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# The light scattering tool box for the characterization of proteins, peptides and others bio-macromolecules:

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## Multiple Angle Light Scattering

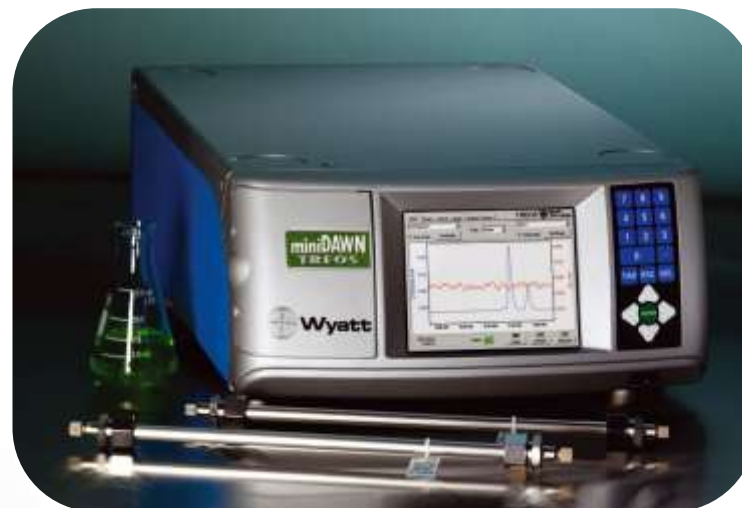
- $M_z$ ,  $M_w$ ,  $M_n$  (= absolute molar masses)
- RMS radius (= radius of gyration)
- $R_h$  (= hydrodynamic radius) if QELS
- Conformation
- Branching
- Online or in batch mode
- Stoichiometry of complexes
- +4°C to +80°C temp control in option



**DAWN Heleos II (18 angles)**



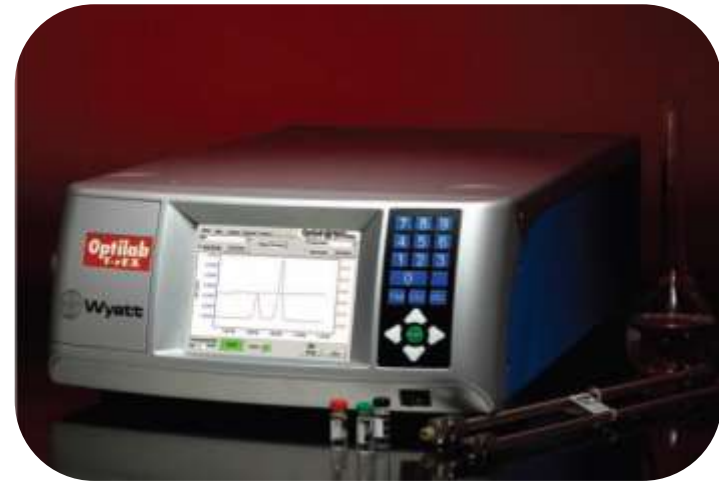
**WyattQELS (=DLS)**



**miniDAWN Treos (3 angles)**

## RI & IV

- $C$  (= concentration)
- $dn/dc$
- Temp control  $+4^{\circ}\text{C}$  to  $+65^{\circ}\text{C}$
- UV extension coefficient in solution from RI peak
- $\eta$  (= Intrinsic viscosity)
- $K$  &  $a$  (=Mark-Houwink-Sakurada coefficients)
- $Rh_v$  (= hydrodynamic radius from visco)
- Temp control  $+4^{\circ}\text{C}$  to  $+65^{\circ}\text{C}$



