

# Characterization of Alternan, a high molar mass polysaccharide from *Leuconostoc mesenteroides*, by aFFFF-MALS



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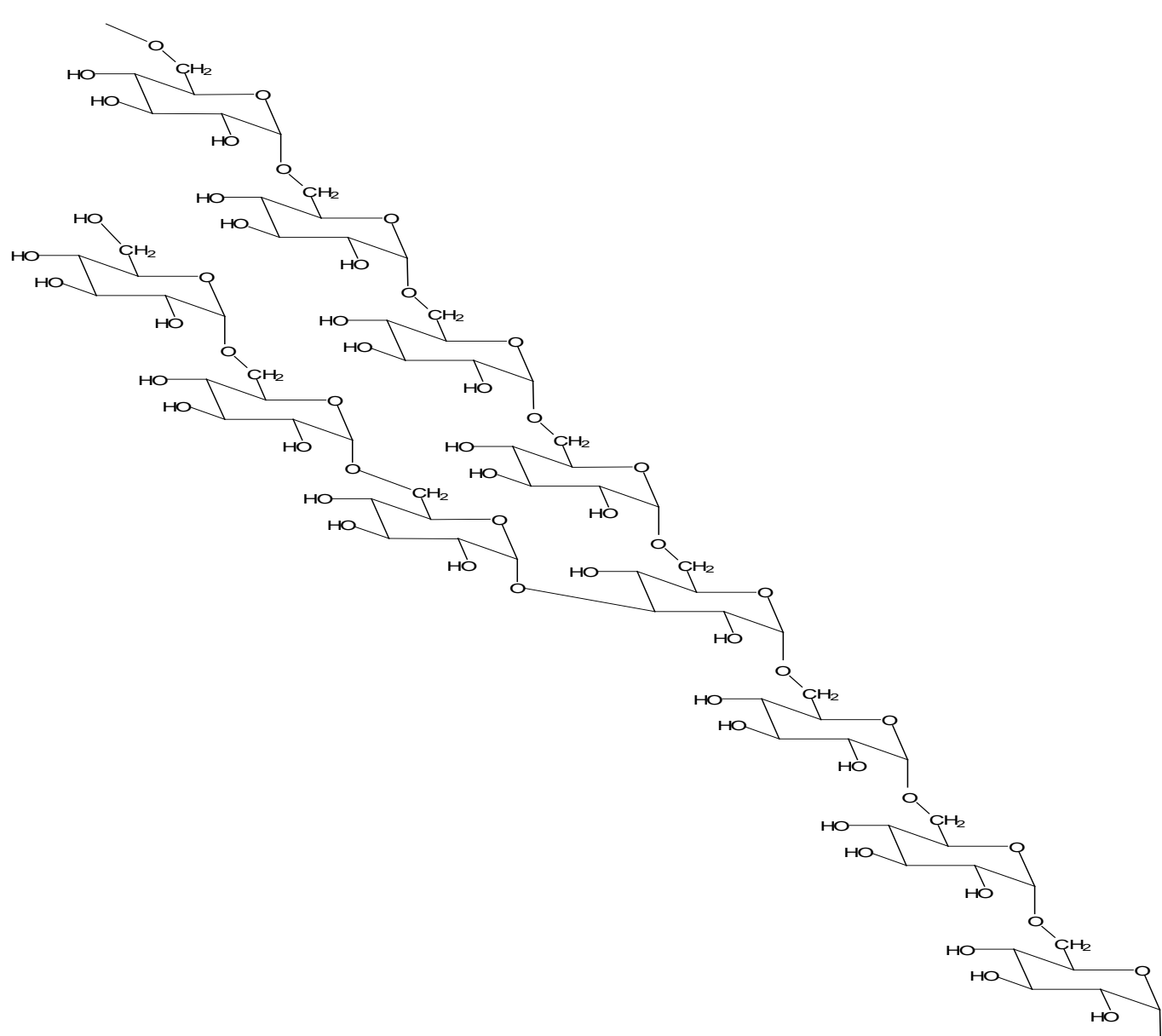


