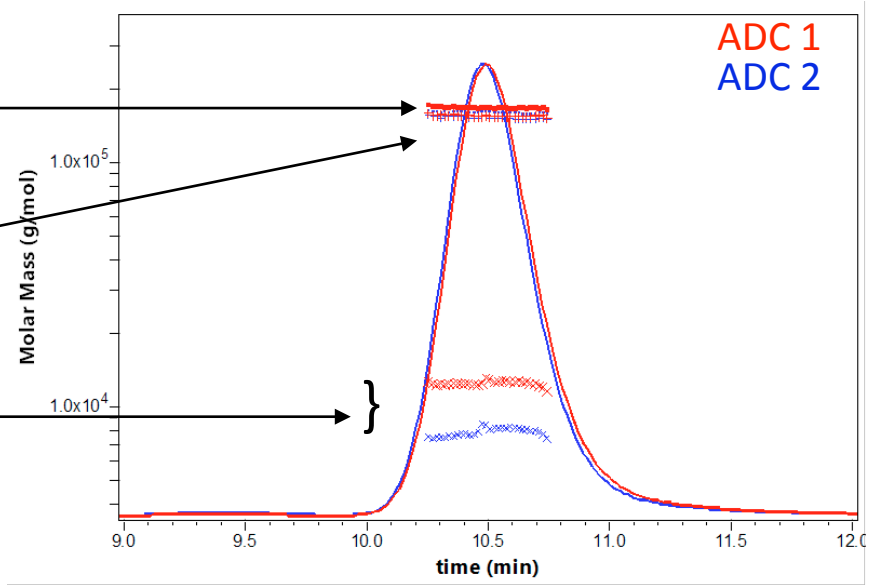
- Fragments of this IgG protein were separated (300 mm column) and peaks were identified by both **MW** and **UV extinction coefficient**, calculated by the ASTRA software.

Antibody Drug Conjugates

M_w of ADC

M_w of antibody fraction

M_w of *total* modifier

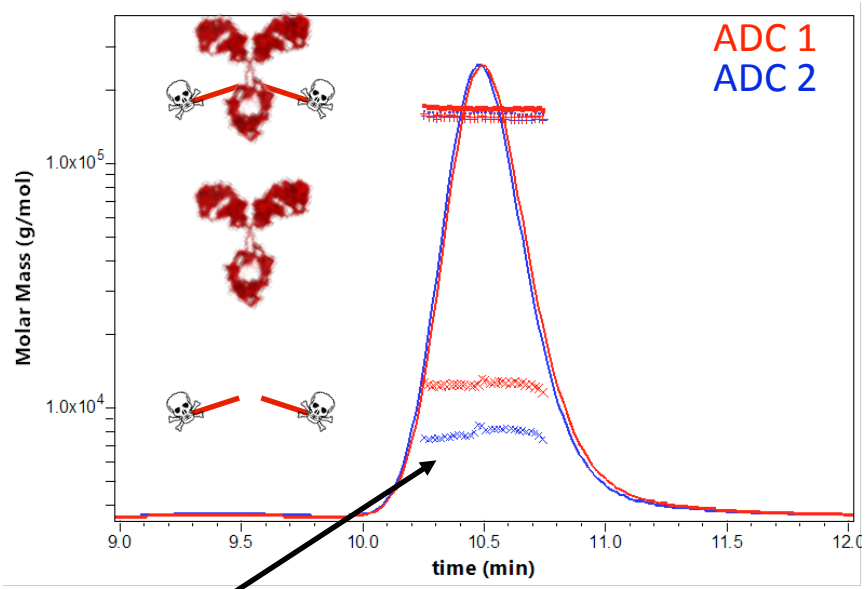


Antibody Drug Conjugates

Assessing Drug Antibody Ratio (DAR)

	M_w (kDa)			
	Complex	mAb	Modifier	DAR
ADC 1	167.8 (±1.2%)	155.2 (±1.8%)	12.6	**10.1**
ADC 2	163.7 (±1.2%)	155.6 (±1.2%)	8.1	**6.5**