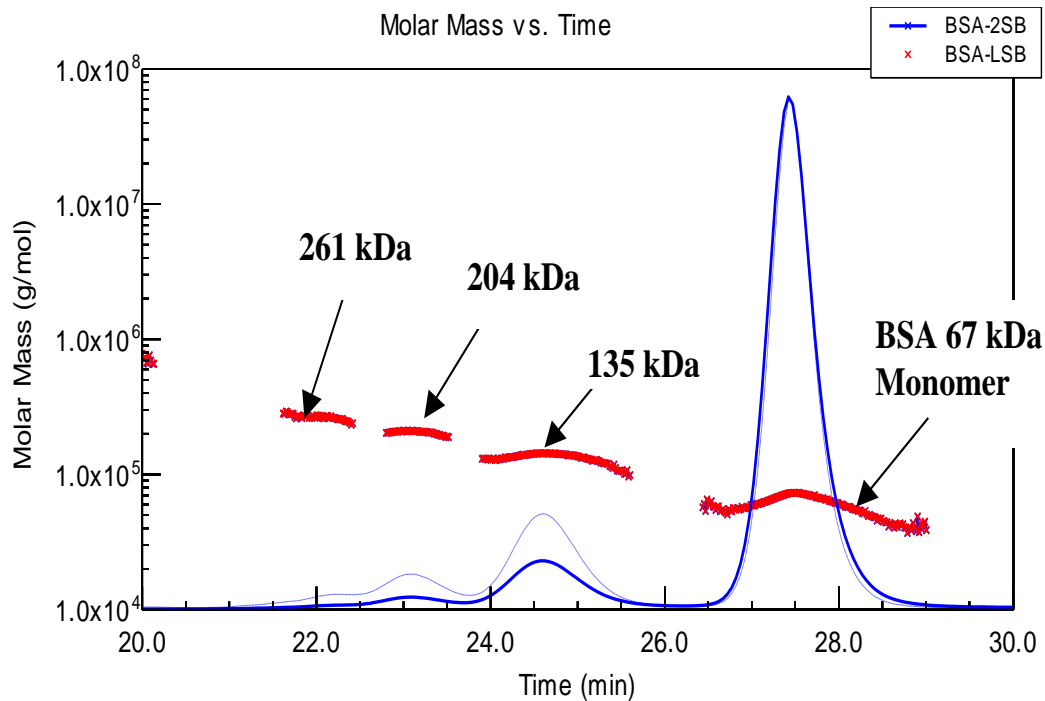
**Optilab TrEX**



**ViscoStar II**

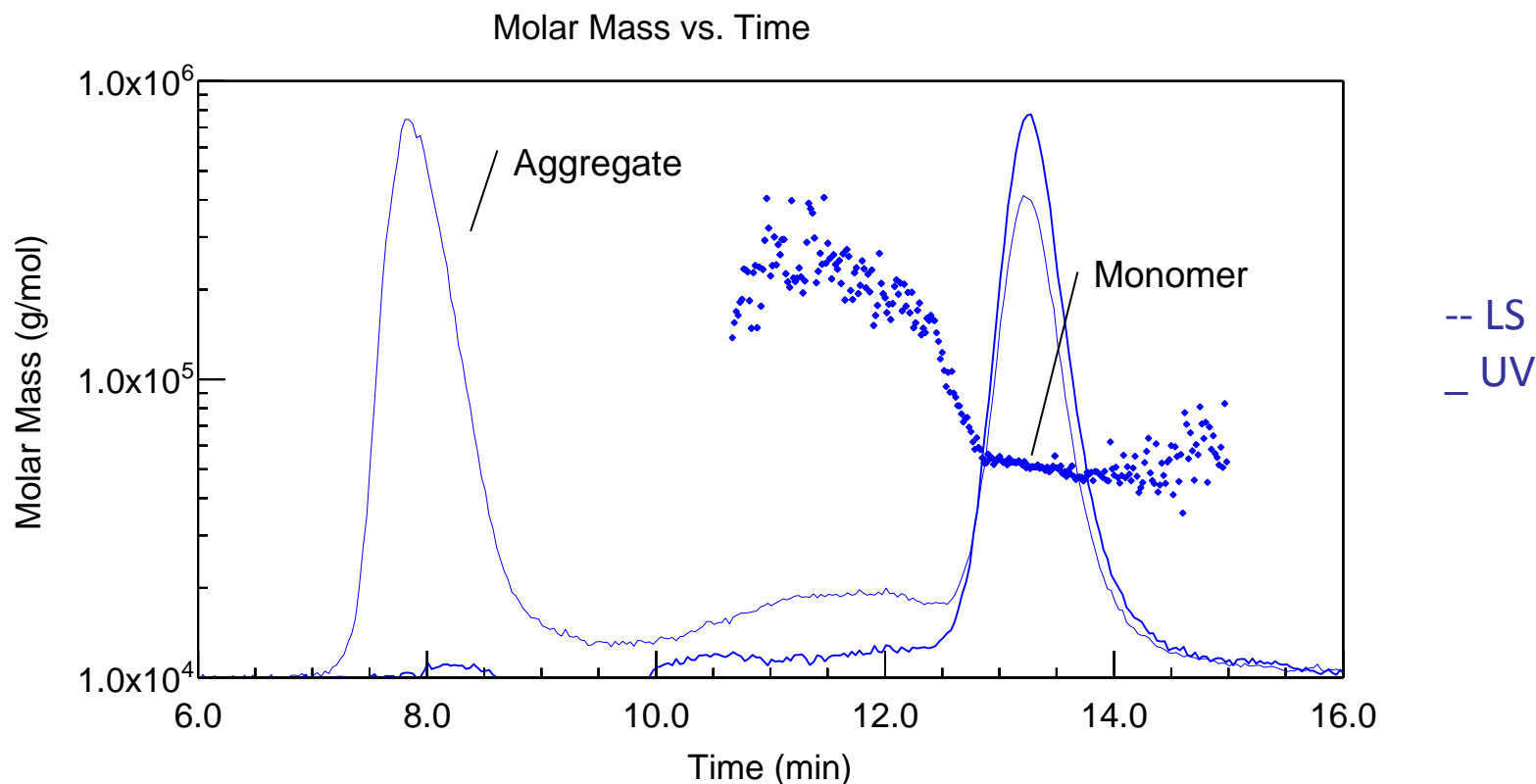
# Oligomer „hunting“

- Protein aggregates well characterize.
- High sensitivity of MALS to aggregates.
- MALS-UV/RI allows stoichiometry determination.



Aggregates	%	Molar Mass [kDa]
<i>Monomer</i>	<b>92.4</b>	<b>66.8</b>
<i>Dimer</i>	<b>6.65</b>	<b>135</b>
<i>Trimer</i>	<b>0.95</b>	<b>204</b>
<i>Tetramer</i>	<b>0.53</b>	<b>263</b>

