WYATT TECHNOLOGY

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

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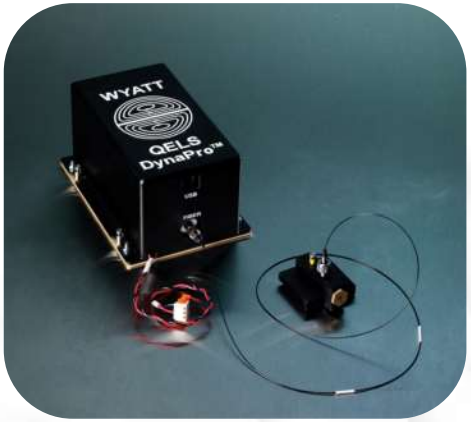
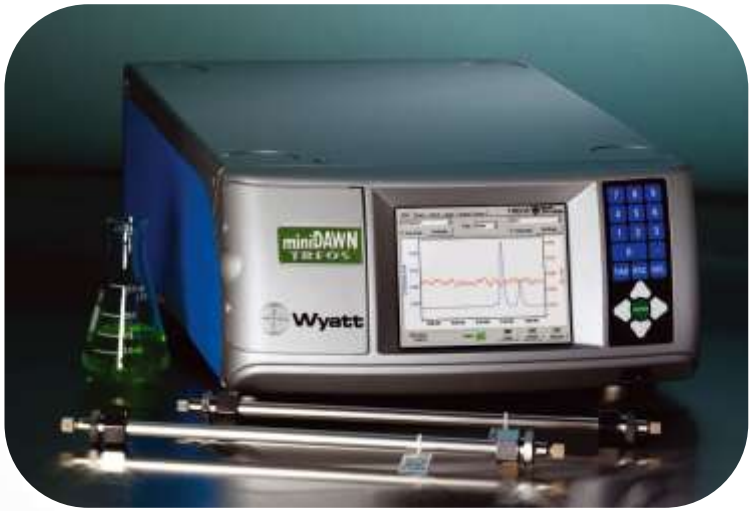
Multiple Angle Light Scattering

- Mz, Mw, Mn (= absolute molar masses)
- RMS radius (= radius of gyration)
- Rh (= hydrodynamic radius) if QELS
- Conformation
- Branching
- Online or in batch mode
- Stoechiometry 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

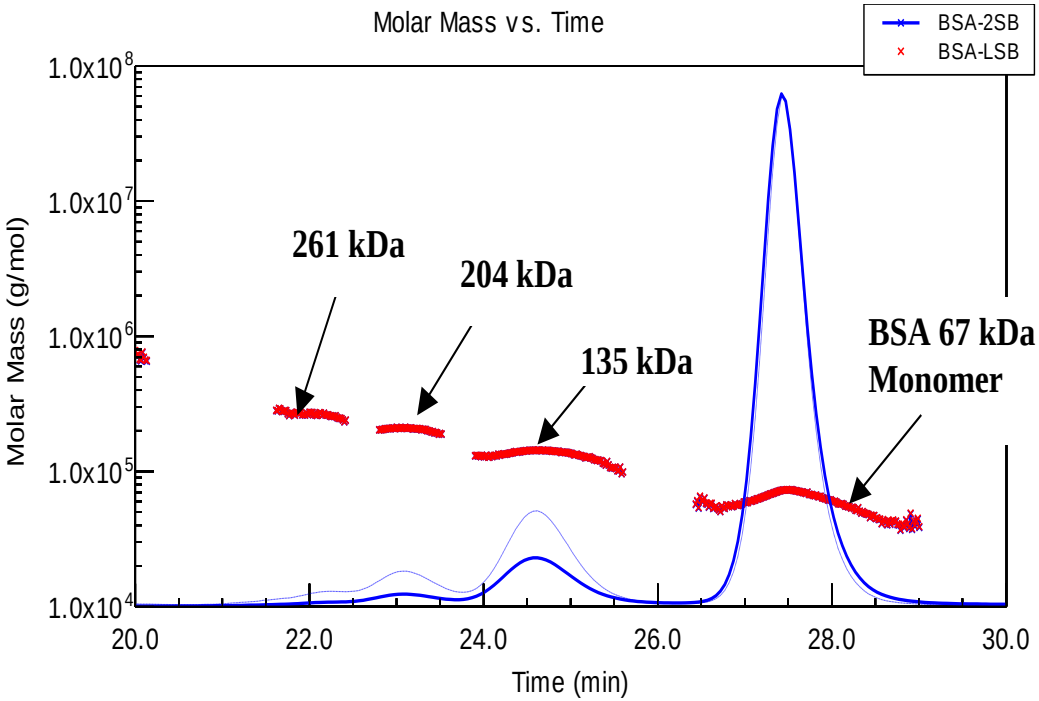
Optilab TrEX

- Ƞ (= Intrinsec viscosity)
- K & a (=Mark-Houwink-Sakurada coefficients)
- Rh_v (= hydrodynamic radius from visco)
- Temp control +4°C to +65°C

