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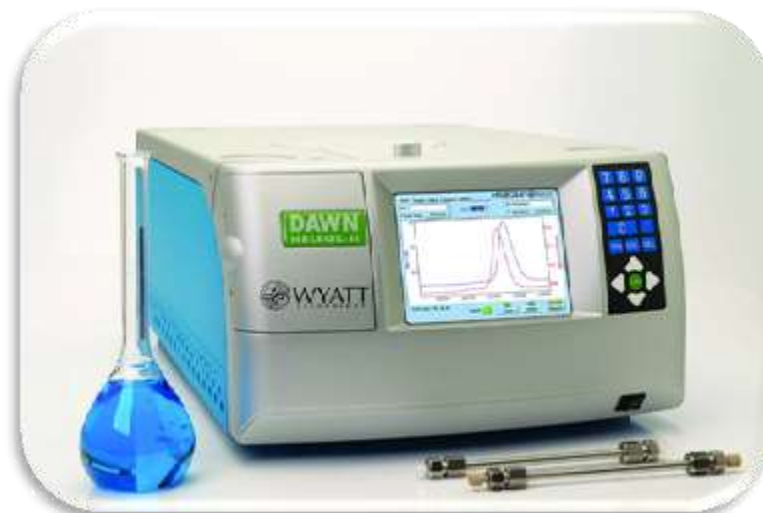
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# The light scattering toolbox for the characterization of proteins, peptides, virus and others bio-macromolecules and nanoparticles

Nicolas Mignard  
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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°C to +65°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°C to +65°C

