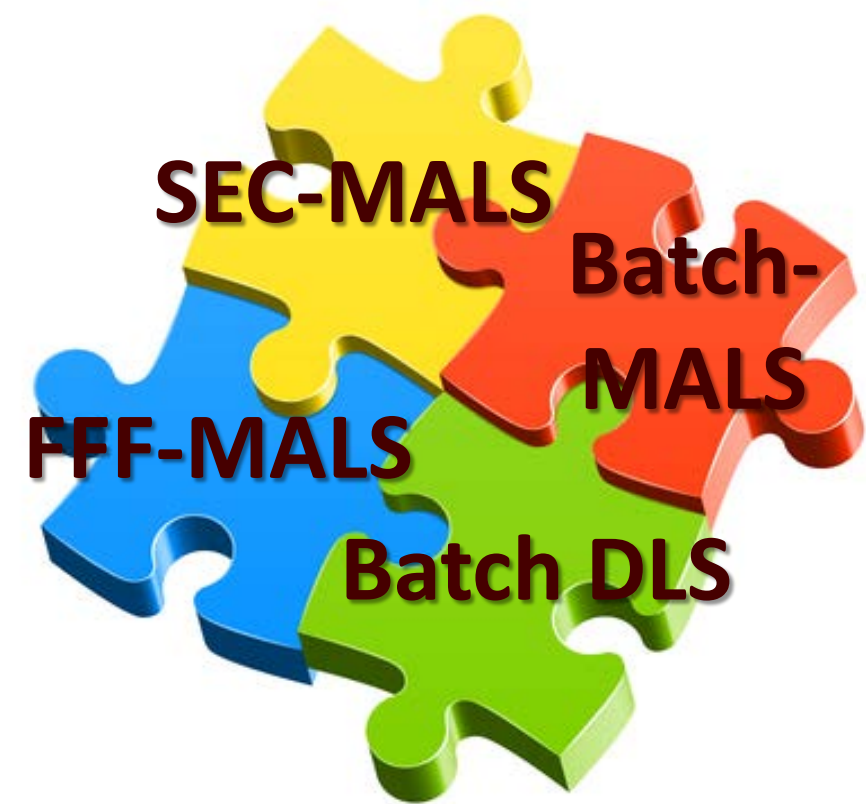


A Case Study with Orthogonal LS Approaches



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Abstract

Size exclusion chromatography (SEC) combined with a multi-angle light scattering (MALS) and a concentration detector (either UV or differential refractive index) is commonly used to detect and quantify aggregates present in a protein solution. However, concerns about potential removal of large aggregates by the SEC column as well as sample alteration due to dilution and solvent exchange call for methods orthogonal to SEC. We will discuss the necessity and benefits of using orthogonal light scattering approaches through a case study of two different lots of Pierce BSA.

Comparison of four orthogonal methods containing light scattering techniques:

	Strengths	Limitations
SEC-MALS	<ul style="list-style-type: none"> Readily available Good resolution 	<ul style="list-style-type: none"> Removal of HMW aggregates Dilution and solvent exchange
FFF-MALS	<ul style="list-style-type: none"> Versatile Good resolution No stationary phase 	<ul style="list-style-type: none"> Method development needed Dilution and solvent exchange Less protein loading capacity
Batch DLS	<ul style="list-style-type: none"> No dilution or solvent exchange Simple and quick operation 	<ul style="list-style-type: none"> Qualitative Low resolution
Batch MALS	<ul style="list-style-type: none"> No dilution or solvent exchange Suitable for lot-to-lot comparability 	<ul style="list-style-type: none"> No resolution Particle-free solutions required