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**ABSTRACT:** Native alternan is a high molar mass polysaccharide produced by some strains of the bacterium *Leuconostoc mesenteroides*. It consists of D-glucose units that alternate their linkages between alpha-(1-6) and alpha-(1-3) between glucosyl moieties. The glucose units contained in the polysaccharide are derived from the hydrolysis of sucrose in a reaction catalyzed by the enzyme alternan sucrose. The native polymer can be produced *in vivo* by the intact bacterial culture or *in vitro* with cell-free supernatant containing alternan sucrose plus sucrose. The polysaccharide can be purified from cell-free supernatant by several rounds of alcohol precipitation. In this study we used the Wyatt Eclipse Field Flow Fractionation System (aFFFF) to fractionate the polysaccharide and multi-angle light scattering (MALS) coupled with a refractive index detector to measure the molar mass and size of native alternan and compared these results to traditional SEC-MALS. The results show that native alternan was highly polydisperse, with a weight average molar mass of 50 million Da and an RMS radius of 45 nm. Conformation analysis showed the structure of the polysaccharide to be a random coil. The RMS radius measured by MALS was compared to the hydrodynamic radius ( $R_h$ ) of the polysaccharide as measured by batch dynamic light scattering (DLS) using the Wyatt DynaPro detector; DLS analysis revealed an  $R_h$  of 50 nm that was stable from 25-85°C.

Alternan



The chemical structure of Alternan

## Materials and Methods:

Purified Alternan was prepared in 100 mM NaNO<sub>3</sub> containing 0.02% (w/v) sodium azide as a preservative. It was filtered through a 0.45  $\mu$ m syringe filter prior to use. For both FFF-MALS and SEC-MALS, the mobile phase was also NaNO<sub>3</sub>/Na<sub>3</sub>. For analysis of Mw and RMS radius, a Wyatt DAWN HELEOS and Wyatt Optilab rEX were used for detection.

The Wyatt Eclipse 3 utilized the 18-cm channel equipped with a 10 kDa MWCO, regenerated cellulose membrane with a 350  $\mu$ m tall spacer. The channel flow rate was 1 ml/min. During elution, the cross flow was initially set at 1.0 ml/min, and a 50 minute linear gradient to 0 ml/min cross flow was used.

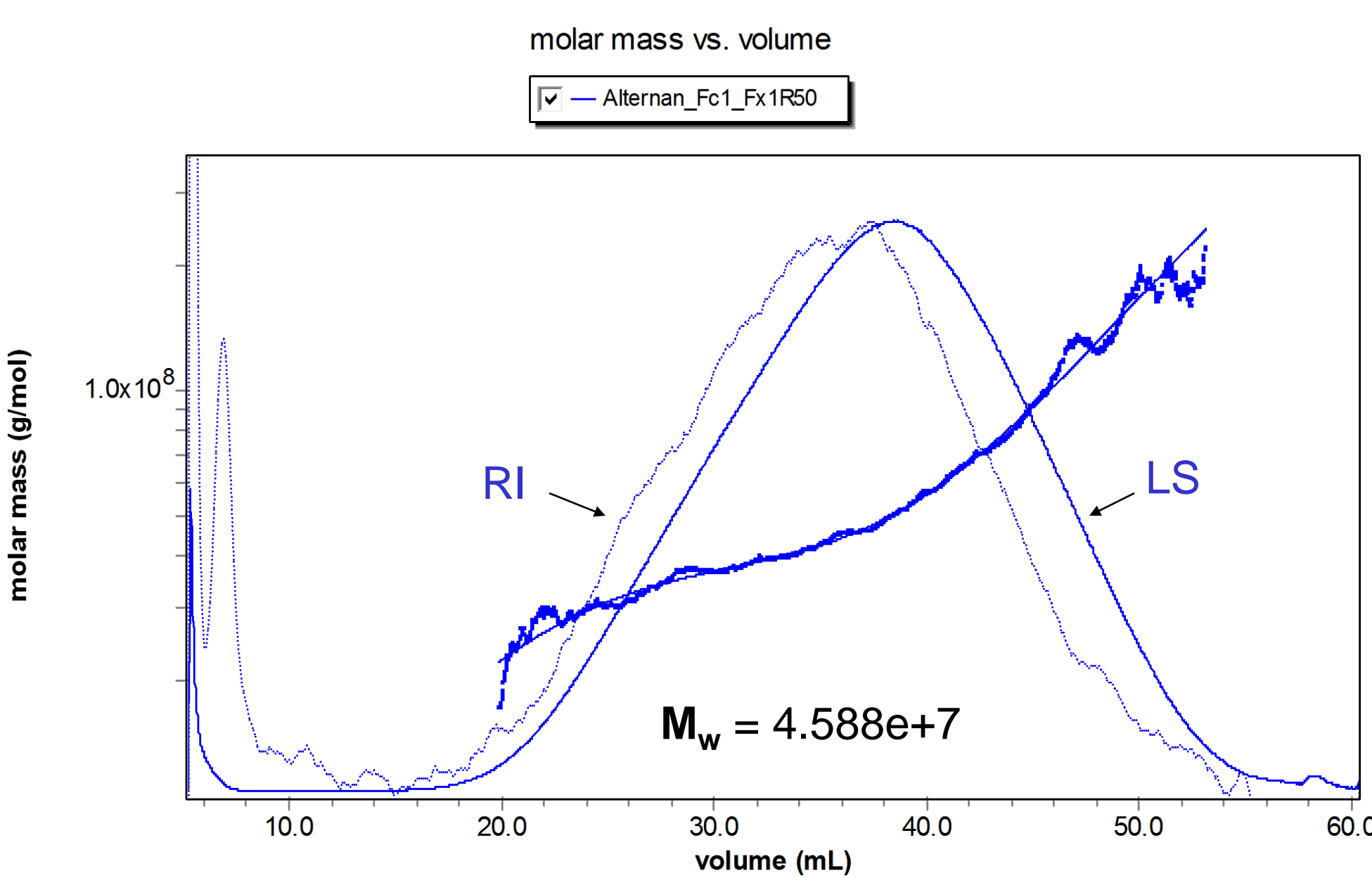
For SEC-MALS, a Shodex SB-807-HQ column equilibrated with the same NaNO<sub>3</sub>/Na<sub>3</sub> mobile phase was used. The flow rate was constant at 0.5 ml/min.