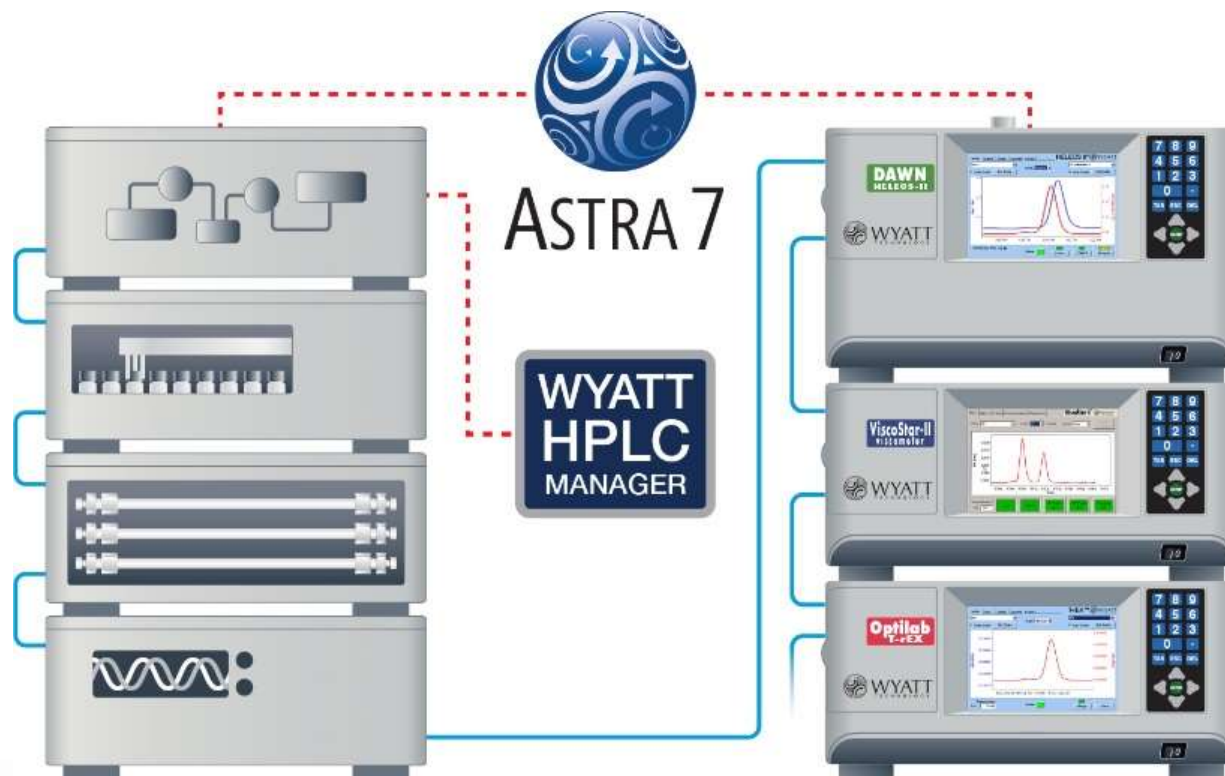


**Optilab TrEX**



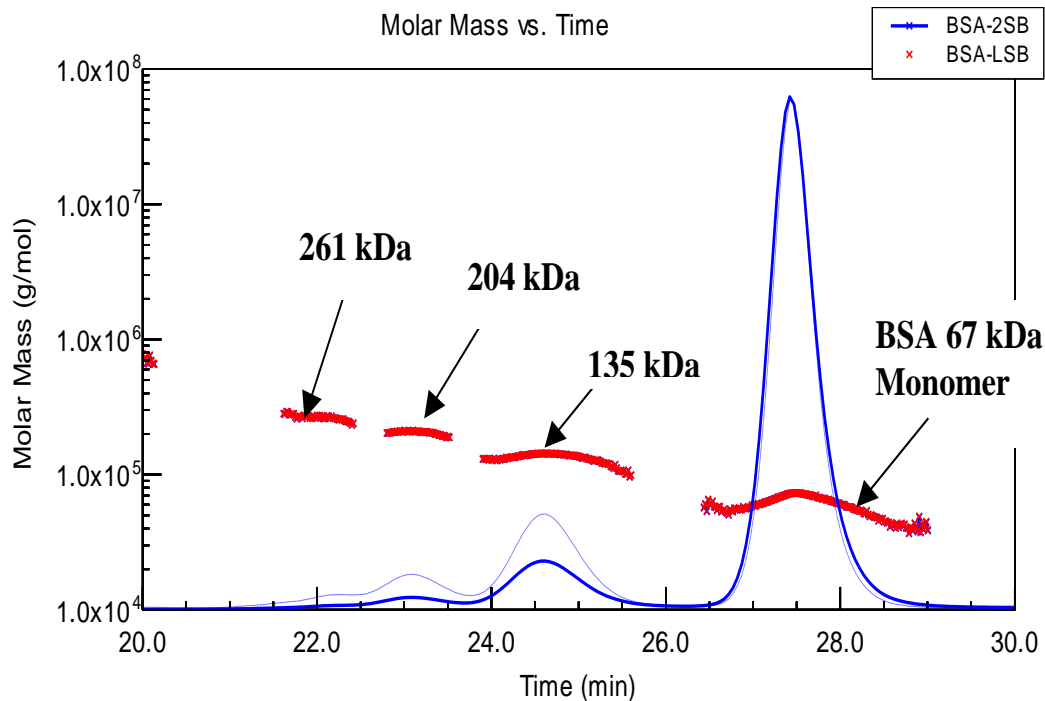
**ViscoStar II**

# On any HPLC/FPLC systems with our Astra 7 software



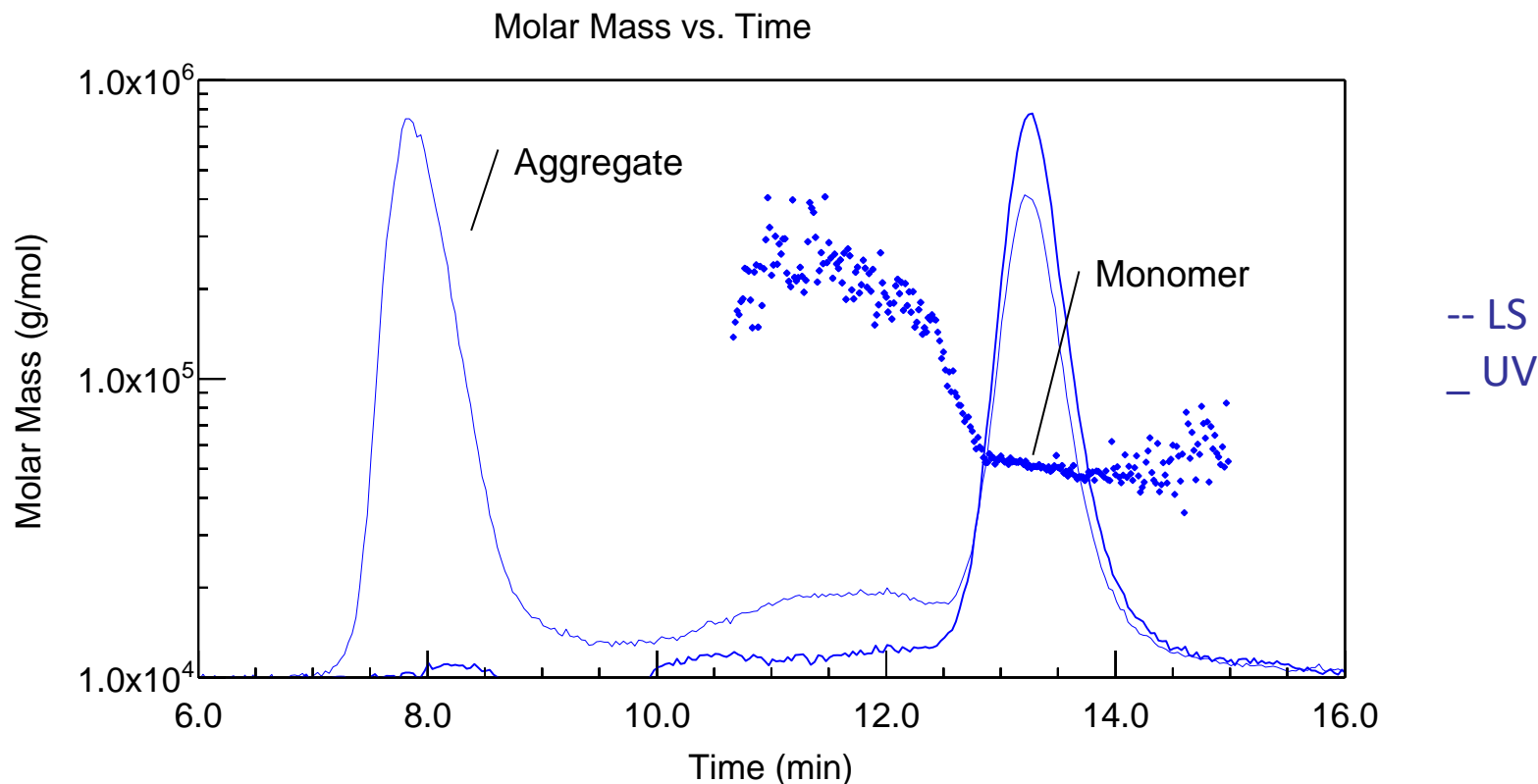
# Oligomer „hunting“

- 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)

## Setup with UHPLC/UPLC-SEC

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

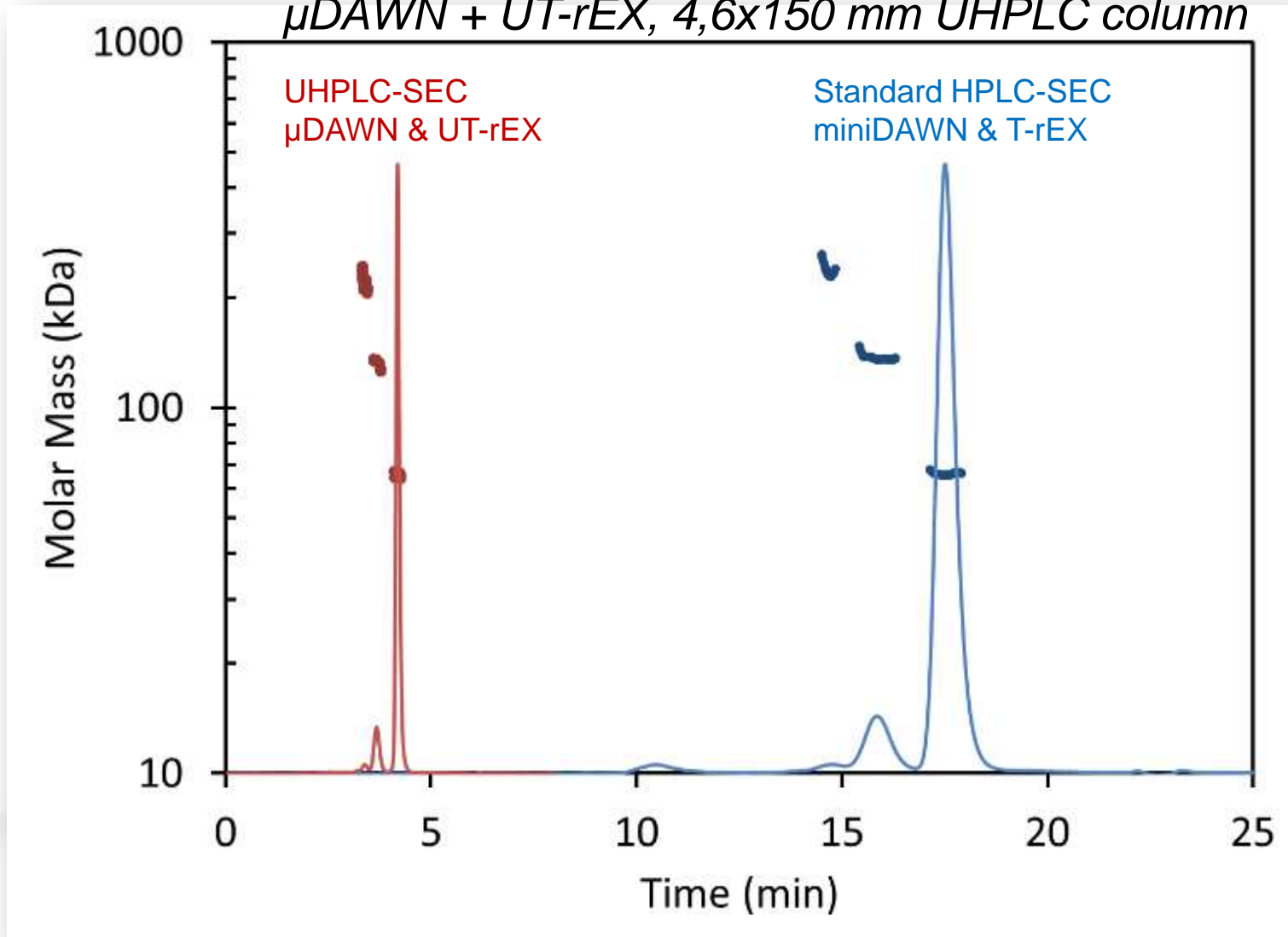


$\mu$ DAWN (3 angles + QELS)