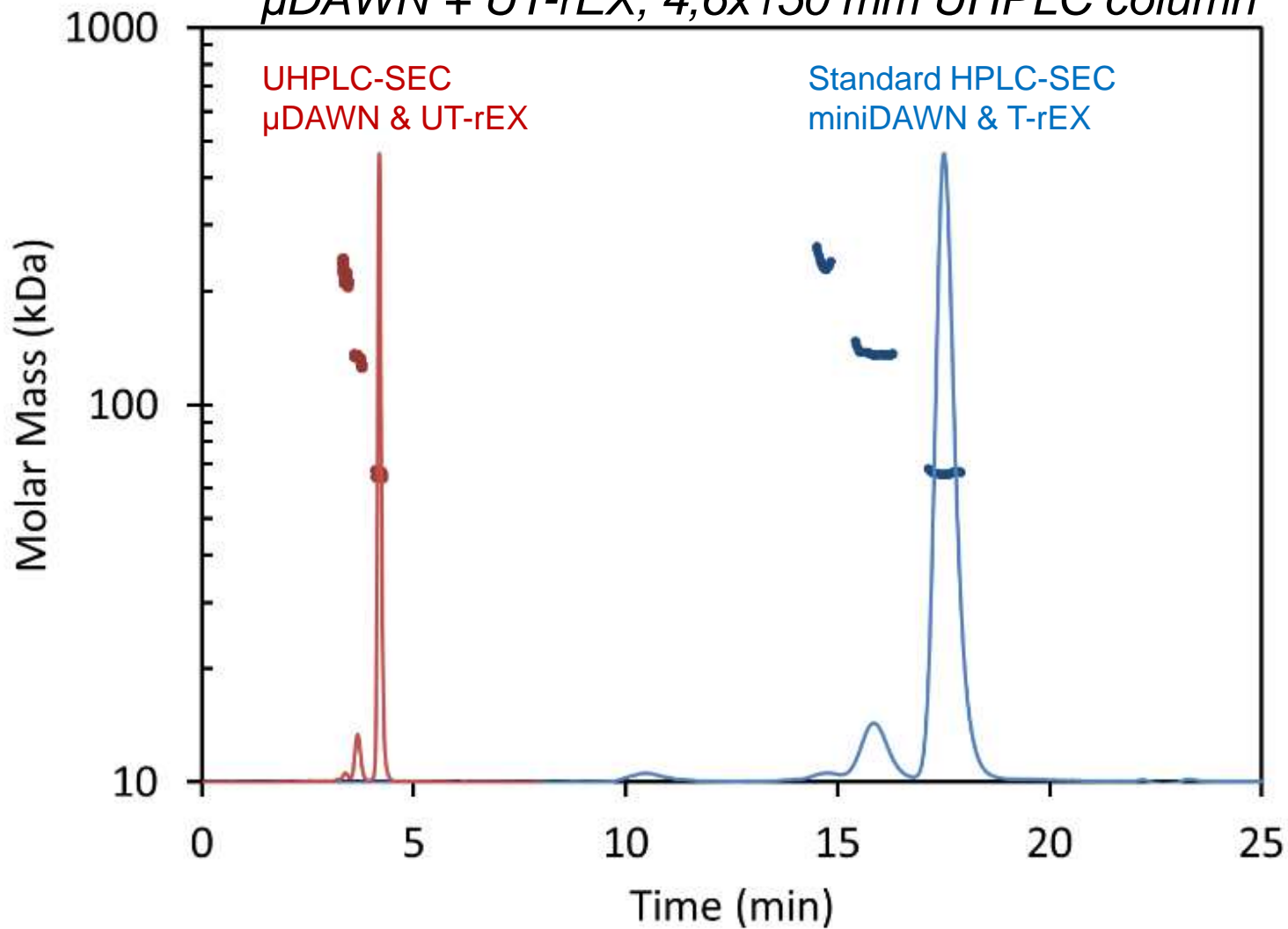
# Aggregates detection



- 6  $\mu\text{g}$  of aggregates found by MALS
- Molar mass of the main peak is measured at 50 kDa
- The protein is with a majority of monomer (theoretical molar mass value is 47.8kD)

# Comparison of $\mu$ SEC-MALS with „classical“ SEC-MALS

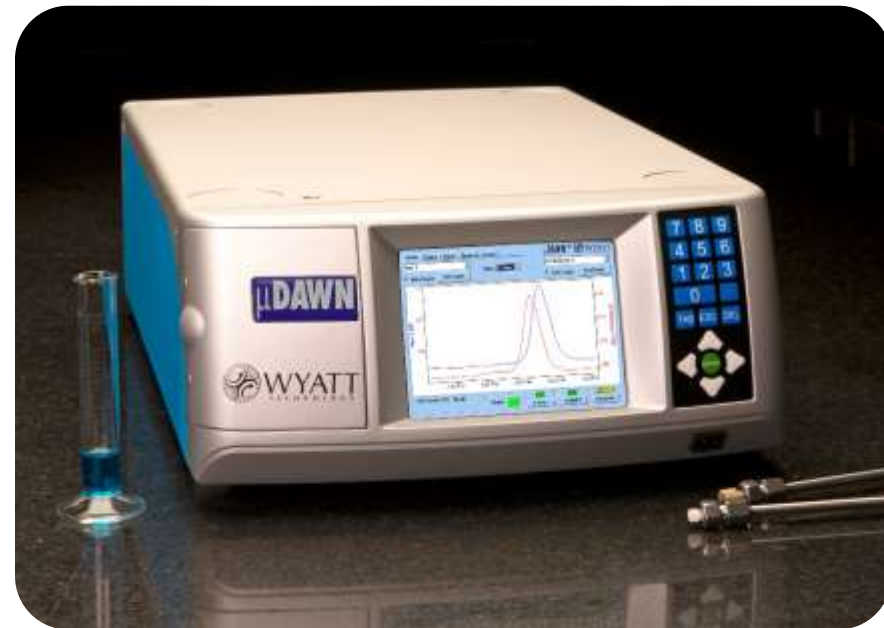
$\mu$ DAWN + UT-rEX, 4,6x150 mm UHPLC column



# μDAWN

- 3 angles at 45°, 90° et 135°
- Laser of 60 mW at 658 nm
- Molar masses from 200 to  $\sim 10^7$  g/mol
- Sizes:  $R_g = \sim 10$  nm à  $\sim 100$  nm  
 $R_h = 1$  nm to 50 nm if QELS
- Cell volume :  $< 10$   $\mu$ L
- Dispersion: UV-MALS  $< 2$   $\mu$ L; MALS-RI  $< 7$   $\mu$ L
- Resolution: 22+ bits (digital), 18 bits (analogue)
- 36 Hz