DAR values calculated based on a modifier M_w of 1250 Da



Horizontal profile indicates homogenous modification

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ADC 2	163.7 (±1.2%)	155.6 (±1.2%)	8.1	**6.5**
ADC 3	159.5 (±8.0%)	155.2 (±8.0%)	4.3*	**~1 - 7**

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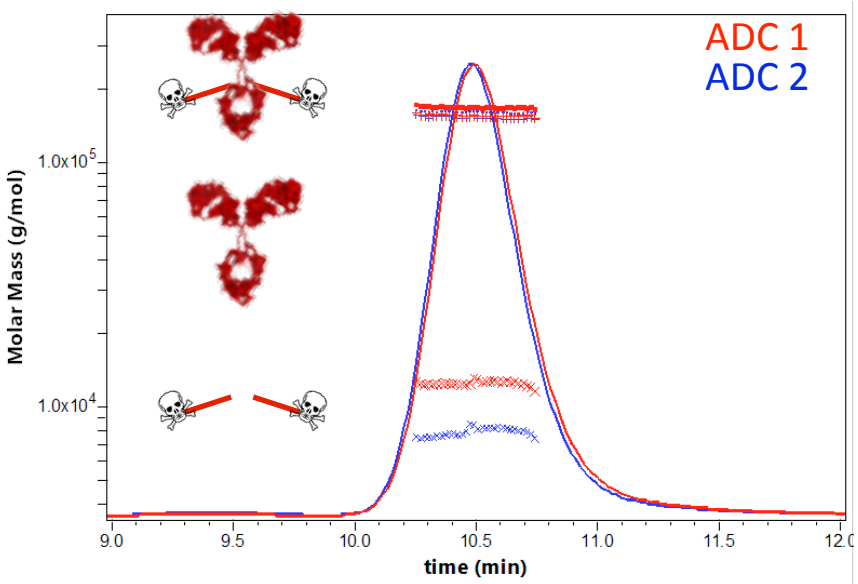
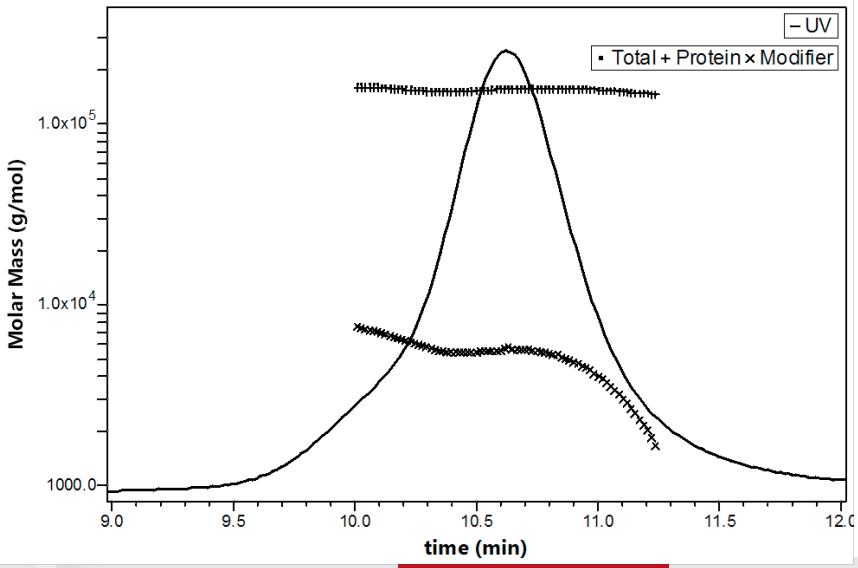


For optimal results the modifier should contain ≥ 3-5 wt% of total conjugate…

…however, lower amounts can be tracked in heterogenous samples.



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- UHPLC coupled with UV MALS DRI
  - Special UHPLC detectors required
  - First test with BEH columns were successful
  - Tests with APC columns are planned for beginning of 2015

- Finally I want to thank
  - My lab team for the experimental data
  - Waters and PSS for the kind invitation
  - **And you for your attention !**