Optilab UTrEX

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

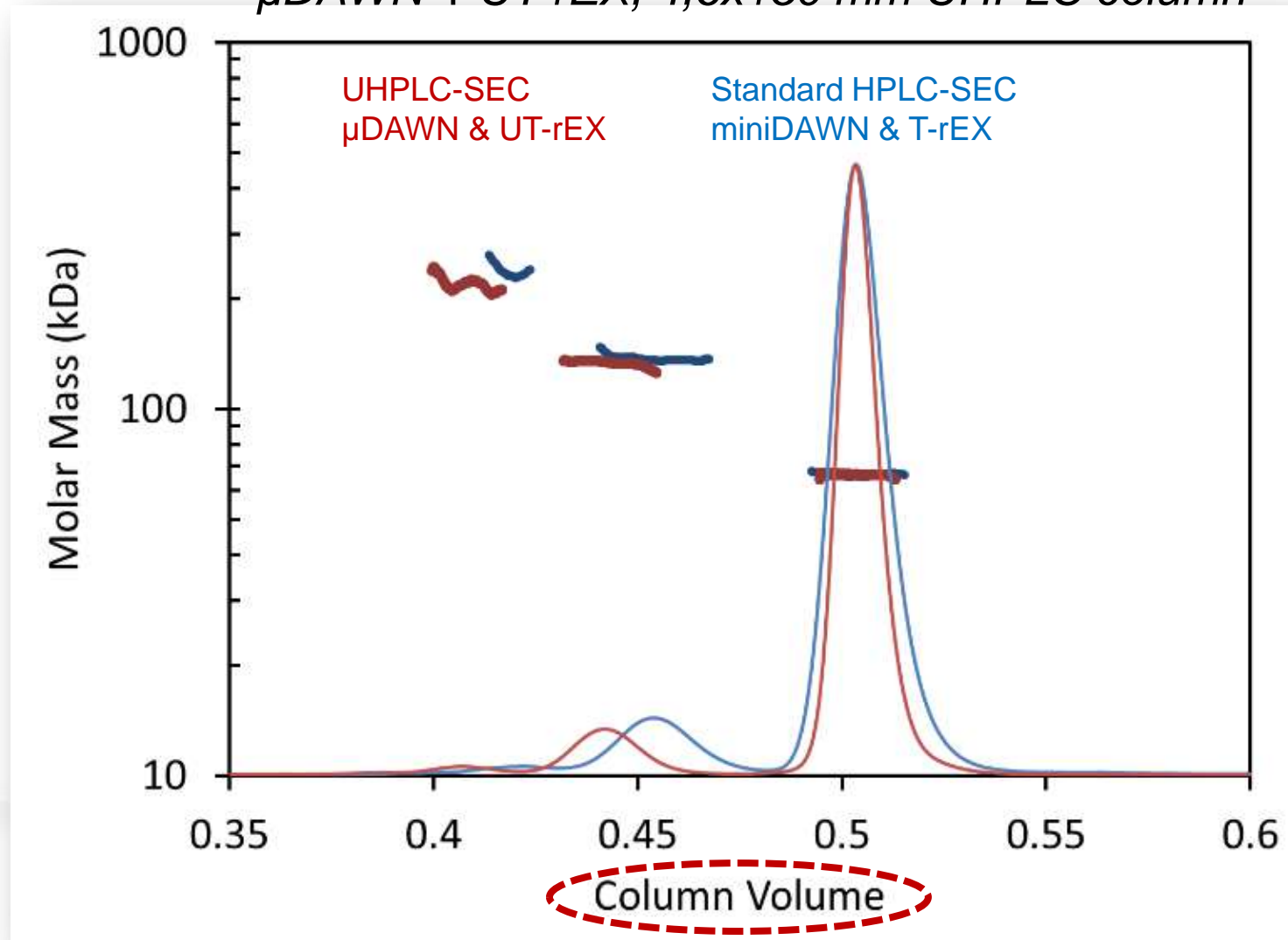
$\mu$ DAWN + UT-rEX, 4,6x150 mm UHPLC column





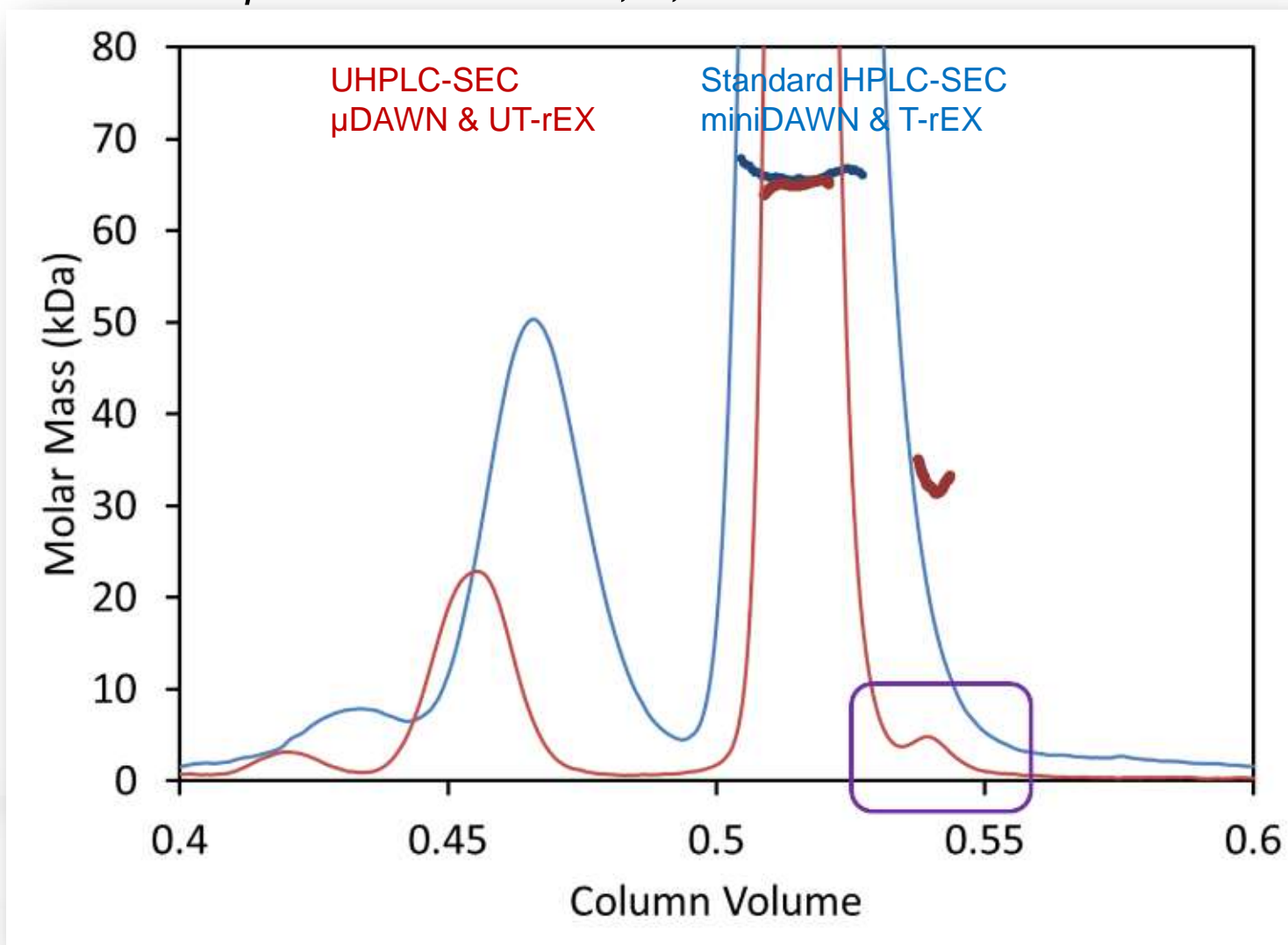
# Comparison $\mu$ SEC-MALS with „classical“ SEC-MALS