- Precision  $\sim 2 - 4$  %
- Repetability  $\sim 1\%$

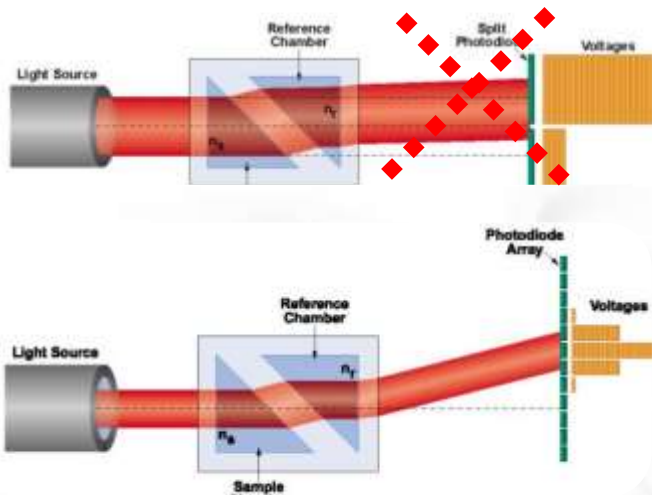


New COMET: online ultrasonic cleaning tool for the measurement cell



# Optilab UTrEX

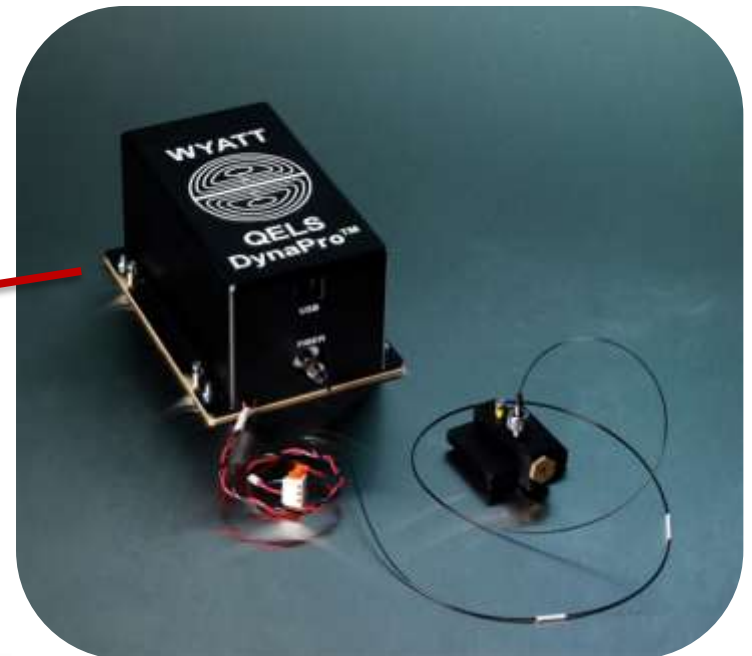
- Differential refractometer at 658 nm
- 512 photodiodes: dynamique range x50
- Absolute refractive index measurement
- dn/dc measurement
- Same wavelength than  $\mu$ DAWN
- Precalibration in factory
- Range: 1,2 – 1,8 RIU
- Sensibility: 0,002 RIU
- Cell volume < 10  $\mu$ L
- Temperature range: +4° / +65°C ( $\pm 0.005$  °C)  
(below +20°C, nitrogen is required to avoid condensation)





# Adding Dynamic Light Scattering : Wyatt QELS

- Online DLS on the same flow cell than  $\mu$ DAWN
- Size range: 1 to 50 nm
- Real time digital correlator
- Hydrodynamic radius for each slice of the chromatogramme