Case Study: Two Lots of Pierce BSA



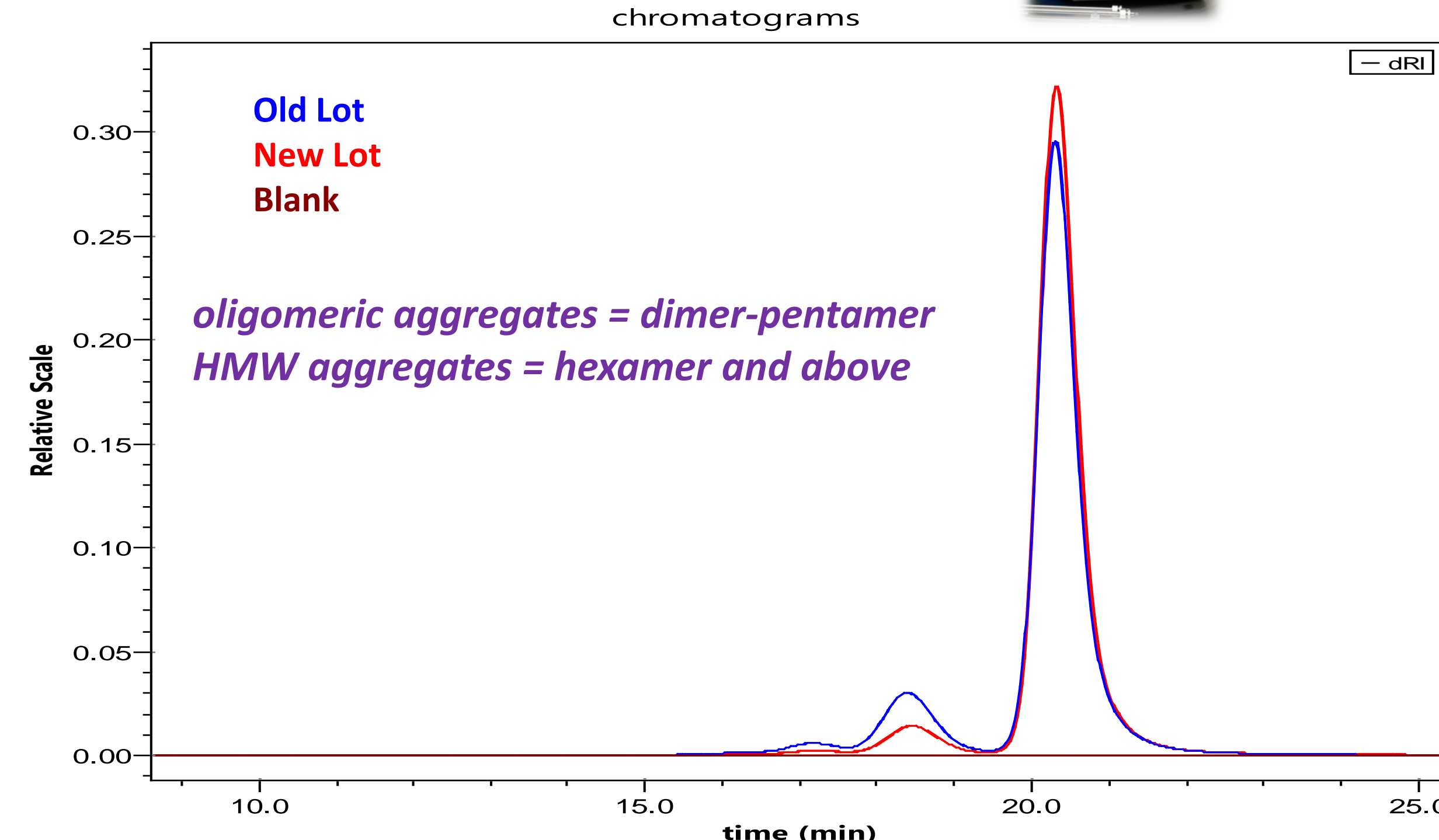
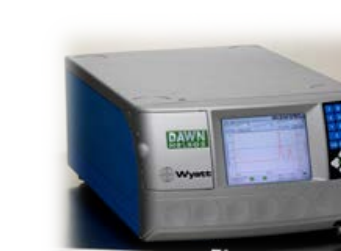
Old Lot: MK164829
New Lot: NG173884

