Aggregation of Antibody-Drug Conjugates: The Light Scattering Toolbox for Screening and Characterization
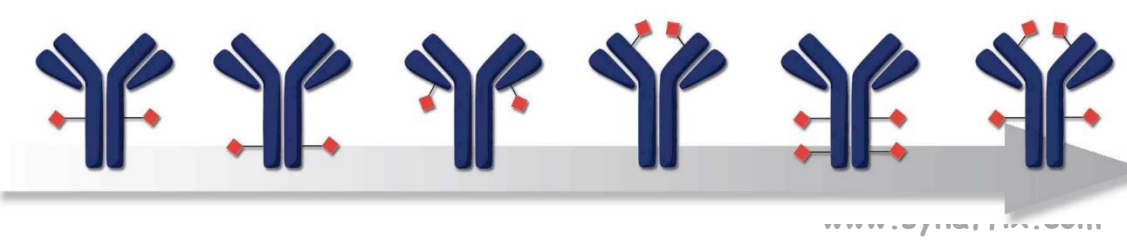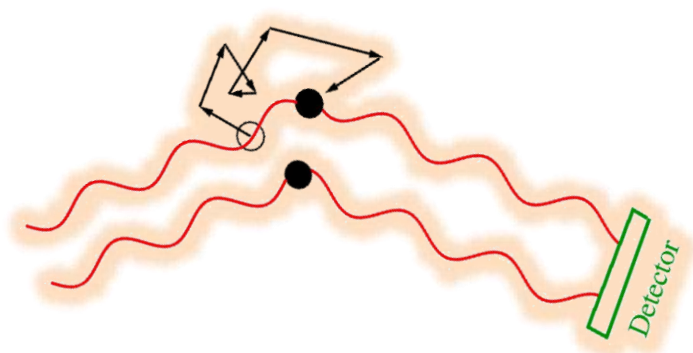
WYATT TECHNOLOGY

SEC-MALS • FFF-MALS • CG-MALS • DLS • MP-PALS
Molar Mass • Size • Charge • Conjugation • Interactions

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Abstract

Antibody-Drug Conjugates (ADCs) show much promise as effective therapeutics for cancer and other diseases. However, they often exhibit an increased aggregation propensity compared to their unmodified counterparts due to non-specific interactions arising from attached drug and linker moieties. Light scattering offers multiple techniques for addressing the challenges of formulation screening, and characterizing both aggregates and propensity for aggregation. We present these tools in the context of ADCs and the dependence of aggregation profiles on linker chemistry.

Conclusions

➤ High-throughput screening via the DLS Plate Reader is essential for assessing stability and aggregation of ADCs, and the impacts of linker chemistry on the onset of aggregation.

➤ FFF-MALS yields high-resolution characterization of aggregates, leading to a better understanding of the aggregation process.

➤ In a separate study, MALS-UV-RI analysis determined drug-antibody ratio (DAR).

➤ The light scattering toolkit contributes to complete characterization of ADCs, leading to an efficient and rapid development timeline.