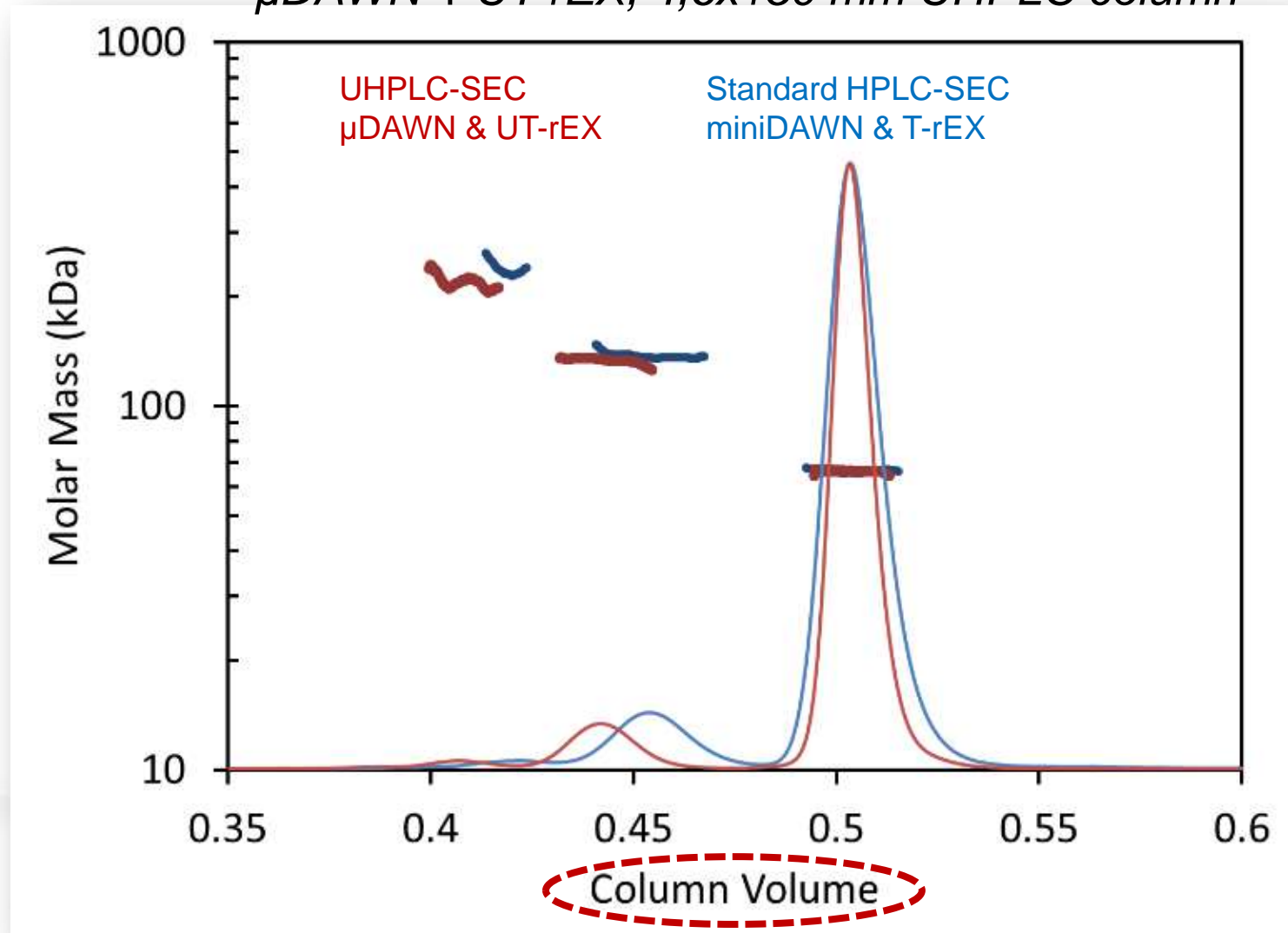
## Setup

# Waters H-Class Bio Inert avec UV (DAD) - $\mu$ DAWN - Optilab UTrEX



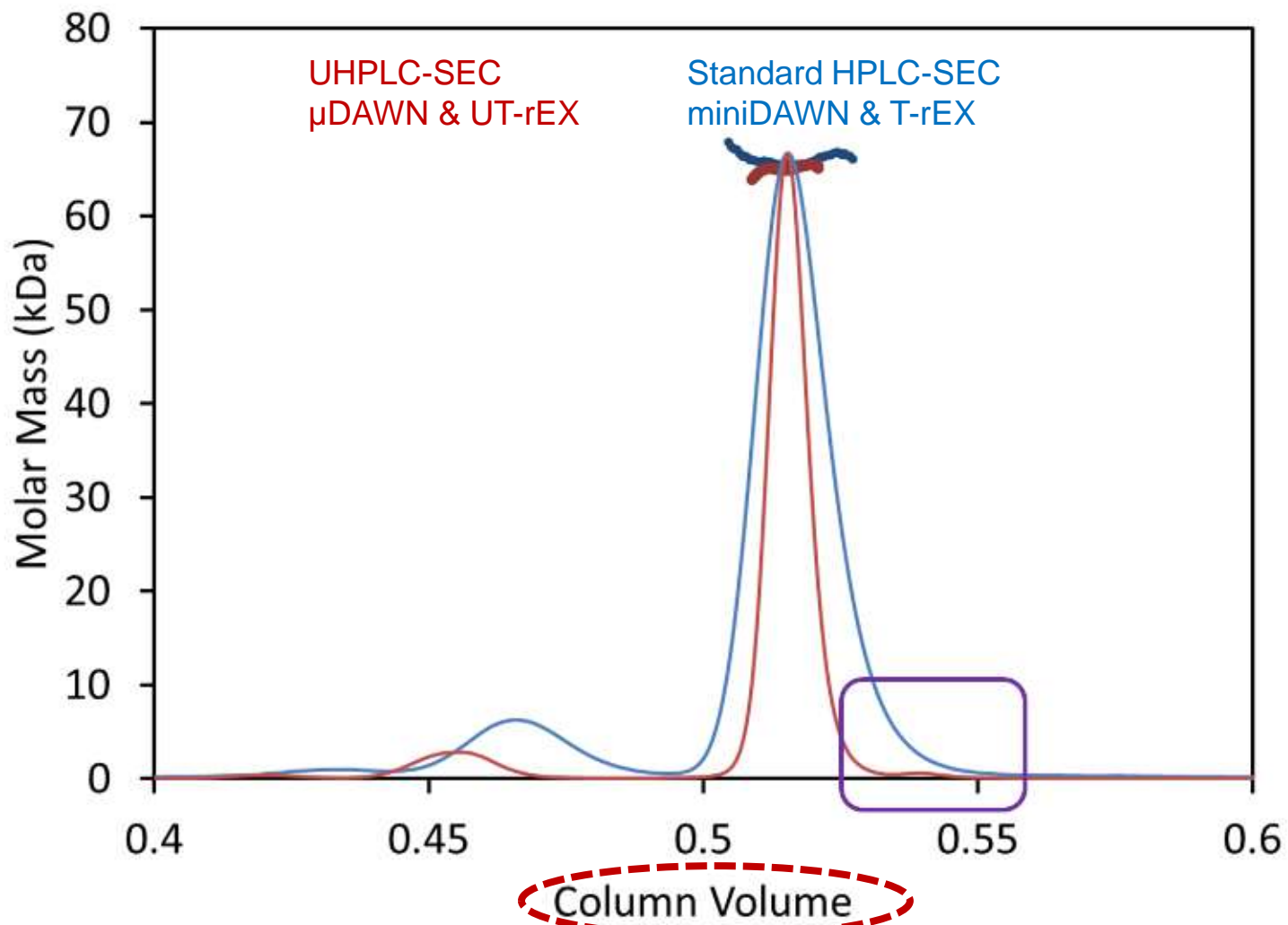
# Comparison $\mu$ SEC-MALS with „classical“ SEC-MALS