For DLS measurement, a Wyatt DynaPro Temperature-controlled Microsampler and cuvette were used. The initial temperature was 25°C, and the  $R_h$  measured at 10°C intervals to 85°C.

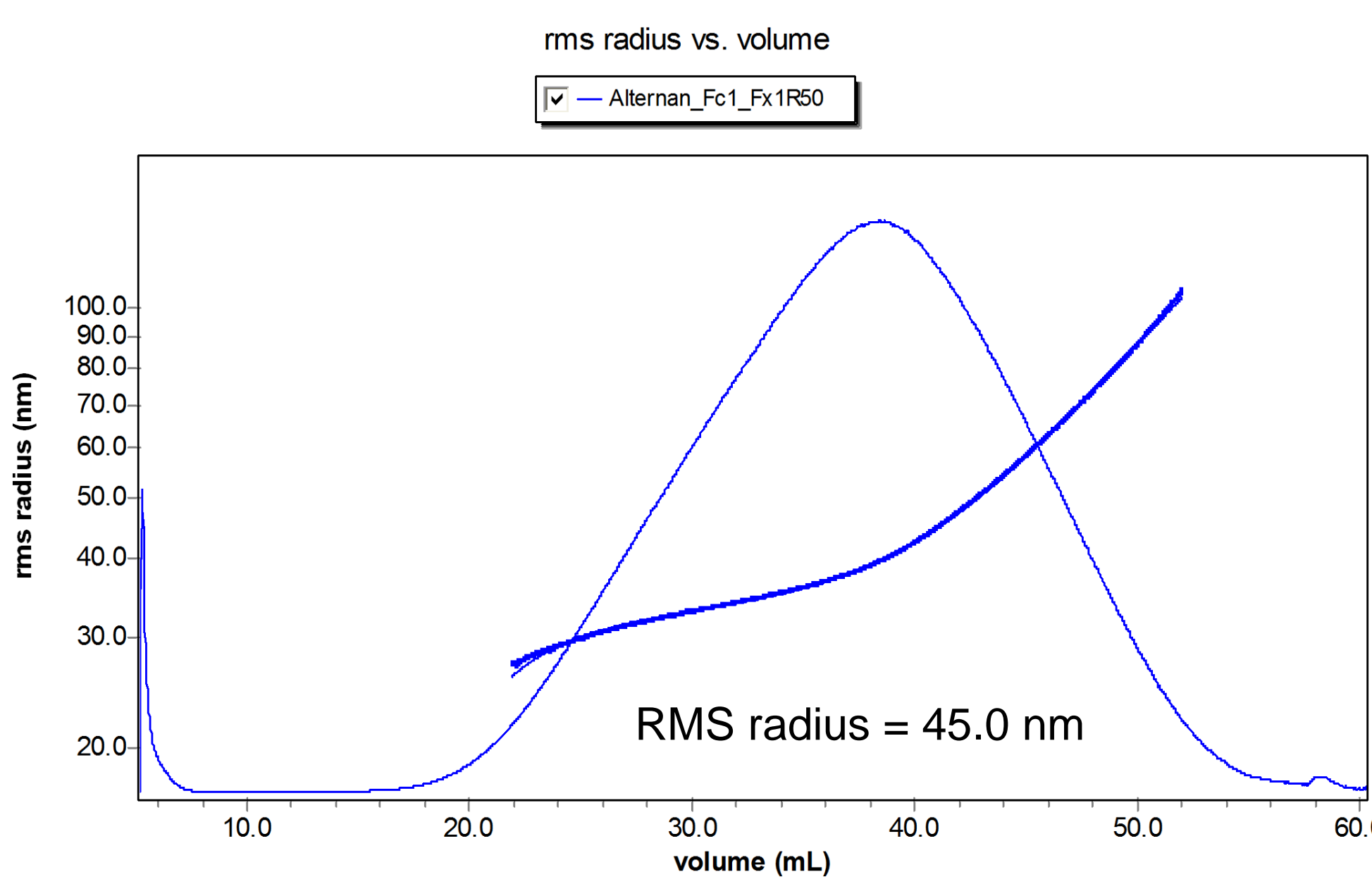
The Wyatt Eclipse aFFFF