ViscoStar II

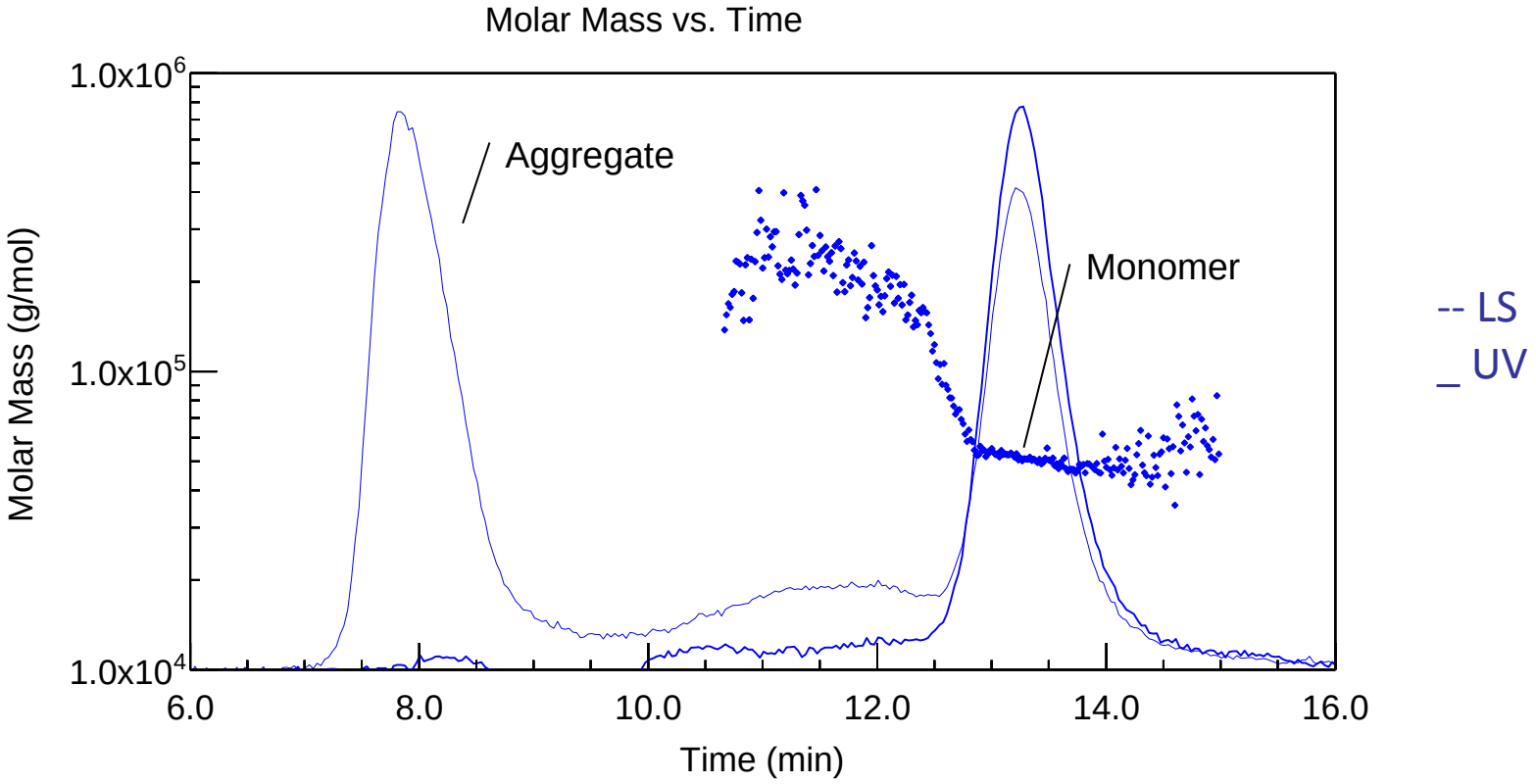
Oligomer „hunting“

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



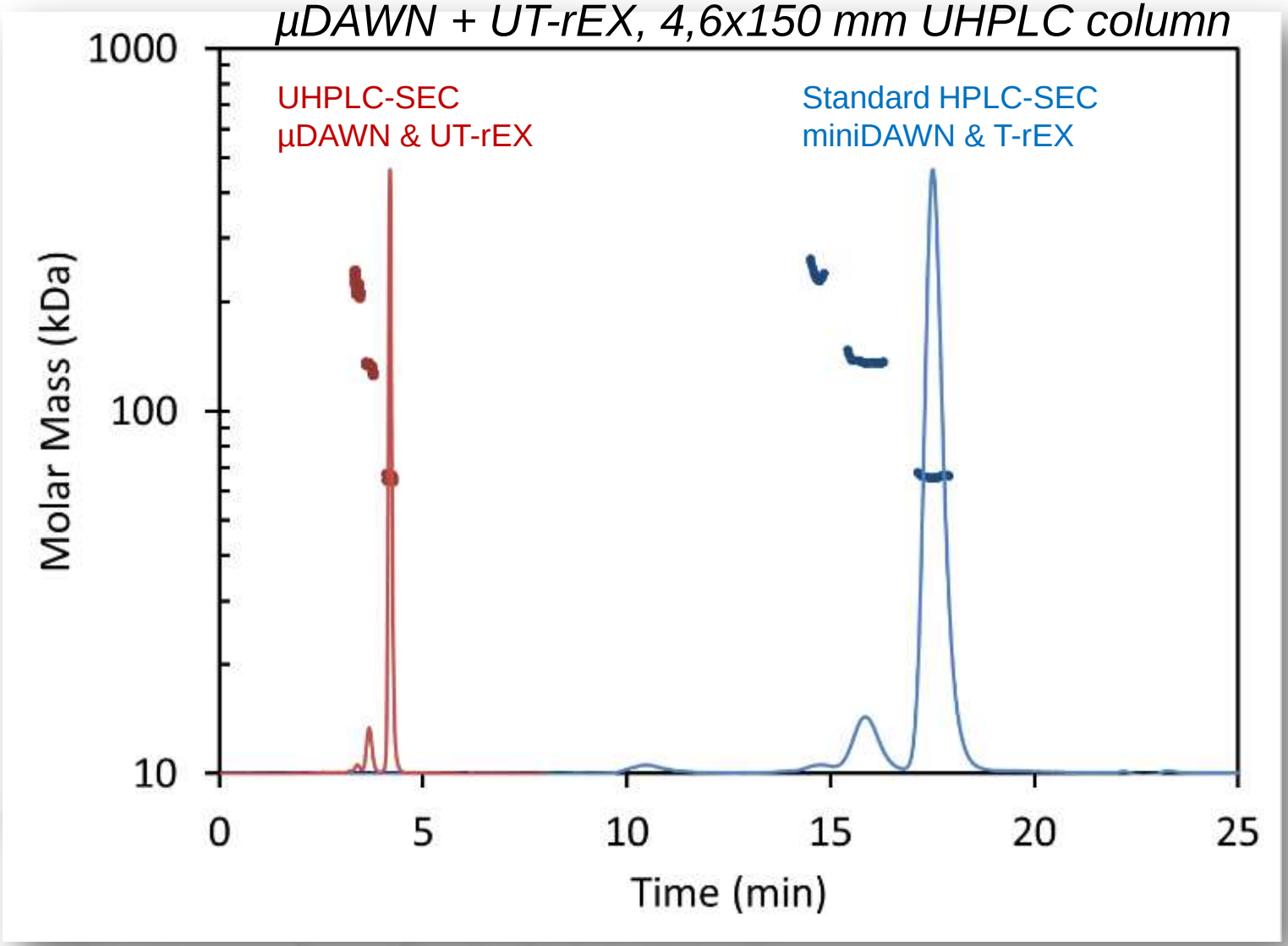
Aggregates	%	Molar Mass [kDa]
Monomer	**92.4**	**66.8**
Dimer	**6.65**	**135**
Trimer	**0.95**	**204**
Tetramer	**0.53**	**263**