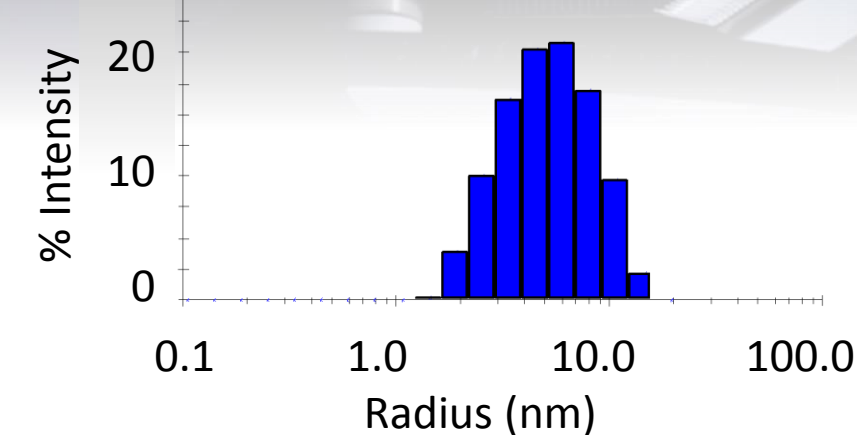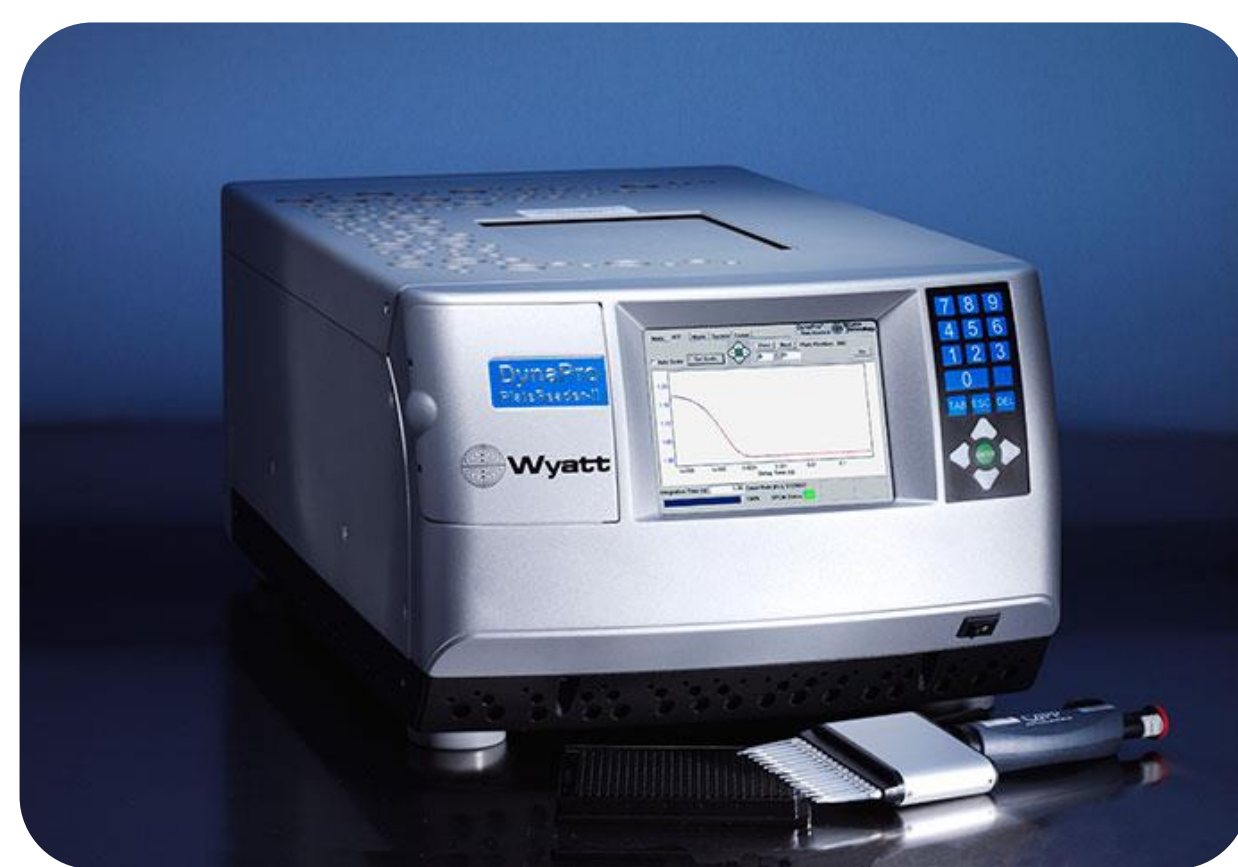## High-Throughput Dynamic Light Scattering

Dynamic light scattering (DLS) analyzes Brownian motion via *the intensity fluctuations* of light scattered by macromolecules and nanoparticles in solution. DLS determines directly **diffusion coefficients D_t**. **Particle radius R_h** is calculated from the Stokes-Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D_t}$$