*$\mu$ DAWN + UT-rEX, 4,6x150 mm UHPLC column*



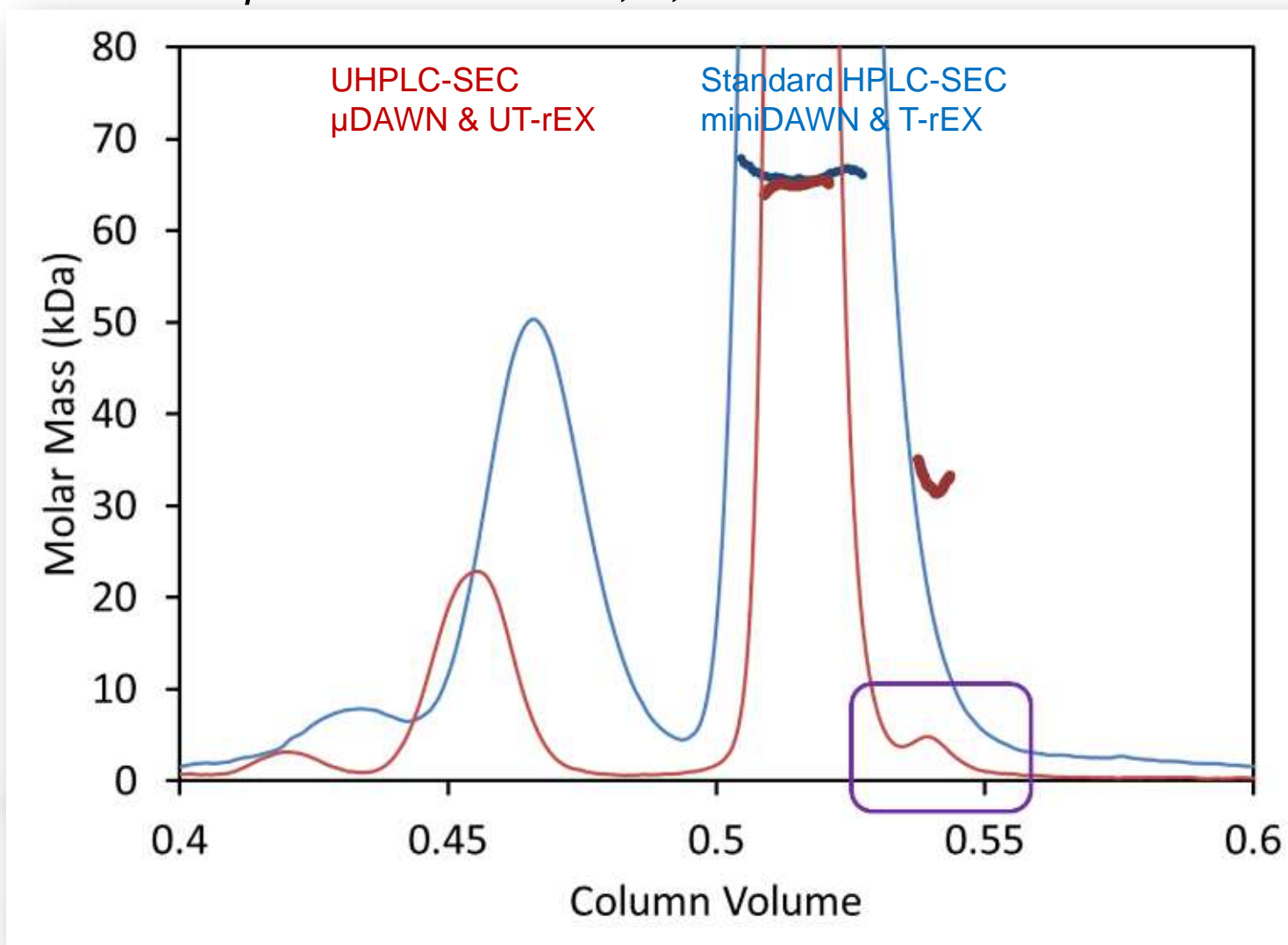
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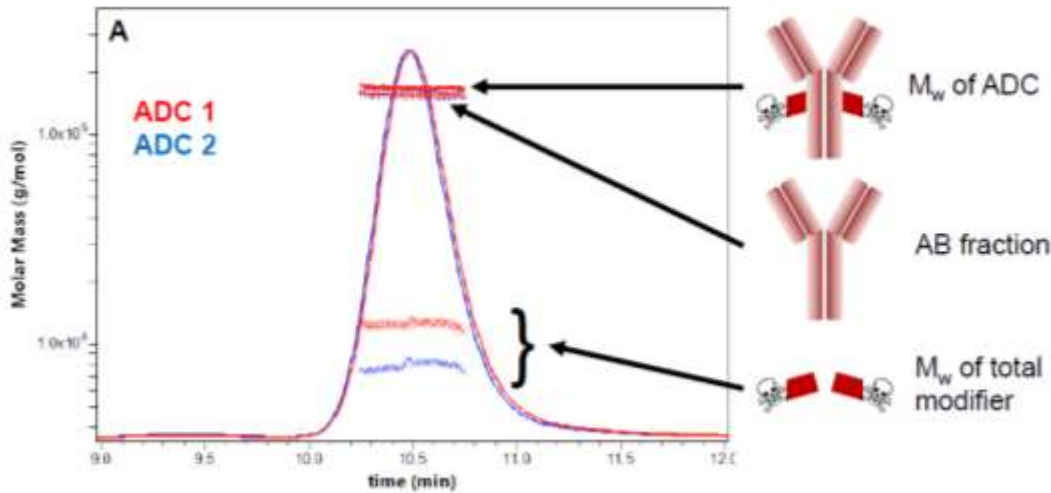
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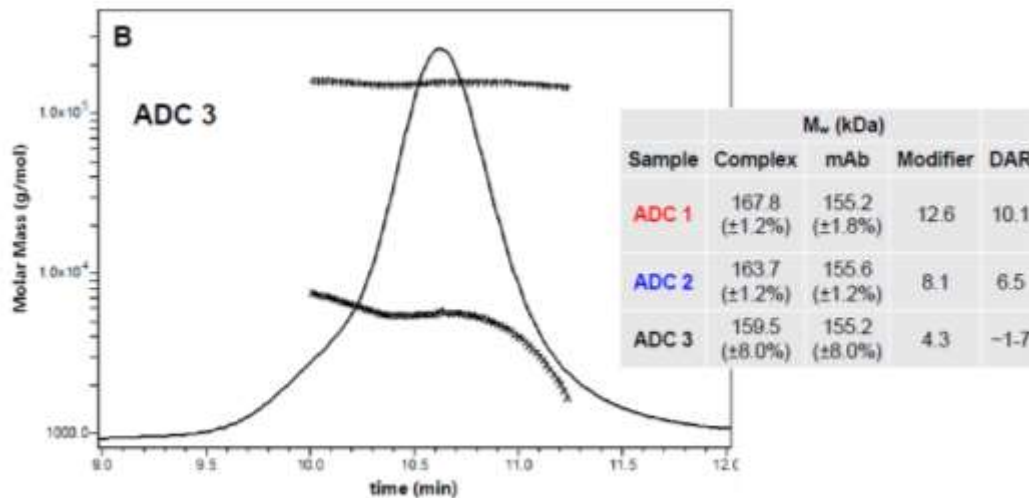