Aggregates detection



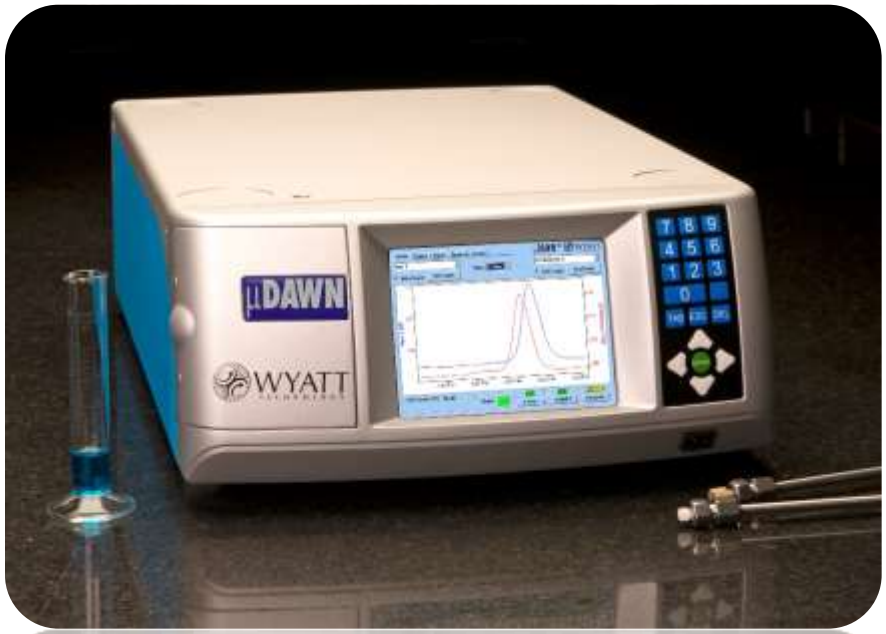
- 6 µg of aggregates found by MALS
- Molar mass of the main peak is measured at 50 kDa
- The protein is with a majority of monomer (theoretical molar mass value is 47.8kD)

Comparison of µSEC-MALS with „classical“ SEC-MALS



µDAWN

- 3 angles at 45°, 90° et 135°
- Laser of 60 mW at 658 nm
- Molar masses from 200 to ~10^7 g/mol
- Sizes: Rg = ~ 10 nm à ~ 100 nm
  Rh= 1nm to 50 nm if QELS
- Cell volume : < 10 µL
- Dispersion: UV-MALS < 2 µL; MALS-RI < 7µL
- Resolution: 22+ bits (digital), 18 bits (analogue)
- 36 Hz
- Precision ~2 - 4 %
- Repetability ~1%



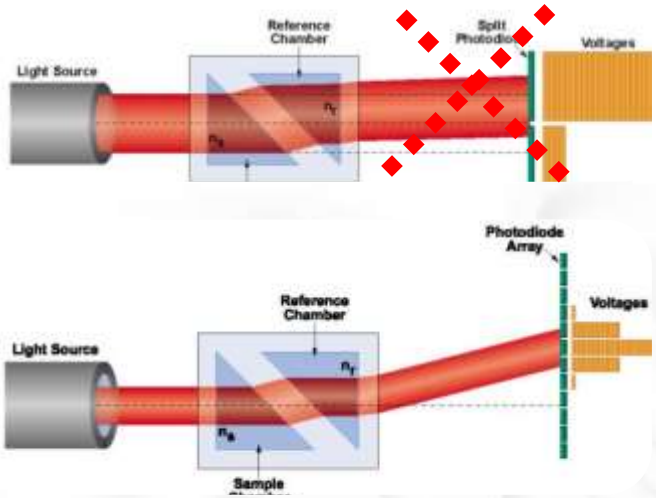
New COMET: online ultrasonic cleaning tool for the measurement cell

Optilab UTrEX

- Differential refractometer at 658 nm
- 512 photodiodes: dynamique range x50
- Absolute refractive index measurement
- dn/dc measurement
- Same wavelength than µDAWN
- Precalibration in factory

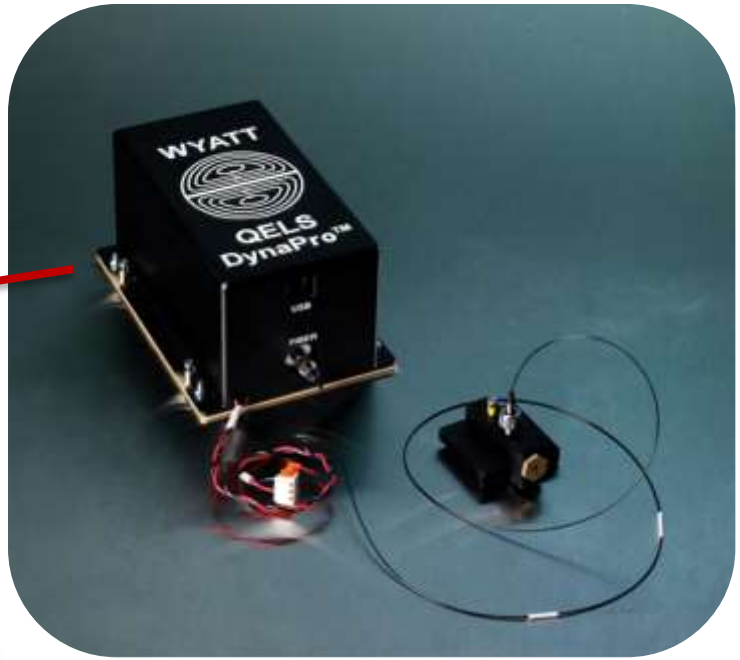
- Range: 1,2 – 1,8 RIU
- Sensibility: 0,002 RIU
- Cell volume < 10 µL
- Temperature range: +4° / +65°C (±0.005 °C)

(below +20°C, nitrogen is required to avoid condensation)