DLS determines **size distributions** without fractionation, providing **polydispersity** estimates. High-throughput screening via the DynaPro® Plate Reader II is a powerful tool for characterizing multiple aspects of biotherapeutic stability:
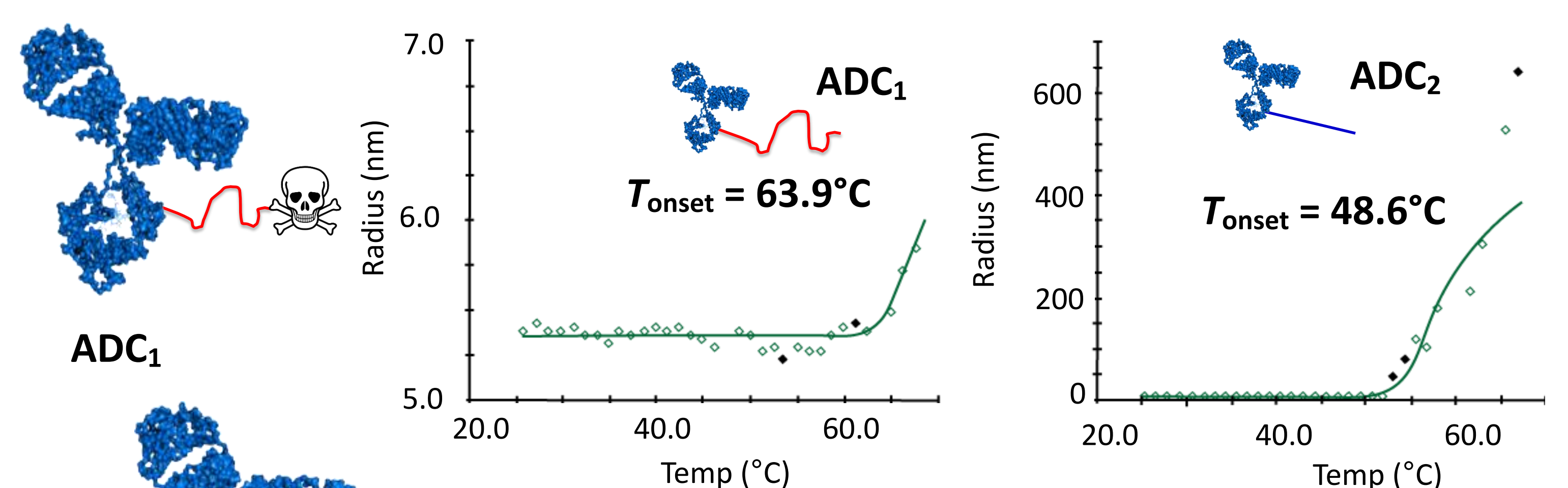
• Degree and type of aggregation
• Thermal conformational stability (T_M, T_{onset})
• Chemical conformational stability
• Colloidal stability

Linker-Induced Instability Studied by DLS

ADC$_1$
T_{onset} = 63.9°C

ADC$_2$
T_{onset} = 48.6°C

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.
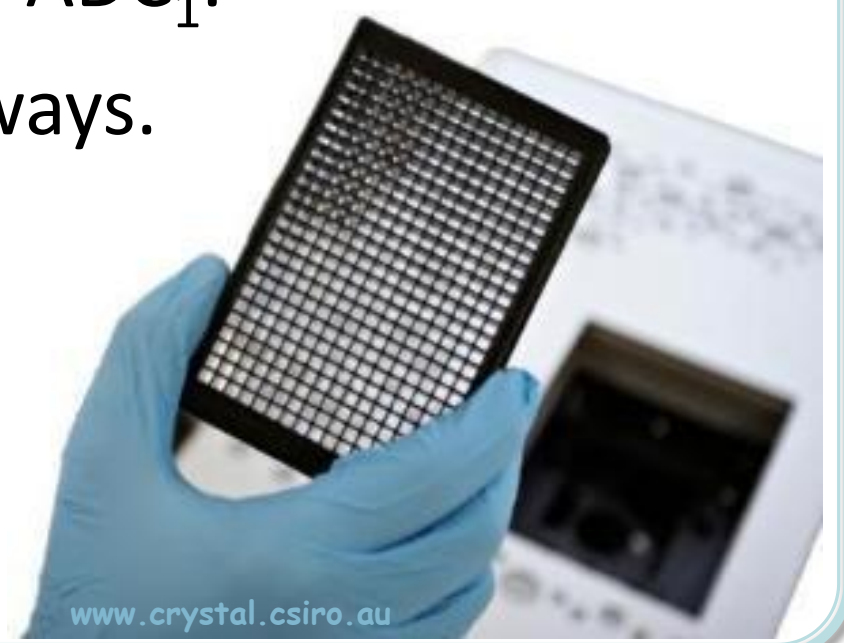
Size Distributions by FFF-MALS

Multi-angle, static light scattering (MALS) analyzes **molar mass M_w** from first principles, via the *average intensity* of scattered light I and concentration c:

$$I_{scattered} \propto M_w c$$

This analysis is *independent of questionable reference standards* and column calibration.