# Proteins conjugates analysis: ADC (Antibody Drug Conjugate)

*UV +  $\mu$ DAWN + UT-rEX, 4,6x300 mm UHPLC column*



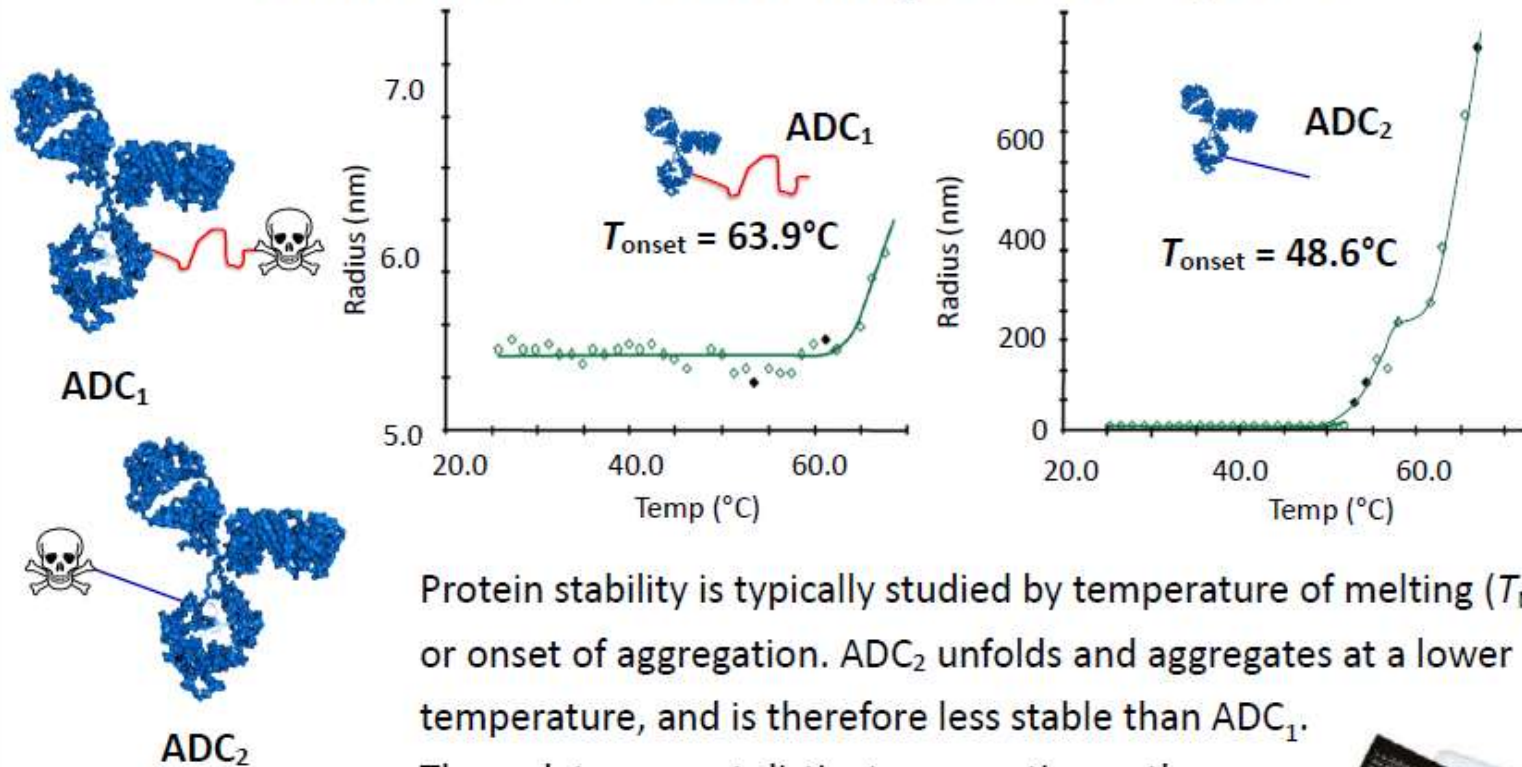
- Drug Antibody Ratio (DAR) measurements





# Stability of ADC (Antibody Drug Conjugate) as function of linker

## Linker-Induced Instability Studied by DLS



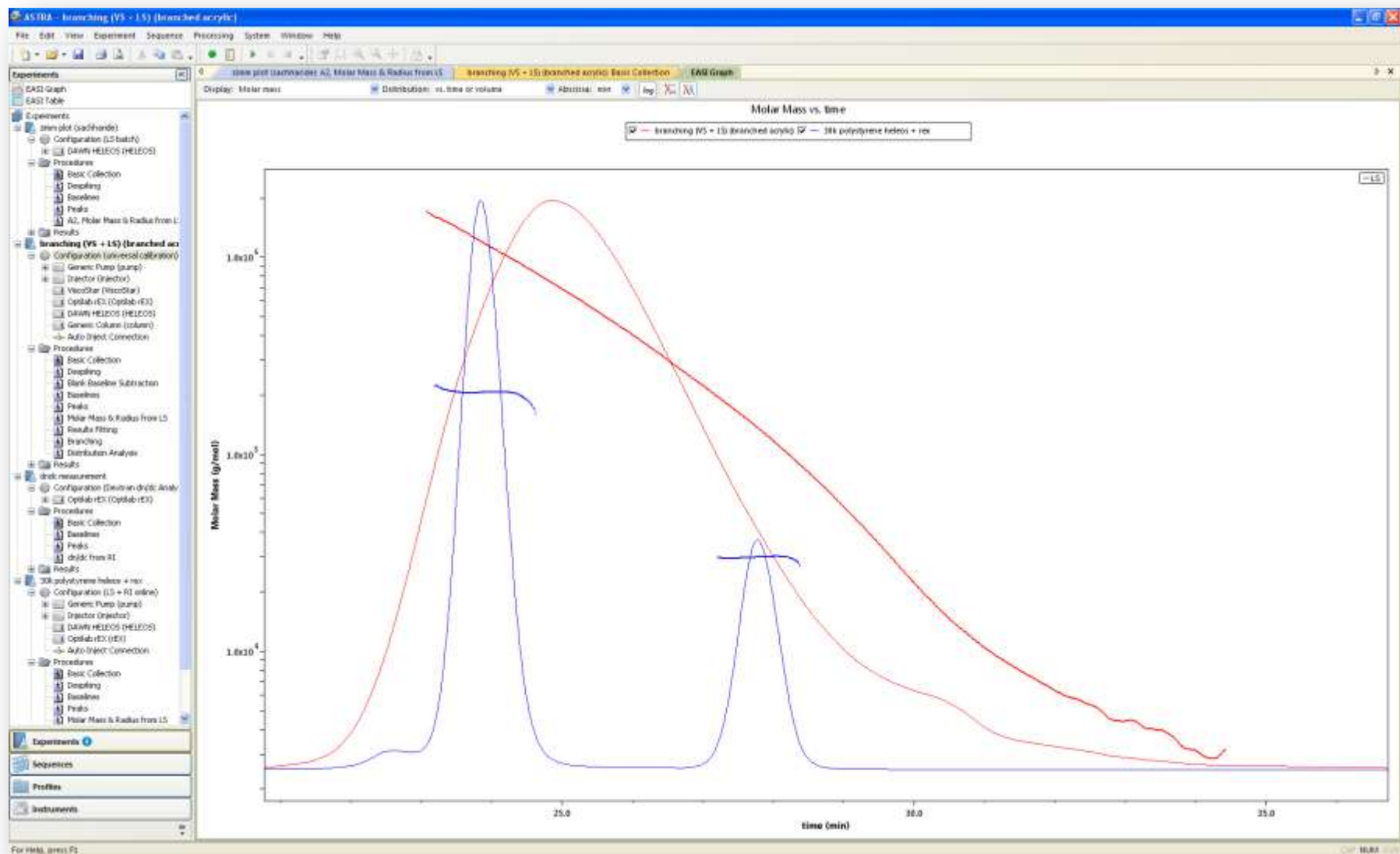
Identical mAb and drug,  
different linkers

Protein stability is typically studied by temperature of melting ( $T_M$ ) or onset of aggregation. ADC<sub>2</sub> unfolds and aggregates at a lower temperature, and is therefore less stable than ADC<sub>1</sub>. These data suggest distinct aggregation pathways.