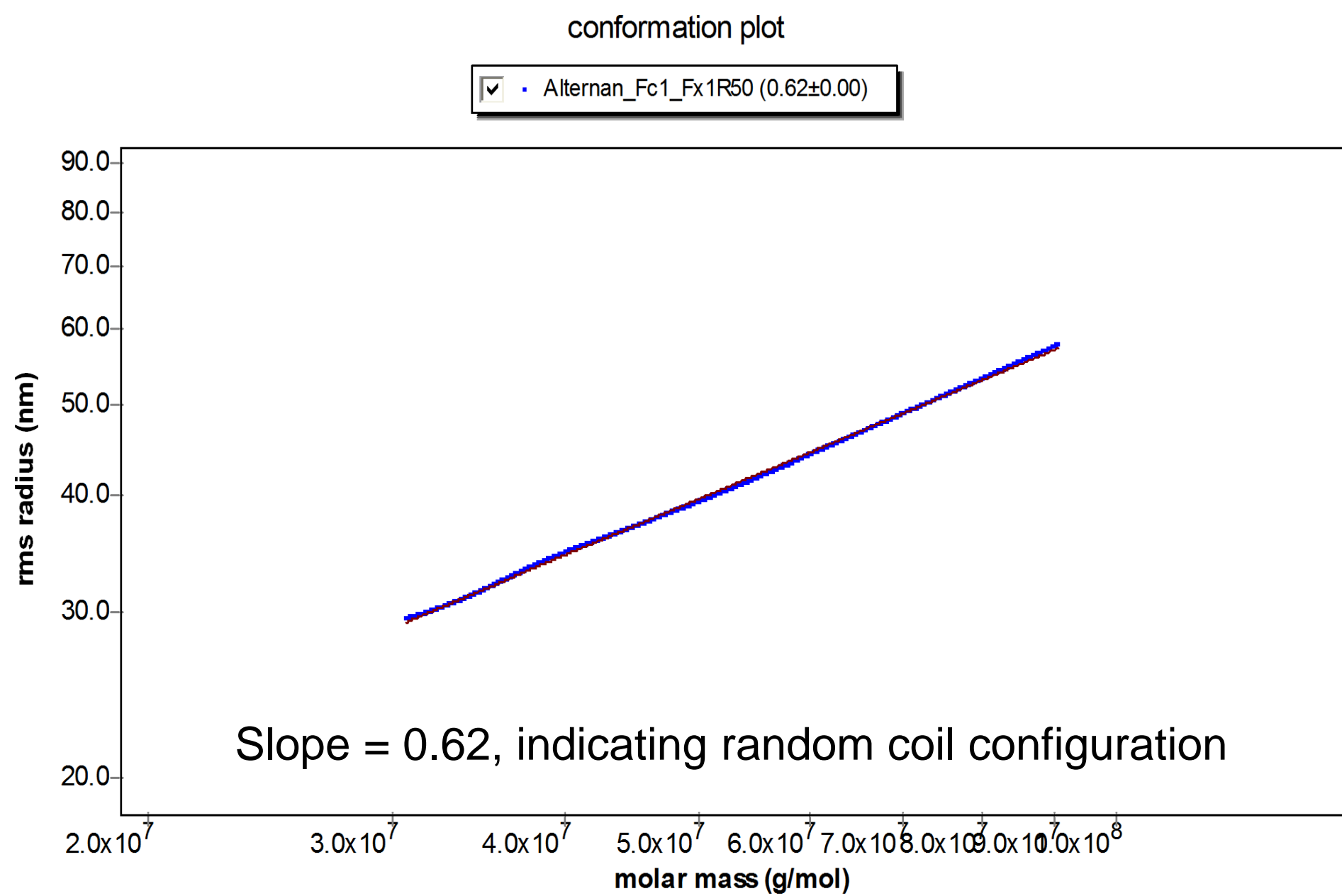
## FFF-MALS of Alternan



M<sub>w</sub> Distribution Plot