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



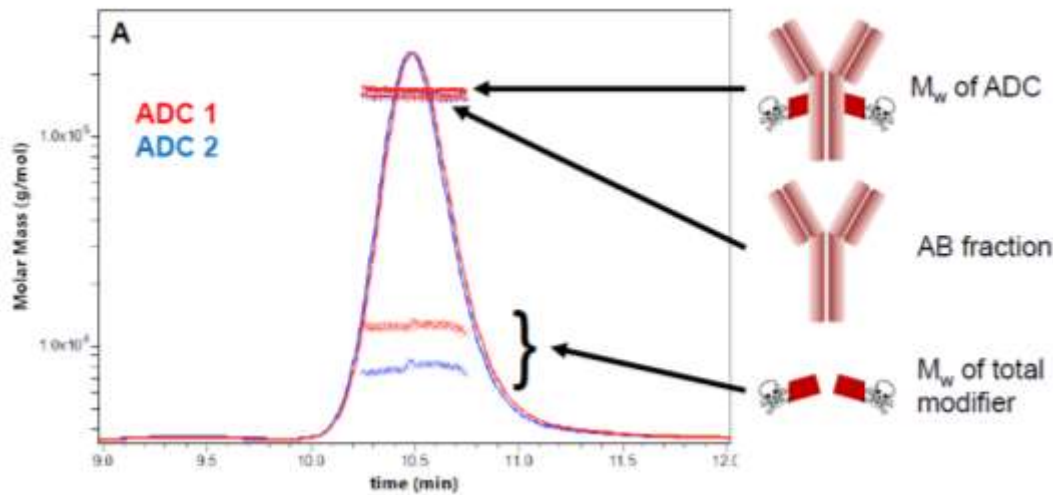
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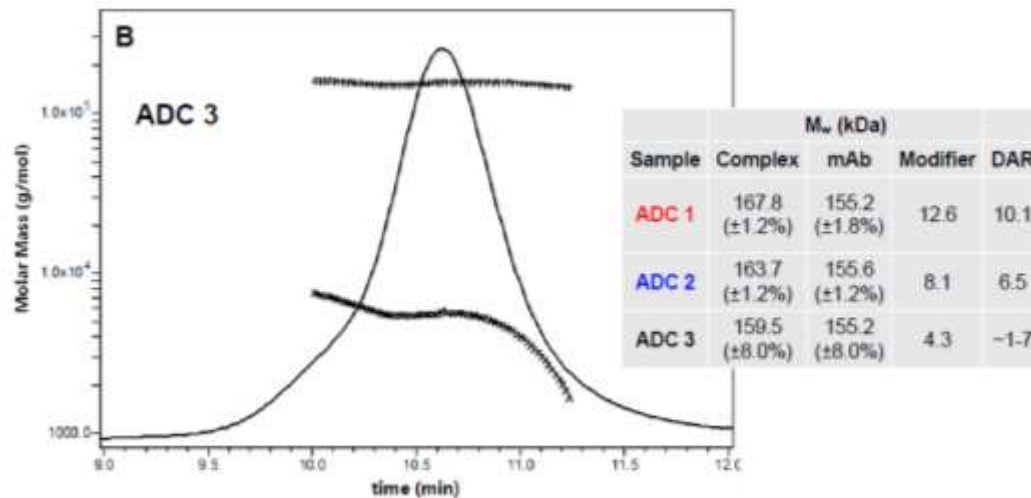


# Proteins conjugates analysis: ADC (Antibody Drug Conjugate)

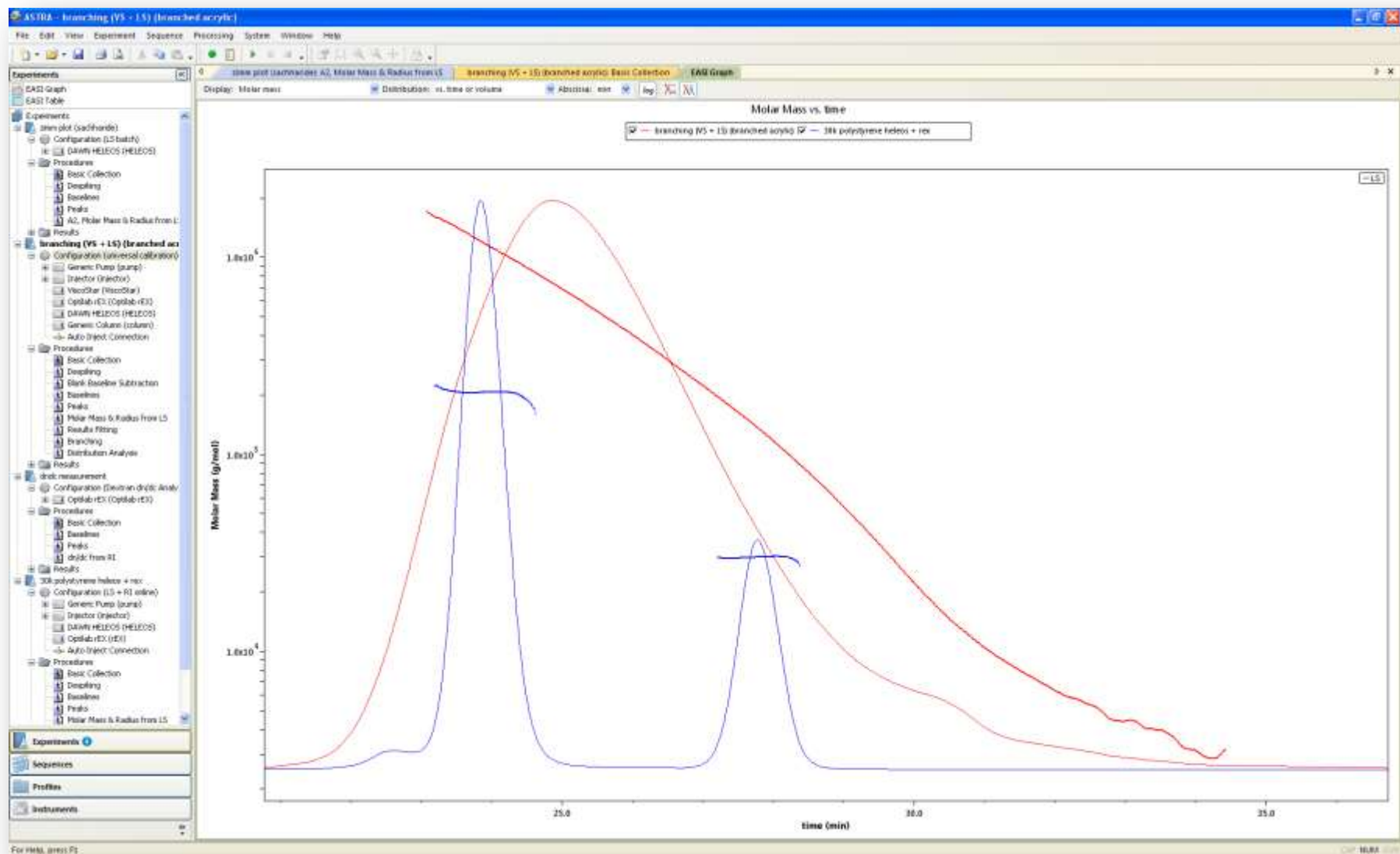
*UV +  $\mu$ DAWN + UT-rEX, 4,6x300 mm UHPLC column*