SLS & DLS by NanoStar



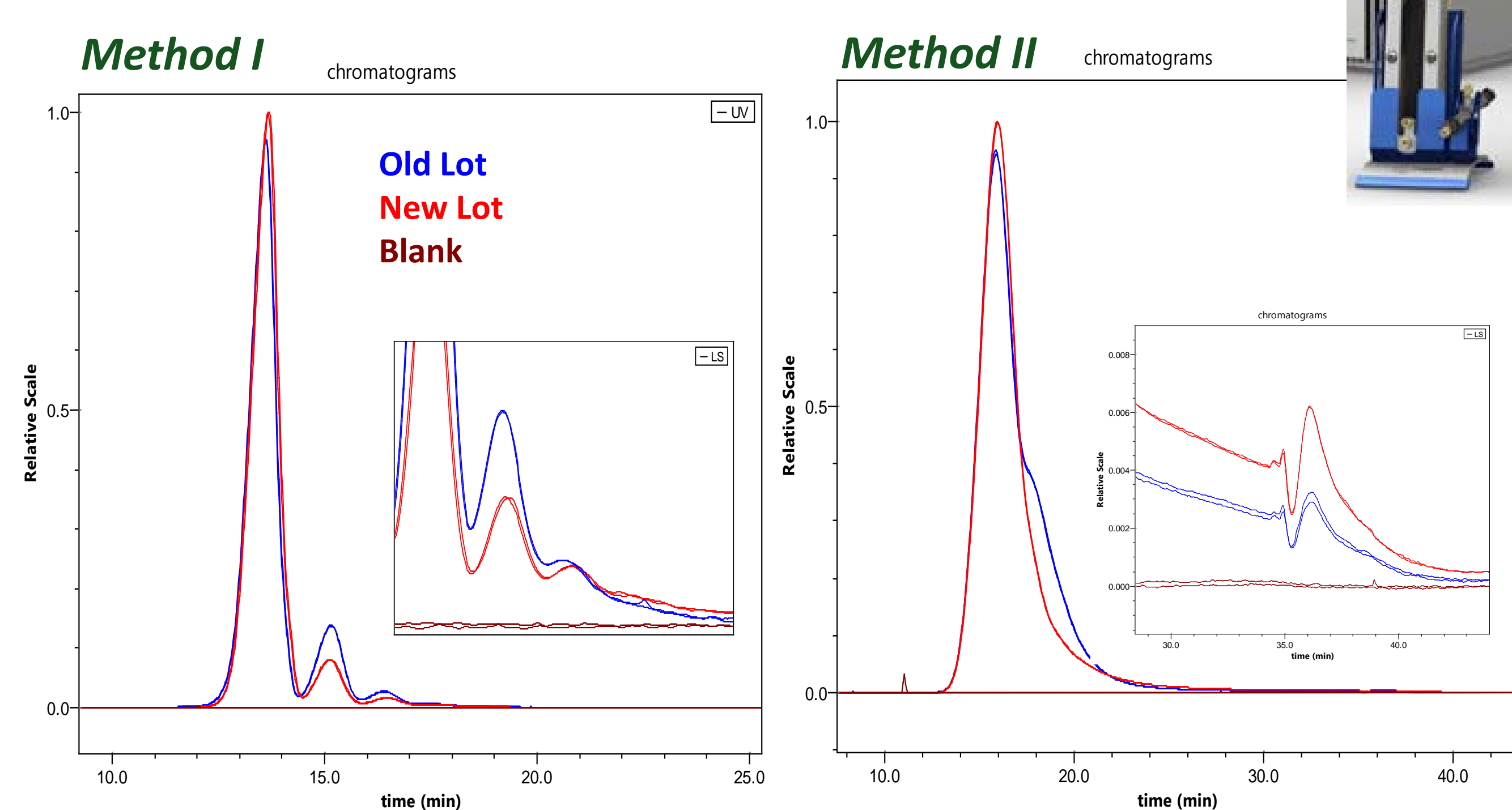
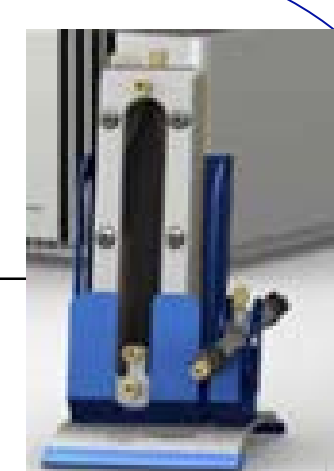
	M_w (SLS) [kD]	R_h (DLS) [nm]
Old Lot	82.3 ± 0.1	3.9 ± 0.1
New Lot	88.6 ± 0.2	4.1 ± 0.1

SEC-MALS



SEC-MALS analysis measures more oligomeric aggregates in the Old Lot and hence higher weight-average MM (M_w) of the Old Lot.