Adding Dynamic Light Scattering : Wyatt QELS

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

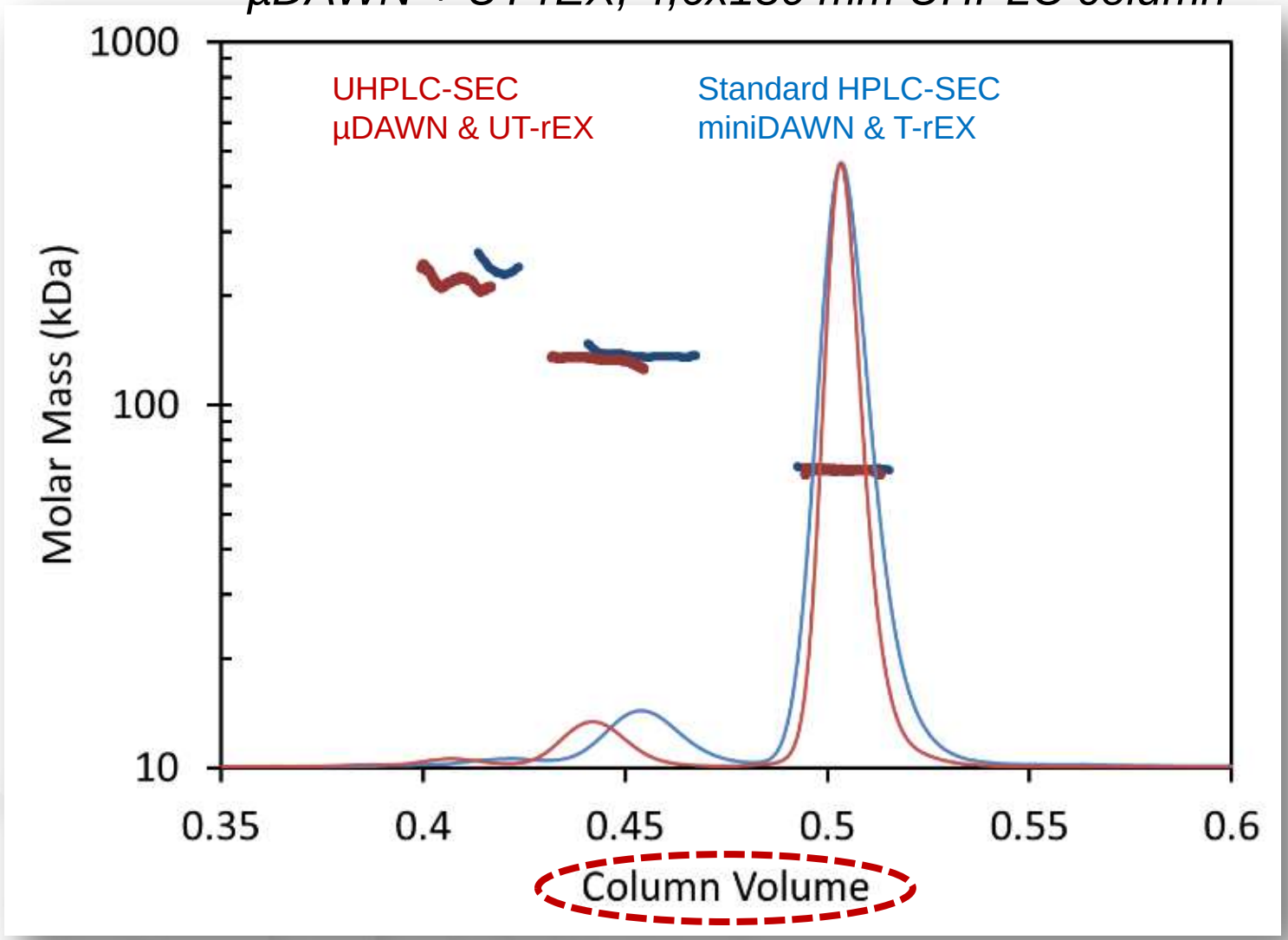


Setup

Waters H-Class Bio Inert avec UV (DAD) - µDAWN - Optilab UTrEX

Comparison µSEC-MALS with „classical“ SEC-MALS

µDAWN + UT-rEX, 4,6x150 mm UHPLC column



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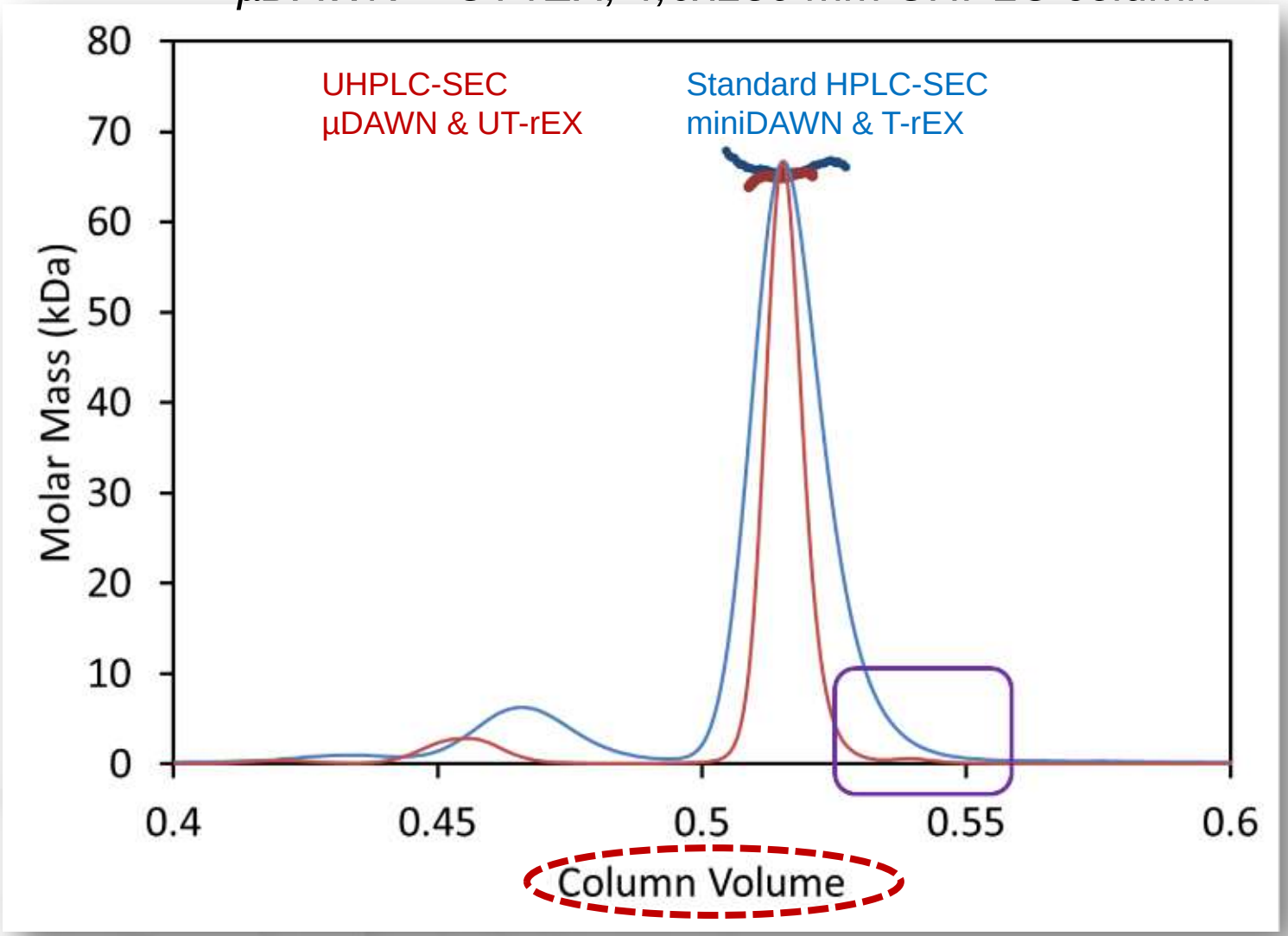