ADC drug and linkers molecules are often hydrophobic, making them incompatible with fractionation via size-exclusion chromatography (SEC). Field flow fractionation (FFF) separates macromolecules and nanoparticles by size *without a stationary phase*, eliminating most of the non-ideal surface interactions prevalent in SEC.

Coupling an Eclipse™ FFF device to a Wyatt DAWN® HELEOS® II MALS detector creates a versatile system for accurate and robust characterization of molar mass and size distributions from 1 nm to 1000 nm.
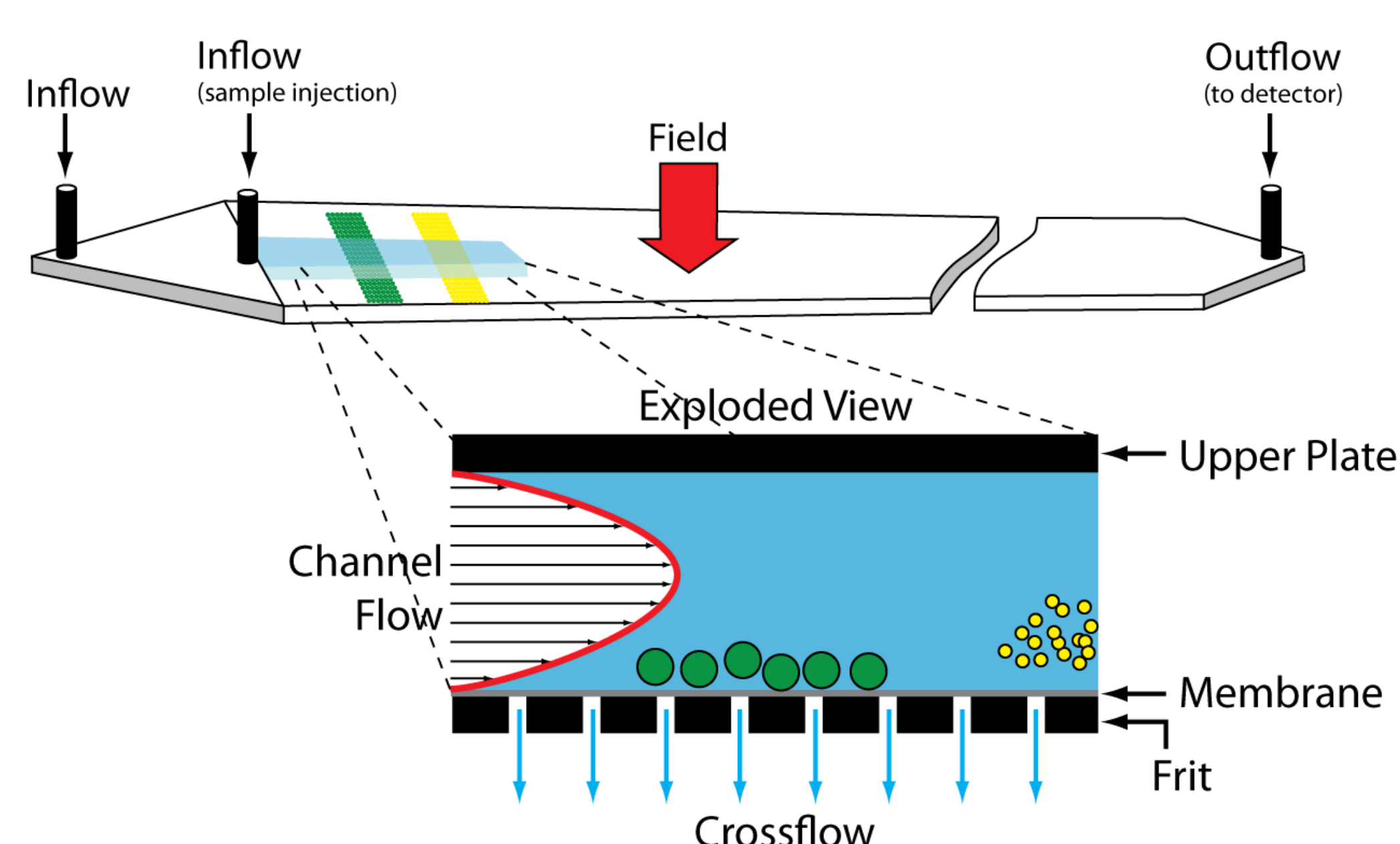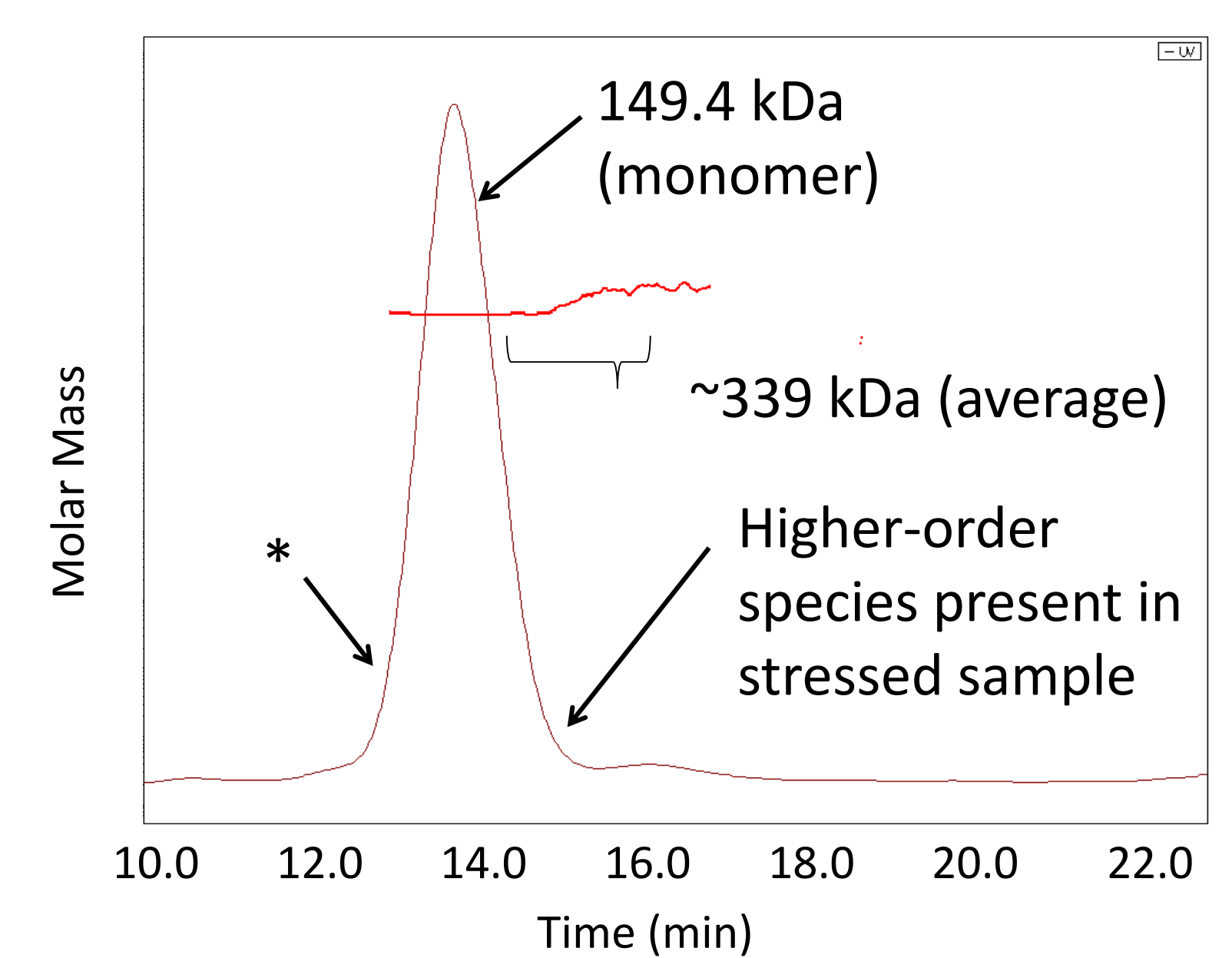
In an Asymmetric-Flow FFF separation channel, macromolecules and nanoparticles are gently pushed against a semipermeable membrane by *crossflow*. Smaller particles diffuse back up towards the center of the channel. Laminar *channel flow* induces a parabolic flow velocity profile, causing smaller particles to elute earlier.