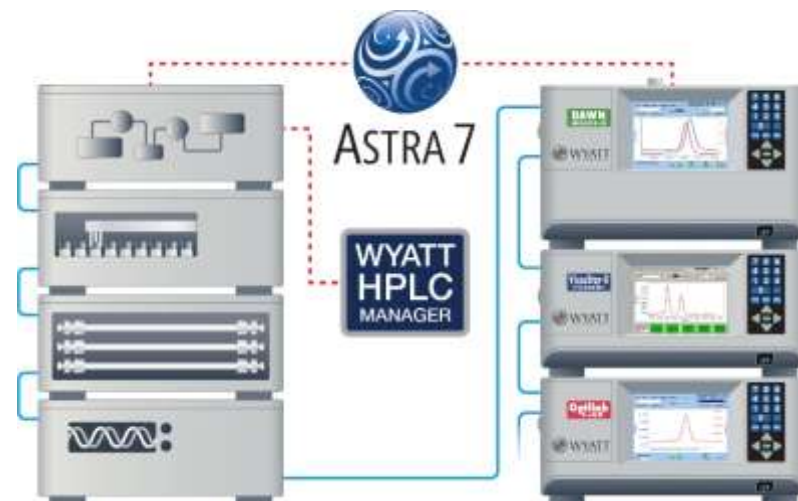
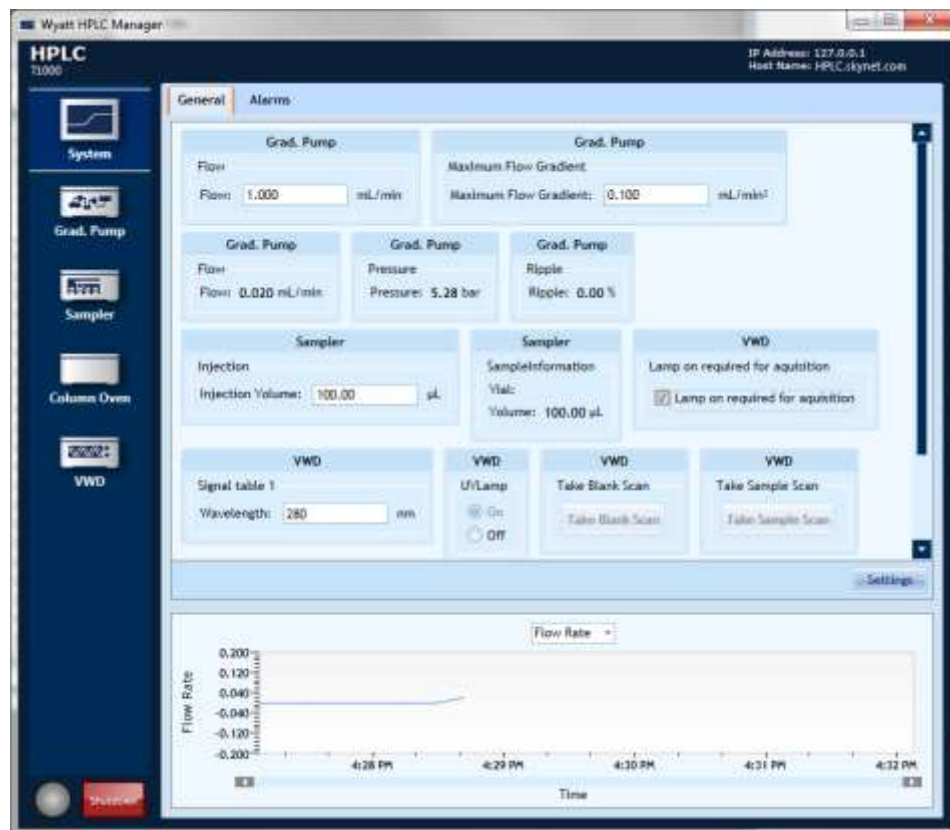
- Drug Antibody Ratio (DAR) measurements



# Astra Software

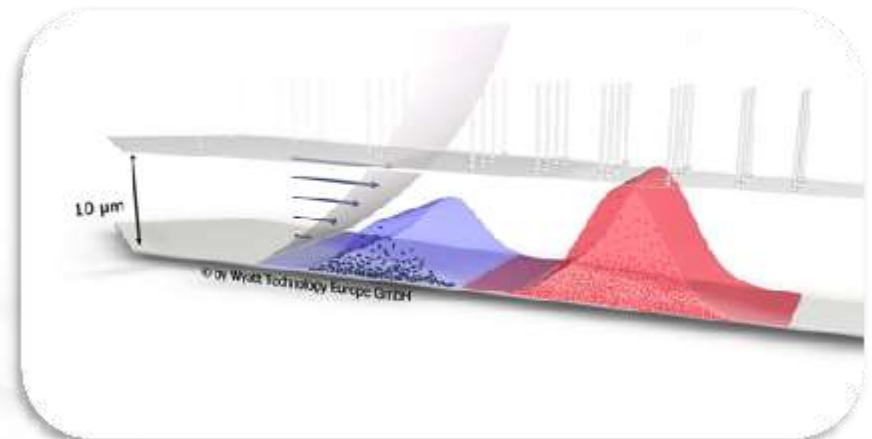
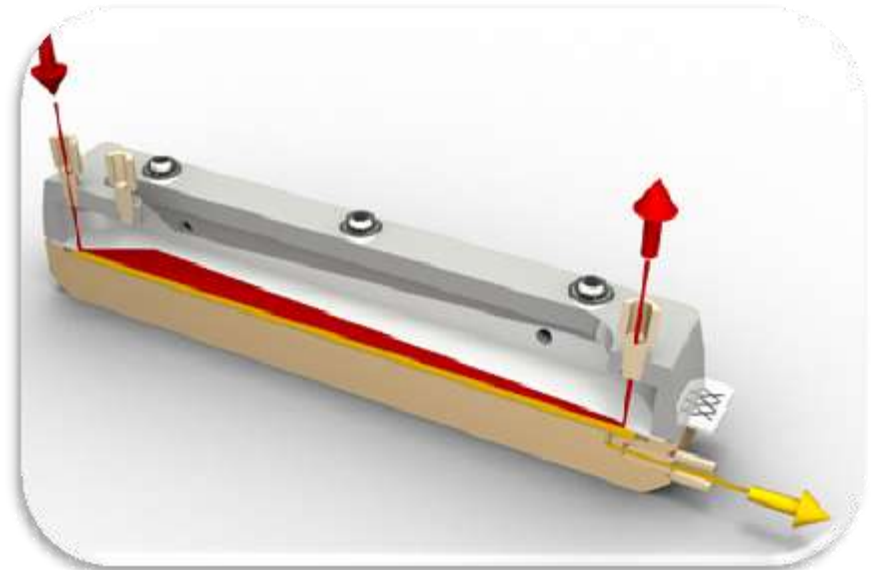