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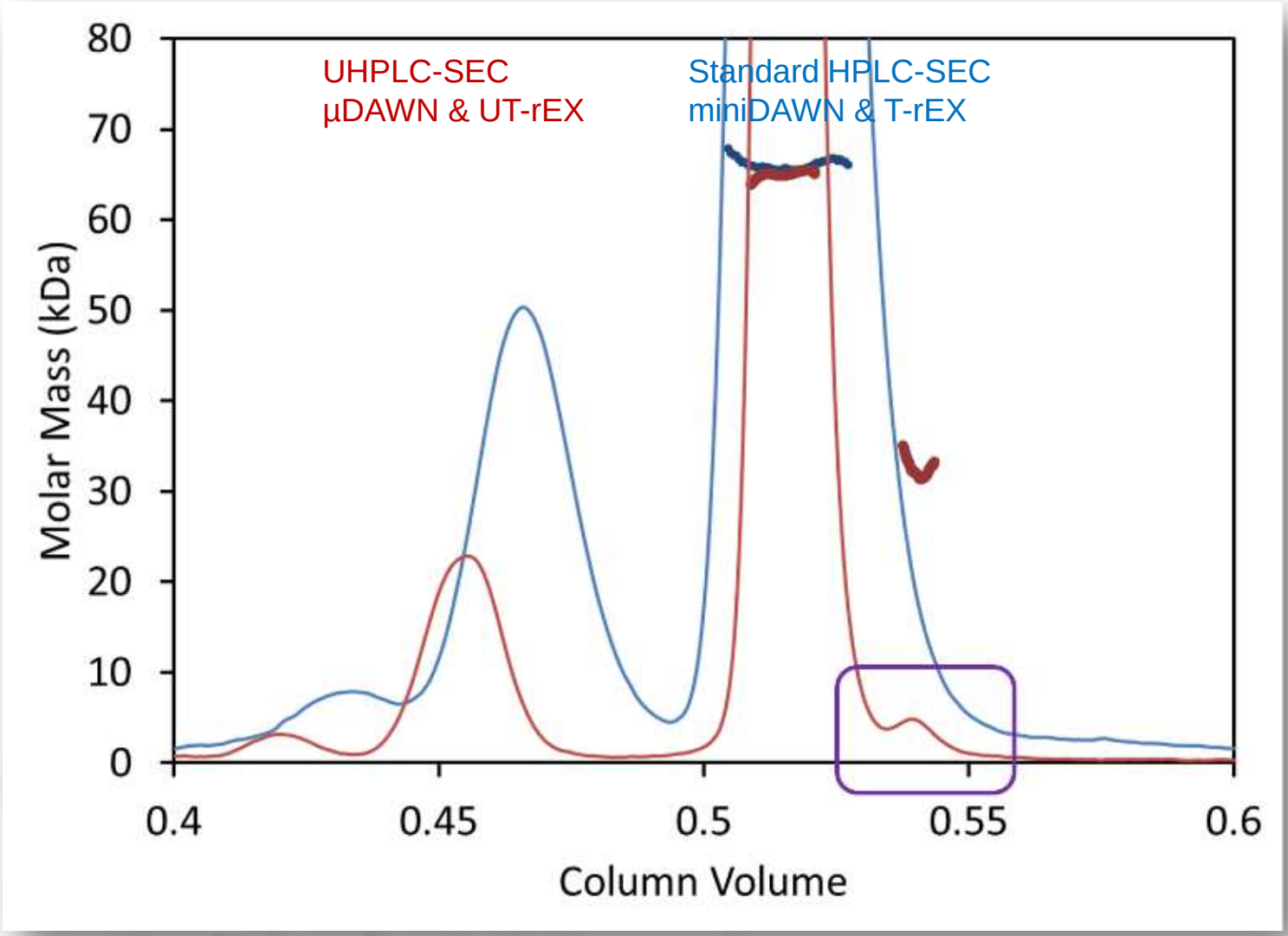
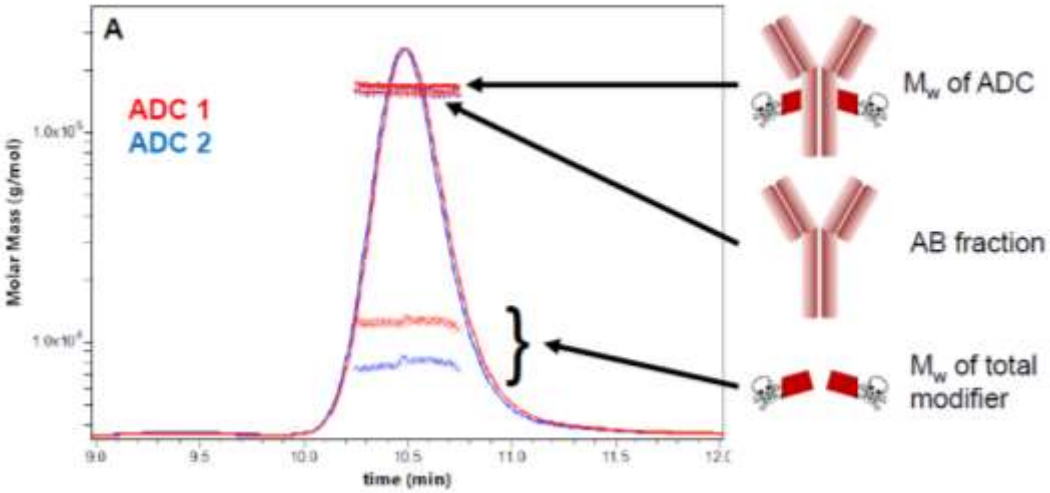
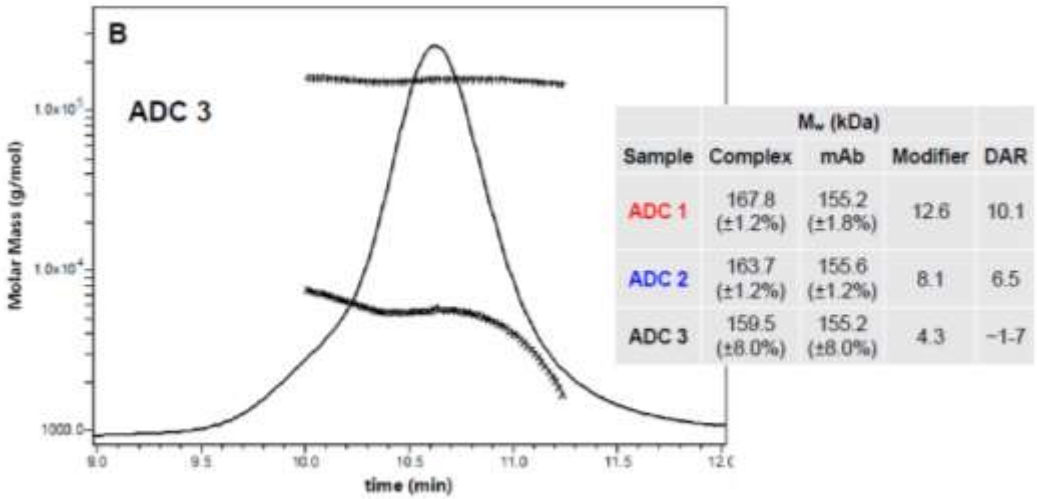