# Astra Software



# Website



**24<sup>th</sup> ILSC** International Light Scattering Colloquium  
 Nov. 3 - 4, 2015 • Santa Barbara, CA  
 Light Scattering in the Nano World  
 Supporting Innovation in Chemistry, Biotechnology & Material Science

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## Dynamic & Electrophoretic Light Scattering

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**INSTRUMENTS**

- Instrument Overview
- MALS
- DAWN HELEOS II
- miniDAWN TREOS
- eDAWN
- DLS & ELS**
- DynaPro Plate Reader II
- DynaPro NanoStar
- MöbiuS
- RelaxoSizer

### DynaPro Plate Reader II

**High-throughput, automated dynamic light scattering.**

The DynaPro Plate Reader II provides unparalleled levels of productivity and flexibility for the most demanding nanoparticle sizing and protein aggregation tasks such as biotherapeutic formulation, screening of promiscuous inhibitors, and optimization of protein crystallization conditions. Measurements are performed *in situ* with no fluid transfer. Select from industry-standard 96-, 384- or 1536-well plates.



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- REFERENCES
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- By Technique**
- SEC-MALS
- FFF-MALS
- CG-MALS
- Batch MALS
- DLS
- ELS
- By Application
- Biotherapeutics
- Proteins
- Biopolymers

**Enter our Application Note Contest for a chance to win a free trip to ILSC!**

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### SEC-MALS

Absolute molar mass and size distributions of macromolecules in solution, combining multi-angle static light scattering (MALS) with size exclusion chromatography (SEC).

### FFF-MALS

Separation and characterization of macromolecules and nanoparticles without chromatographic columns, using the Eclipse Field-Flow Fractionation systems.

### CG-MALS

Biomolecular interactions using the Calypso Composition-Gradient system.



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Why not submit your publication citing a Wyatt DAWN, miniDAWN, Opolab, Eclipse, NanoStar, ViscoStar, DynaPro Plate Reader, Calypso or MöbiuS? Help your colleagues know where to go for the most reliable characterization of macromolecules and nanoparticles in solution! As a token of our appreciation, we'll send you a Wyatt laser pointer, magic mug or t-shirt. E-mail us at [publications@wyatt.com](mailto:publications@wyatt.com).

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Aggregation, stability, molar mass and size distributions of therapeutic biomolecules.

### Proteins

Oligomeric states, protein conjugates, aggregation, colloidal stability and protein-protein interactions.

### Nanoparticles

Size, composition, mass and solution behavior of nanoparticles.

### Characterizing Protein Conjugates and Their Aggregates by Light Scattering

Michelle H. Chen, Ph.D.  
Wyatt Technology Corporation  
www.wyatt.com

### Field Flow Fractionation Combined with Multi-Angle Light Scattering

### Combining Size and Molar Mass Measurements of Protein Solutions and Biomolecules

### High-Throughput Dynamic Light Scattering Using the DynaPro Plate Reader

### Wyatt Technology

Demystifying Light Scattering with Dr. Phillip Wyatt  
Part 2: Multiangle Light Scattering Combined with Fractionation

### The Möbius

Measuring Electrophoretic Mobility, Charge and Zeta ( $\zeta$ -) Potential of Proteins, Biomolecules and Nanoparticles



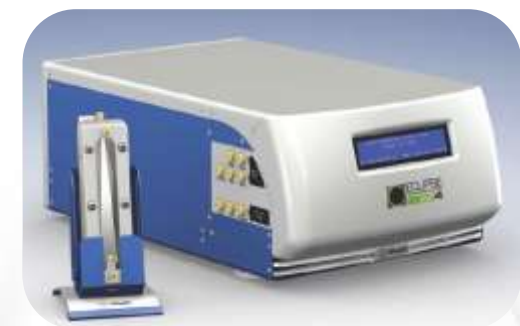
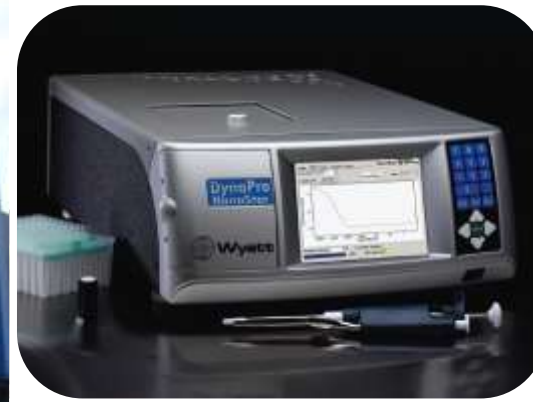
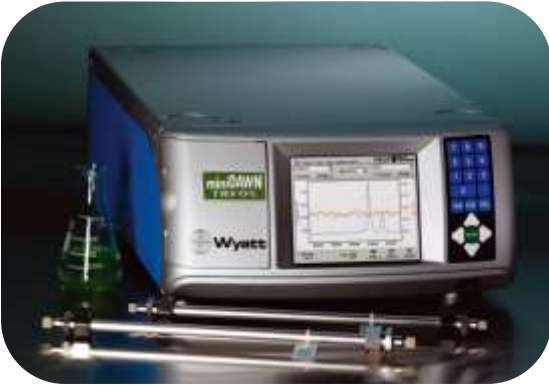
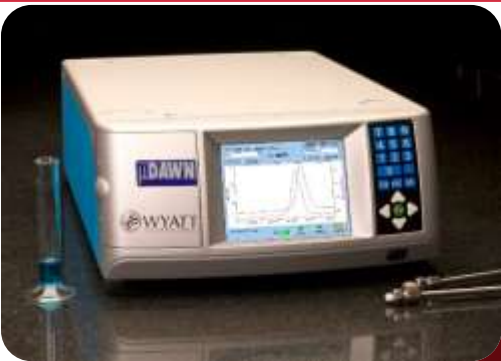
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Demystifying Light Scattering with Dr. Phillip Wyatt  
Part 1: A Brief Introduction to Light Scattering



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**Merci de votre attention**  
**Rendez-vous sur le stand pour les**  
**questions?**

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