# Astra Software: HPLC manager module

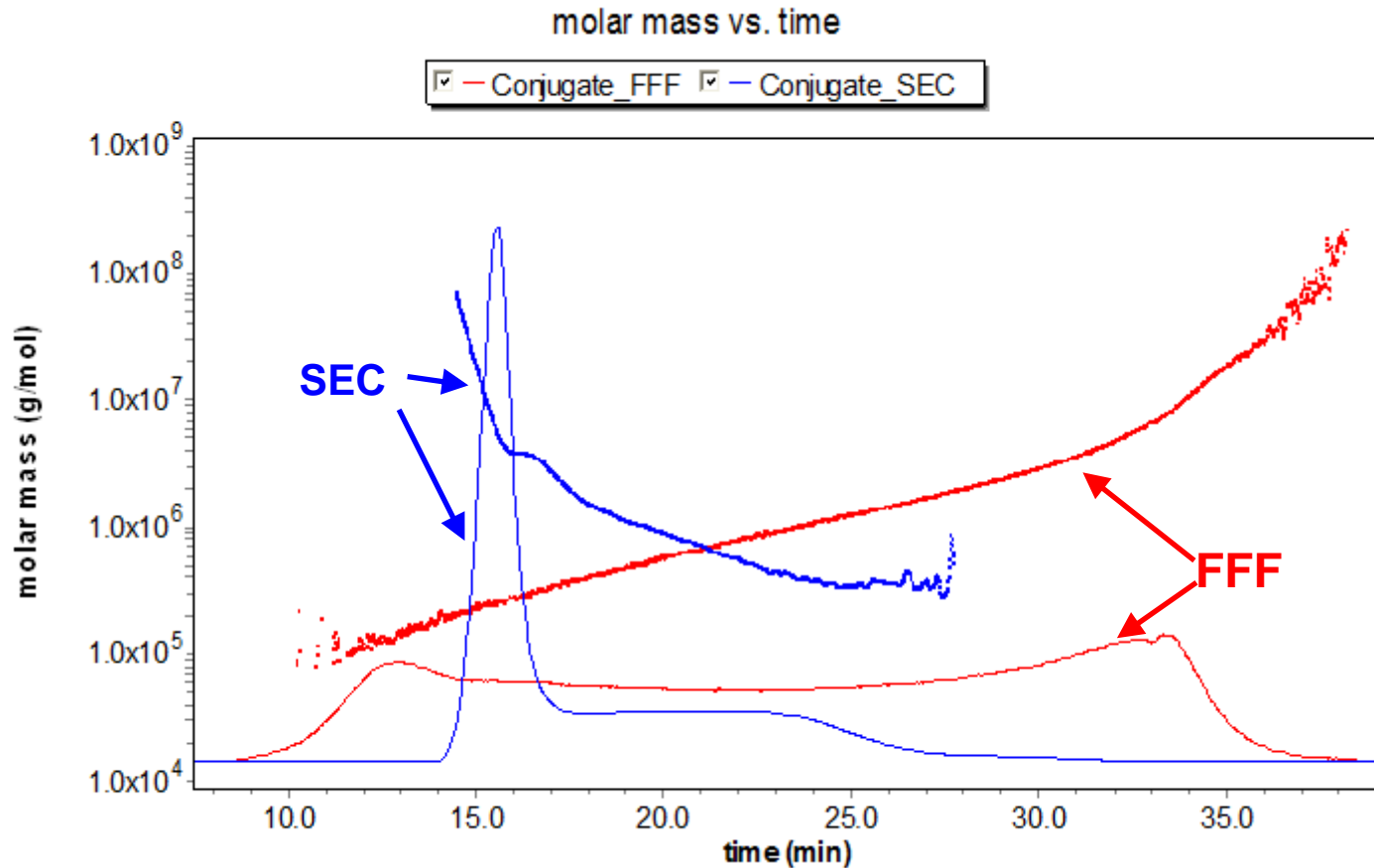


\*Currently only available for Agilent HPLC systems

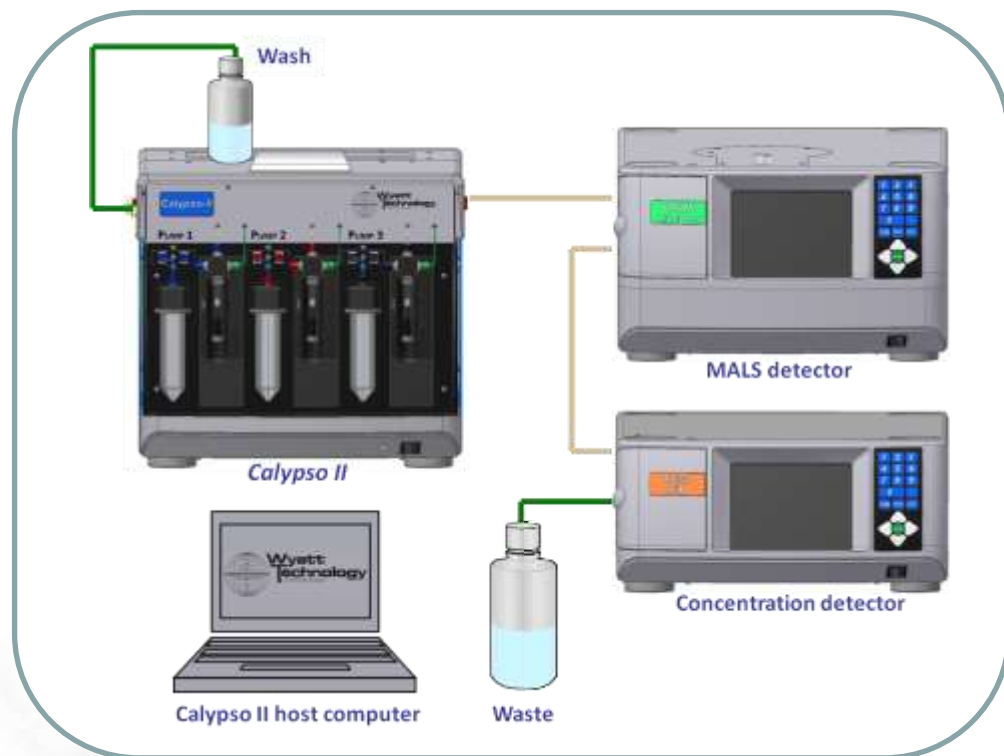
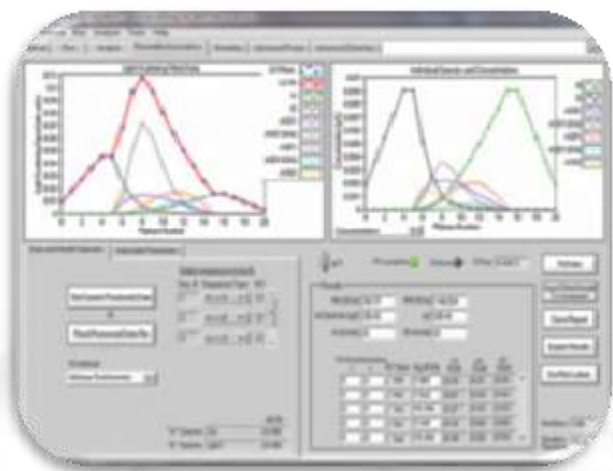
# Field Flow Fractionation (A4F, HF5, ...): Separation without column as function of the hydrodynamic volume of the species



# Field Flow Fractionation (A4F, HF5, ...): Separation without column as function of the hydrodynamic volume of the species



# Calypso: Label-free, immobilization-free characterization of protein-protein and other macromolecular interactions with composition-gradient multi-angle light scattering.