Aggregation Analyses of Stressed and Unstressed ADCs by FFF-MALS

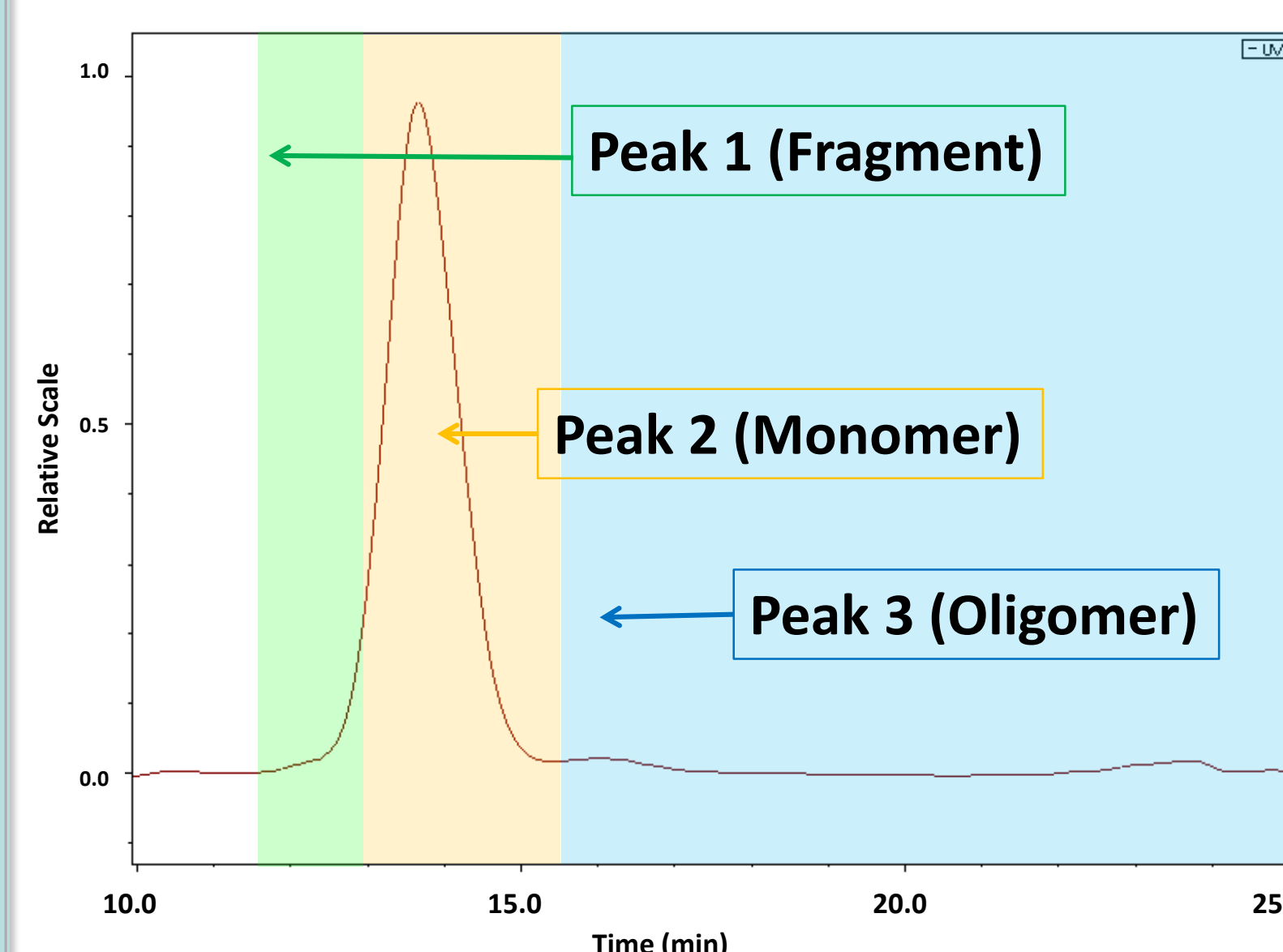Typical Fractogram – Unstressed Sample
M_w = 149.2 kDa from MALS
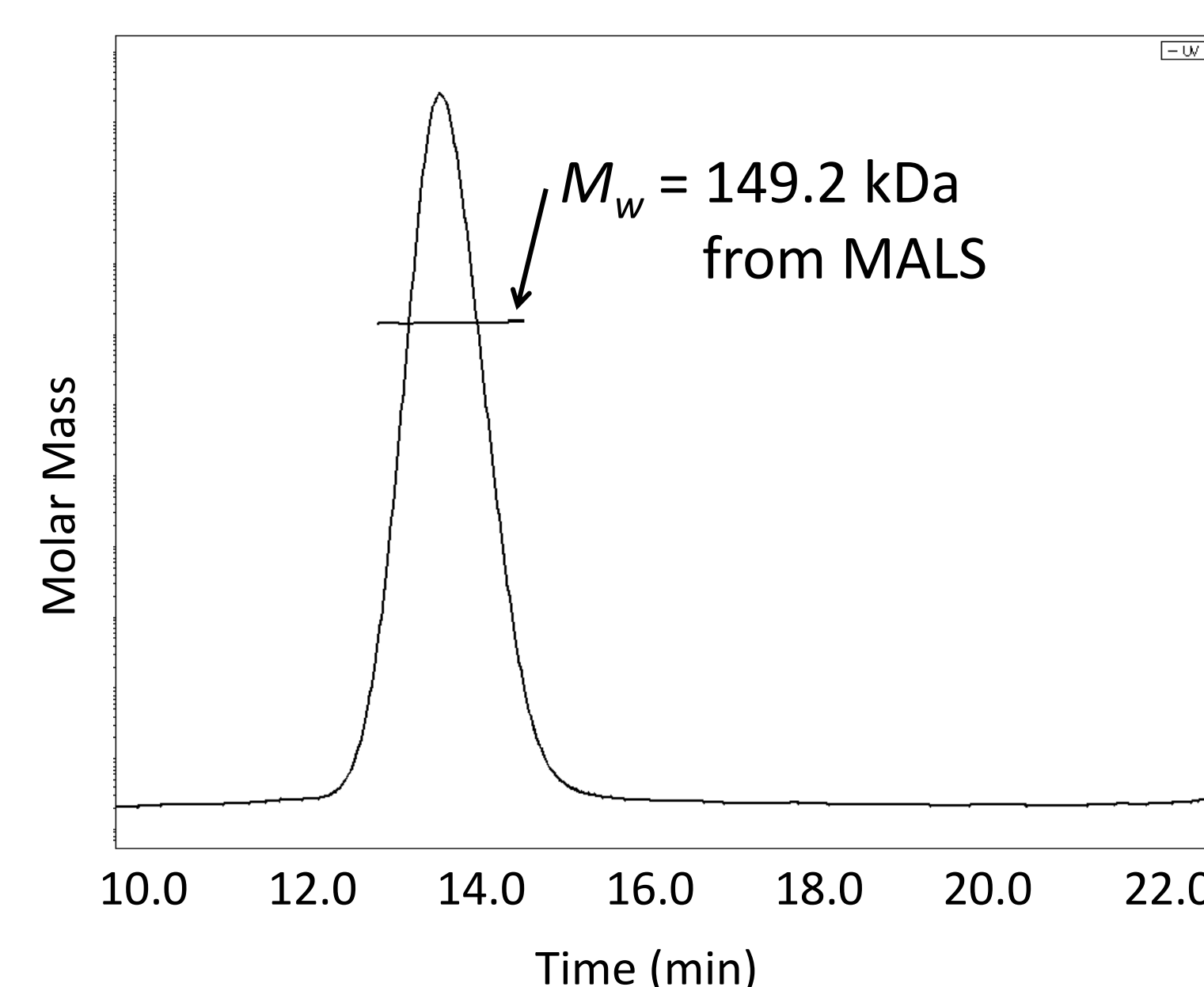
Typical Fractogram – Stressed Sample
149.4 kDa (monomer)
~339 kDa (average)
Higher-order species present in stressed sample

* Small shoulder suggestive of fragmentation. This is present to some extent in all samples.

Average M_w and Mass Fraction (n=2)

	Peak1		Peak 2		Peak 3	
	%	M_w	%	M_w	%	
Unstressed	0.75	148.4	97.75	231.5	1.5	
Stressed	1.40	149.6	95.1	297.1	3.5	

Peak 1 (Fragment)
Peak 2 (Monomer)
Peak 3 (Oligomer)

M_w is determined by the ratio of light scattering signals to UV. Mass *fraction* is determined by integrating UV peaks. FFF-MALS is highly sensitive to small quantities of aggregates due to the dependence of scattered intensity on M_w.

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