Proteins conjugates analysis: ADC (Antibody Drug Conjugate)

UV + µDAWN + UT-rEX, 4,6x300 mm UHPLC column



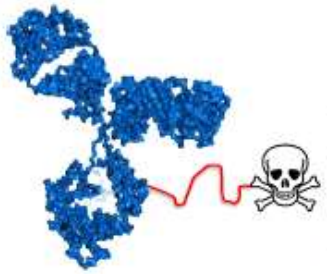


Sample	M_w (kDa) Complex	mAb	Modifier	DAR
ADC 1	167.8 (±1.2%)	155.2 (±1.8%)	12.6	10.1
ADC 2	163.7 (±1.2%)	155.6 (±1.2%)	8.1	6.5
ADC 3	159.5 (±8.0%)	155.2 (±8.0%)	4.3	~1-7

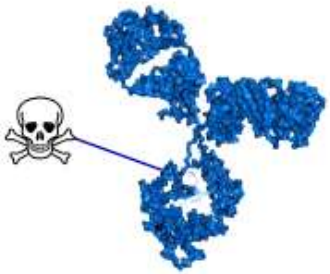
- Drug Antibody Ratio (DAR) measurements

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

Linker-Induced Instability Studied by DLS

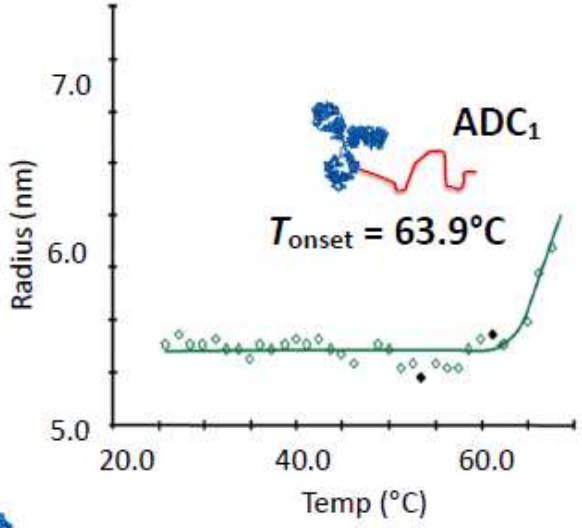


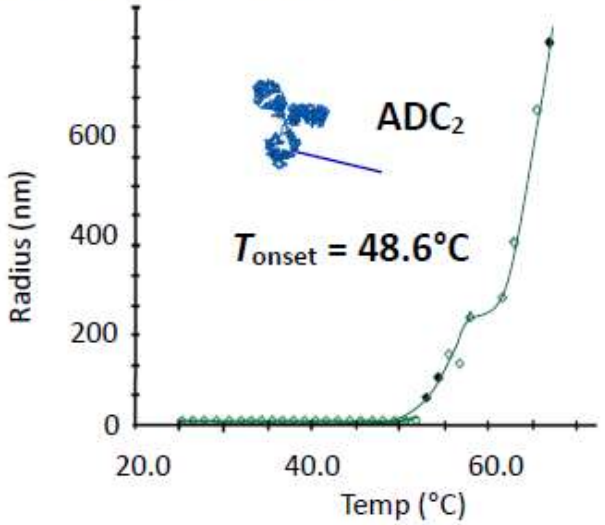
ADC_1



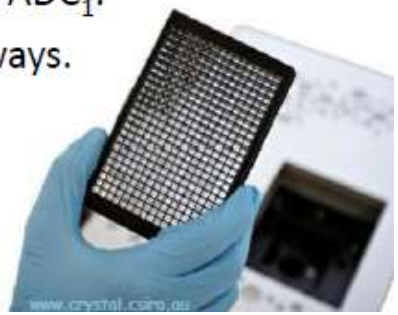
ADC_2

Identical mAb and drug, different linkers

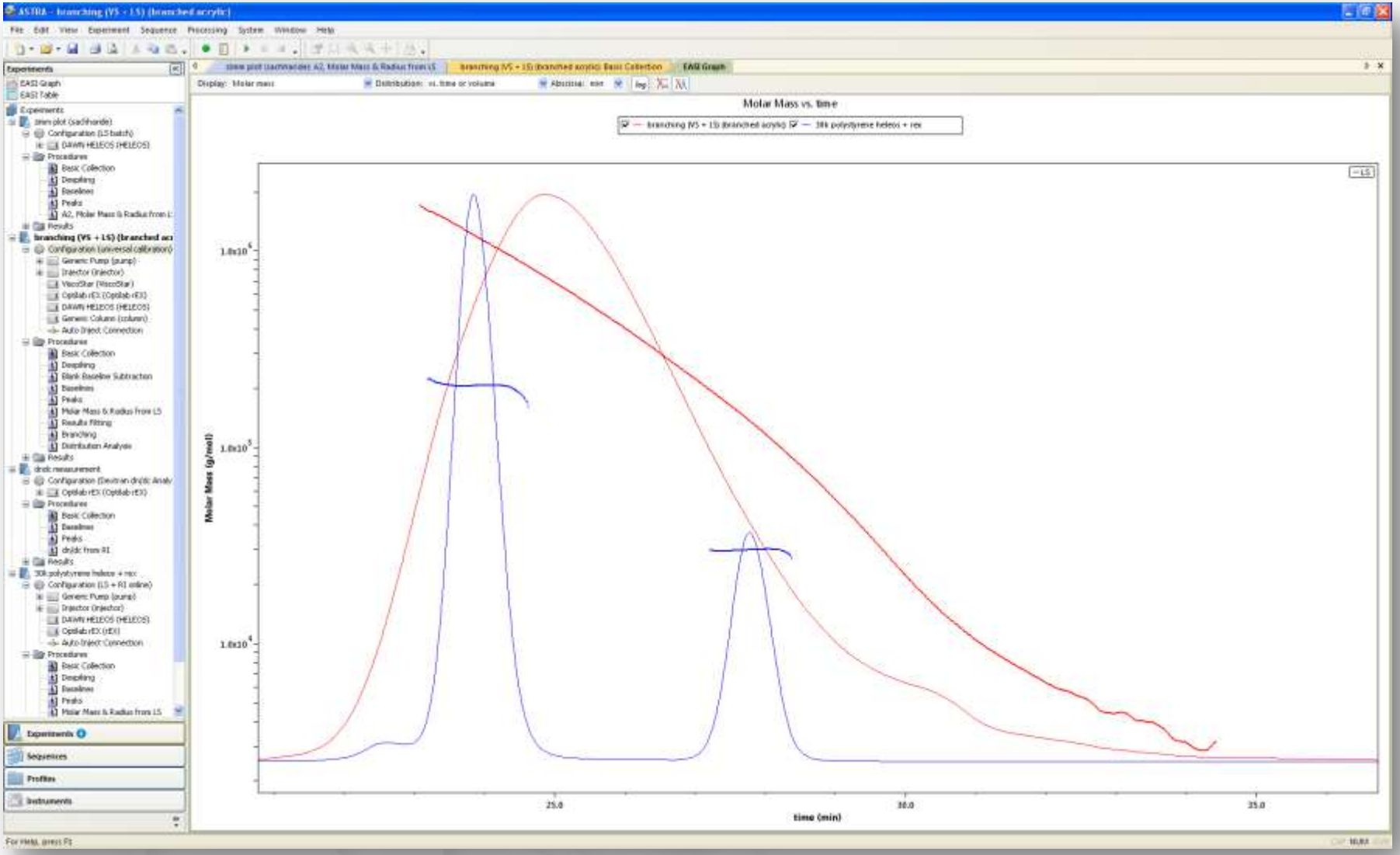




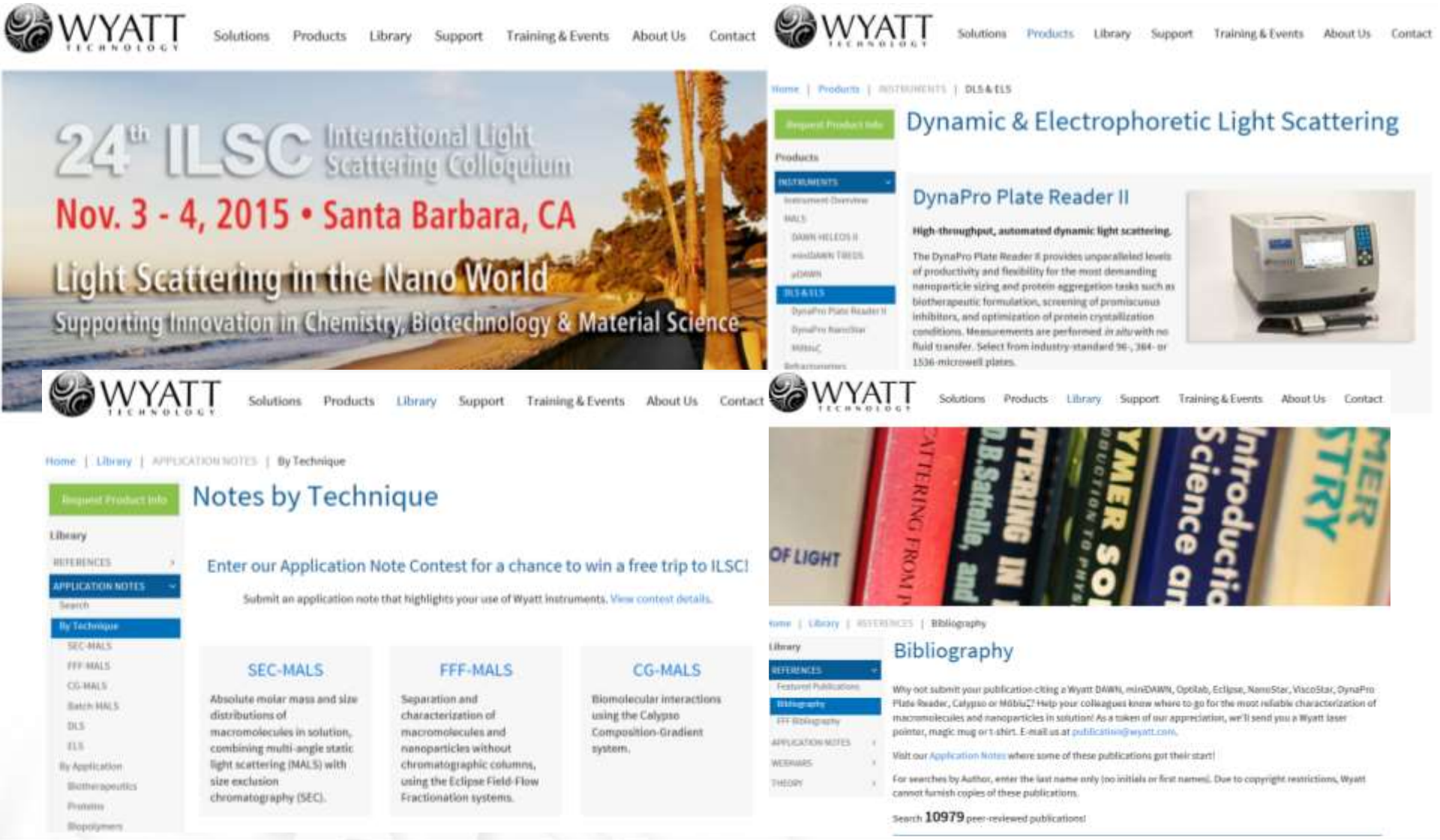
Protein stability is typically studied by temperature of melting (T_M) or onset of aggregation. ADC_2 unfolds and aggregates at a lower temperature, and is therefore less stable than ADC_1. These data suggest distinct aggregation pathways.