FFF-MALS



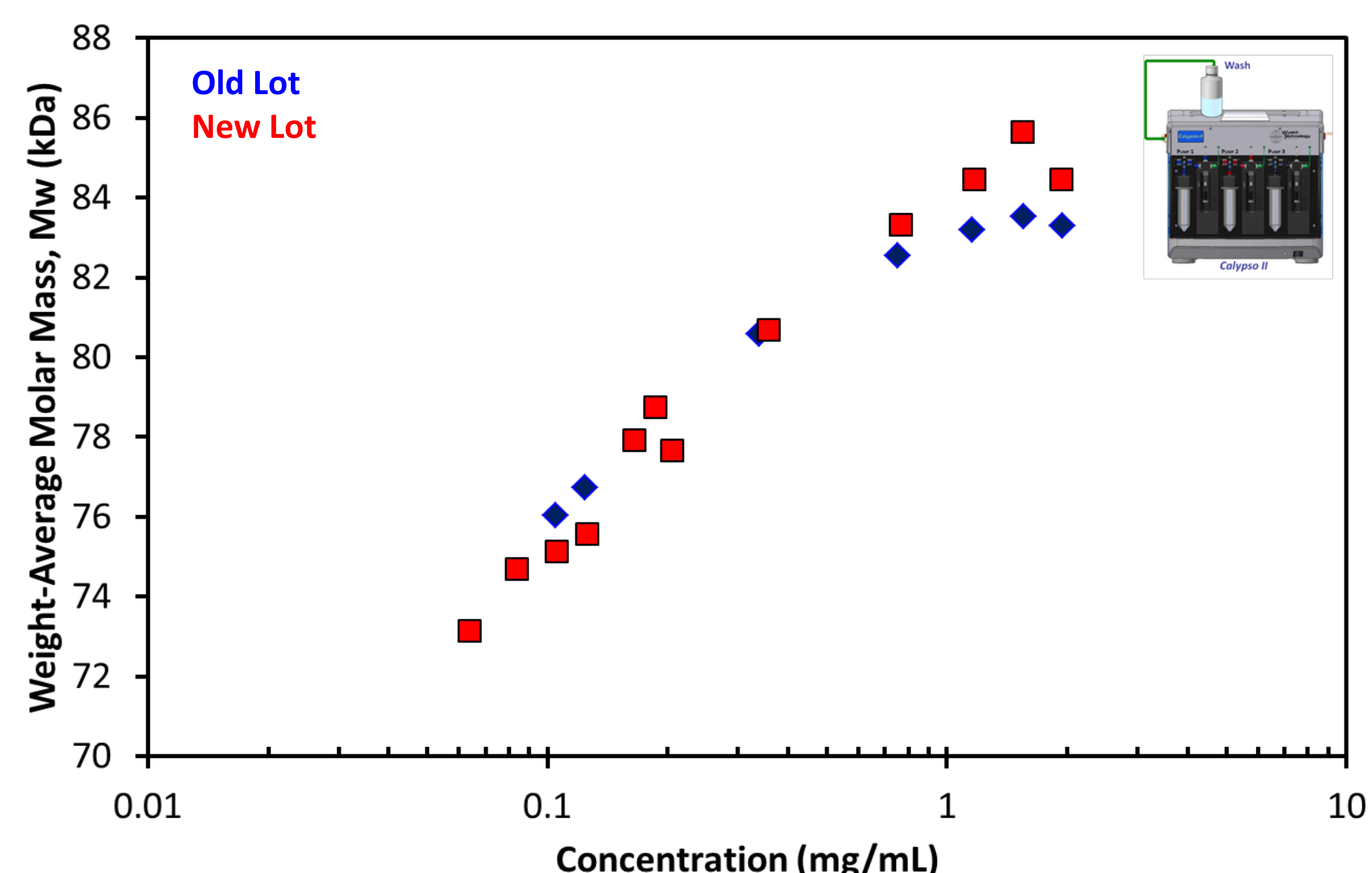
Fractograms obtained from FFF-MALS with "high resolution" method (left) and "HMW aggregate hunting" method (right) supported SEC-MALS data, but also revealed more HMW aggregates in the New Lot.

Mw Results from SEC & FFF

	M_w (SEC) [kDa]	M_w (FFF I) [kDa]	M_w (FFF II) [kDa]
Old Lot	77.6	79.1	83.5
New Lot	71.9	75.5	79.7
Monomer Apex Conc. [mg/mL]	0.65	0.03	0.40

M_w values were higher from FFF than from SEC even at lower eluted concentrations. This suggests possible removal of large aggregates by SEC.

Batch MALS from Calypso



Batch MALS at different concentrations revealed inversion of M_w order as Pierce BSA solutions were diluted.

Conclusions

- Old Lot has more oligomeric aggregates.
- New Lot has more HMW aggregates.
- The oligomeric aggregates are likely irreversible as their percentage did not reduce with dilution in SEC and FFF; whereas, the HMW aggregates are reversible as their percentage decreased with concentration in FFF (data not shown here).
- SEC dissociated and removed HMW aggregate fraction at least partially.
- FFF only dissociated HMW aggregates.
- Batch MALS provides true M_w at the concentration of interest.
- SEC and FFF resolve different species.