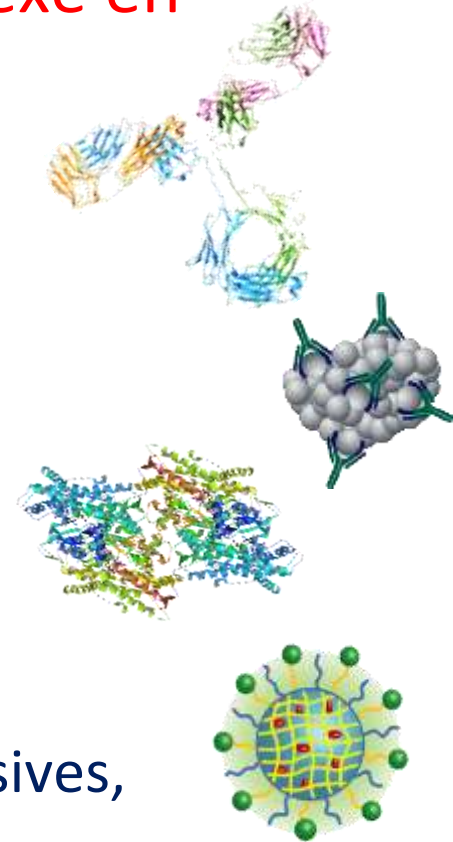


CG : Gradient automatisé, mesures à débit nul ("stopped flow")  
 MALS : mesure de  $M_w$ , dépendant de la composition → Interactions

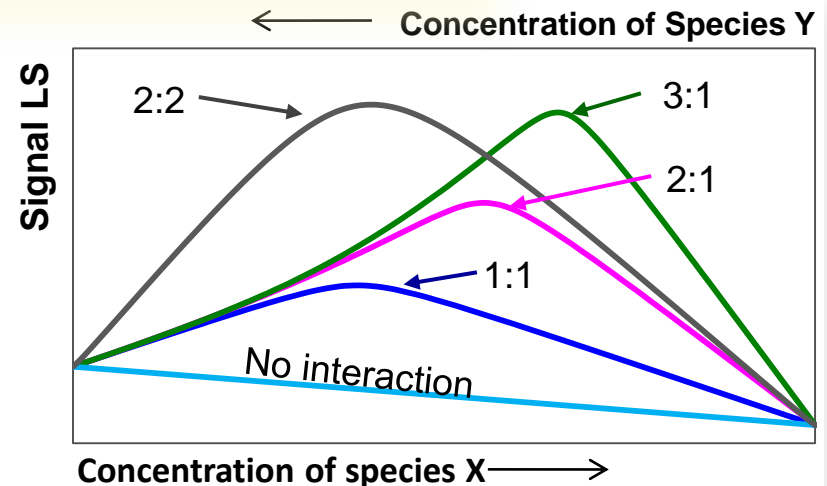
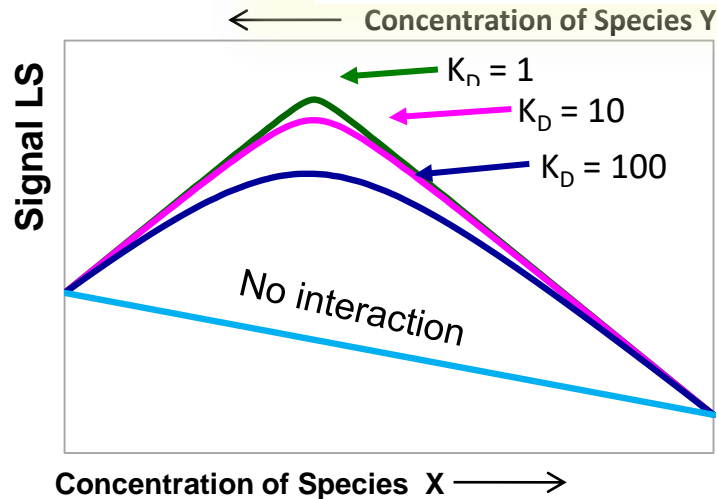
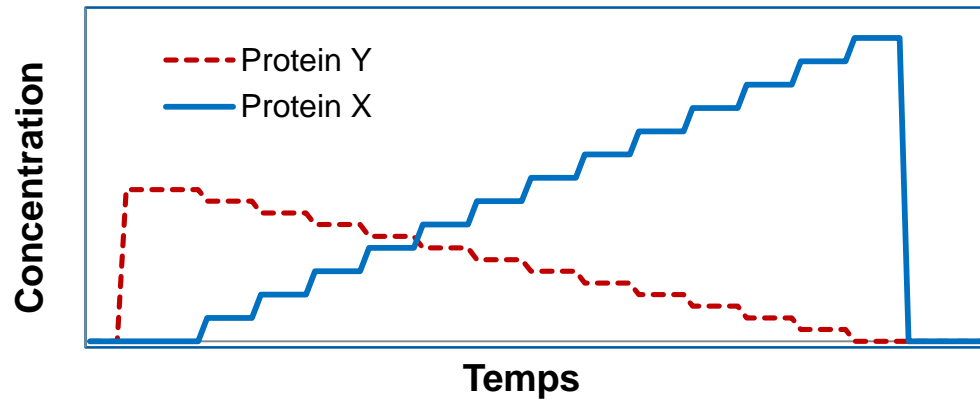
## Une mesure *directe* de la formation du complexe en solution, sans marquage ni immobilisation

Pratiquement pas de développement de méthode,  
 de consommables et de préparations de l'échantillon  
 Complémentaire et orthogonal aux autres méthodes

- Interactions spécifiques / équilibres réversibles: affinité de liaison,  $K_D$  de  $\sim 100$  pM à qq mM
- Stœchiométrie absolue (1:1  $\neq$  2:2)
- Équilibres et cinétiques
- Interactions non-spécifiques: attractives ou répulsives, coefficient du viriel  $A_2$  ("self") et  $A_{11}$  ("cross")

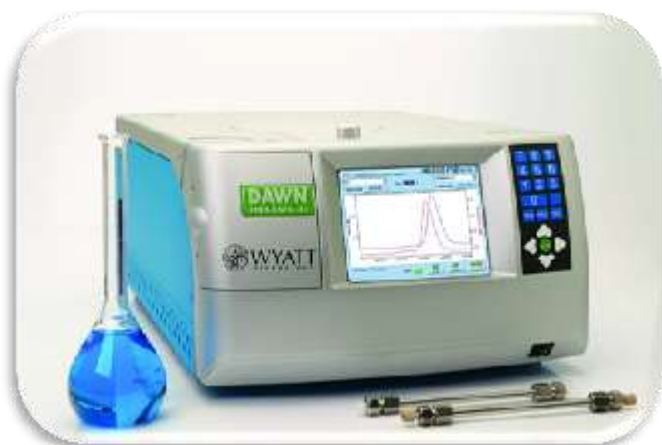


# Calypso: Label-free, immobilization-free characterization of protein-protein and other macromolecular interactions with composition-gradient multi-angle light scattering.