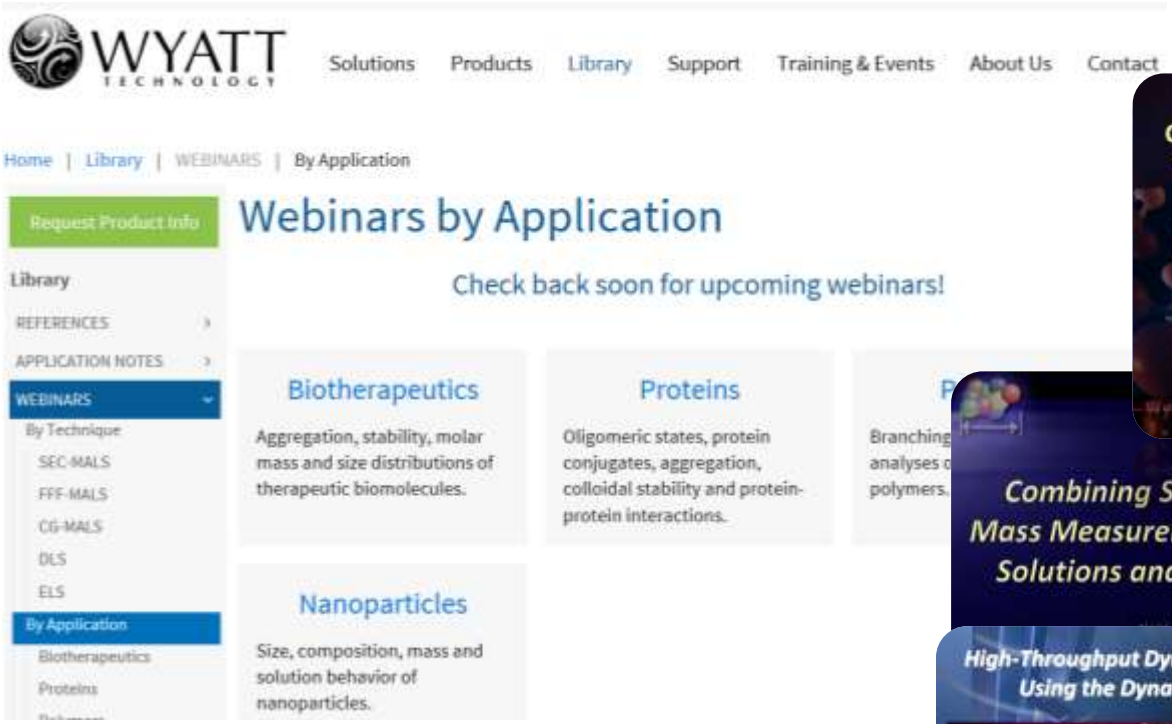
Astra Software

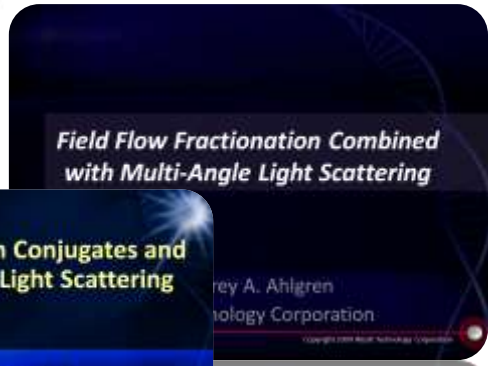


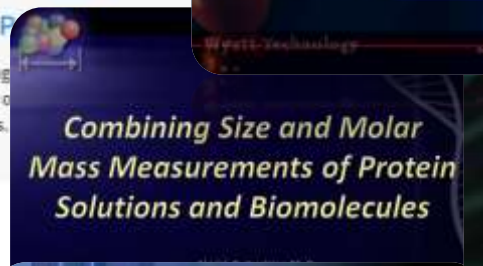
Website

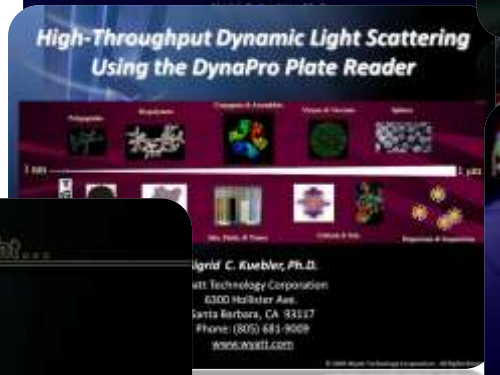


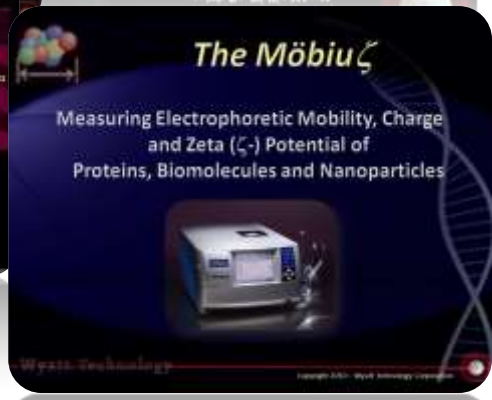
Website : Webinars

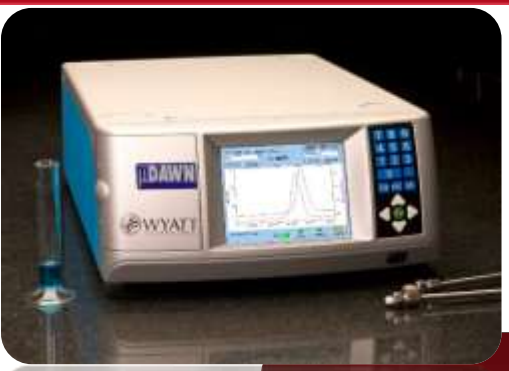












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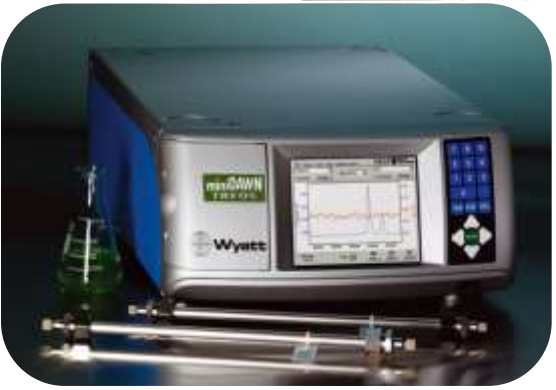

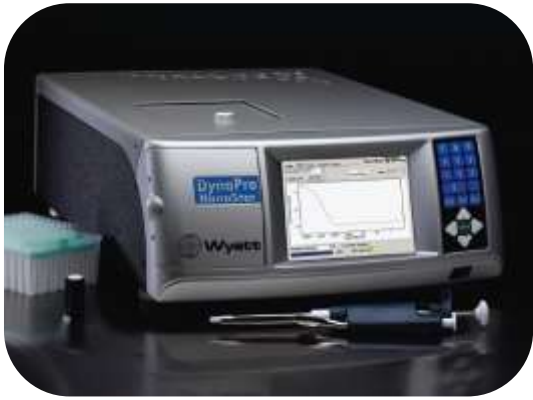

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Merci de votre attention

Rendez-vous sur le stand pour les questions?

Votre contact:

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