Shape and height of LS signal is function of  $K_D$  and stoichiometry

## Batch analysis: DLS & SLS



**DAWN Heleos II (17 angles)**  
with WyattQELS



**DynaPro Plate Reader II**



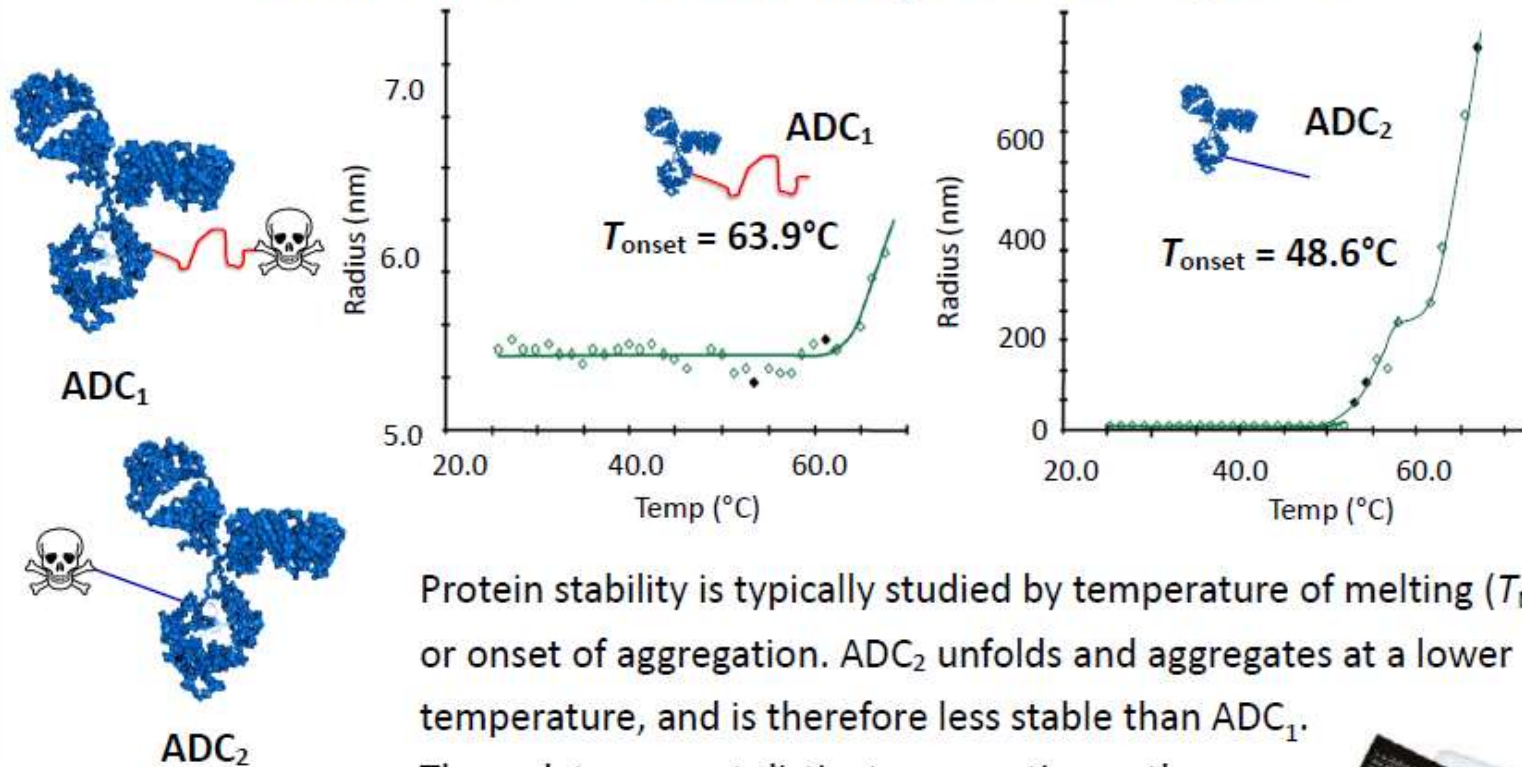
**DynaPro NanoStar**  
Single quartz or disposable microcuvette



**Möbius**  
Size & zeta potential

# 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.



# Website



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with ASTRA 7's new HPLC Control Module. Control a complete Wyatt/Agilent SEC-MALS setup within ASTRA!

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MALS and DLS: Multi-angle and dynamic light scattering instruments for absolute molecular weight, size and more

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- Molar Mass
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- Charge

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

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

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Part 2: Multiangle Light Scattering Combined with Fractionation

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

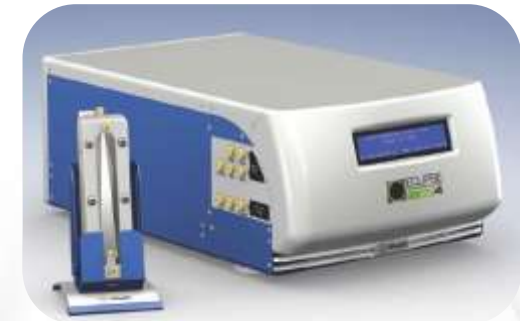
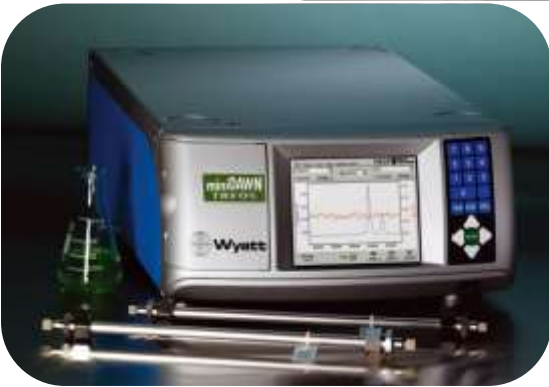
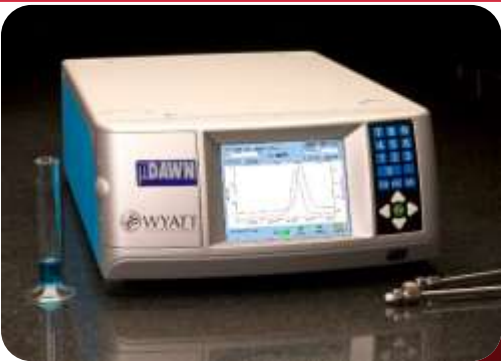


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Demystifying Light Scattering with Dr. Phillip Wyatt  
Part 1: A Brief Introduction to Light Scattering



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

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