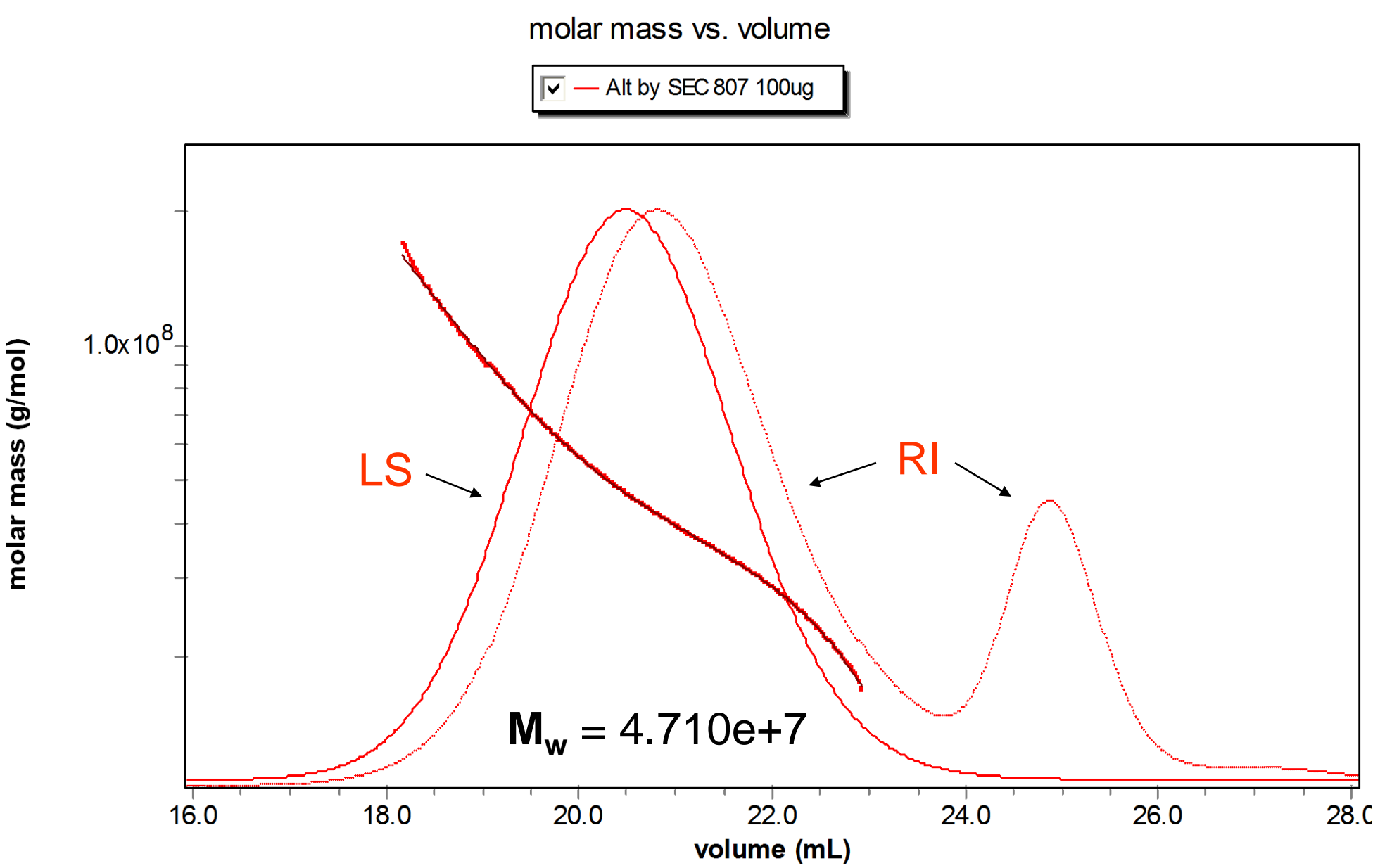


RMS Radius Distribution Plot

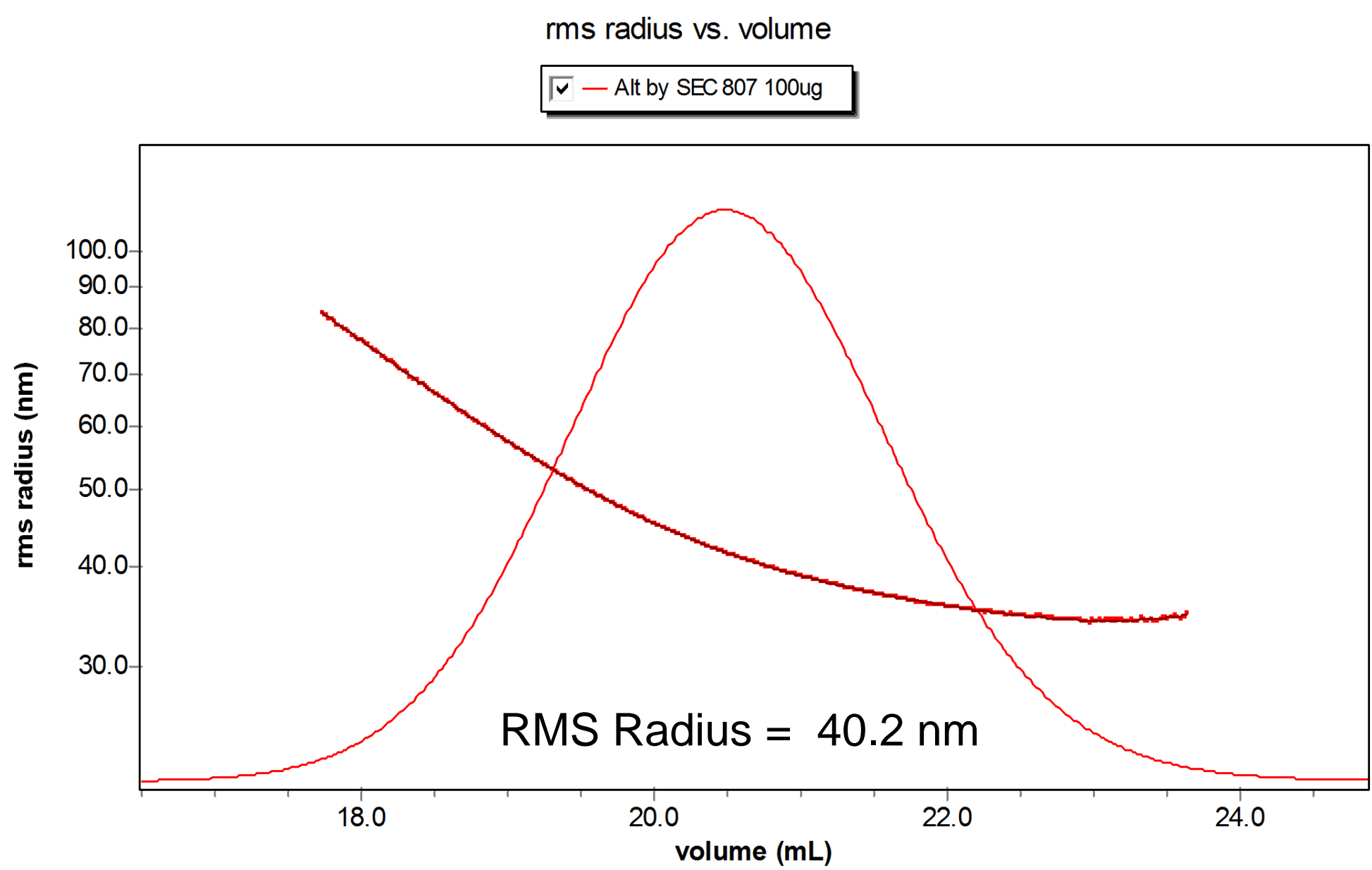


Conformation Plot

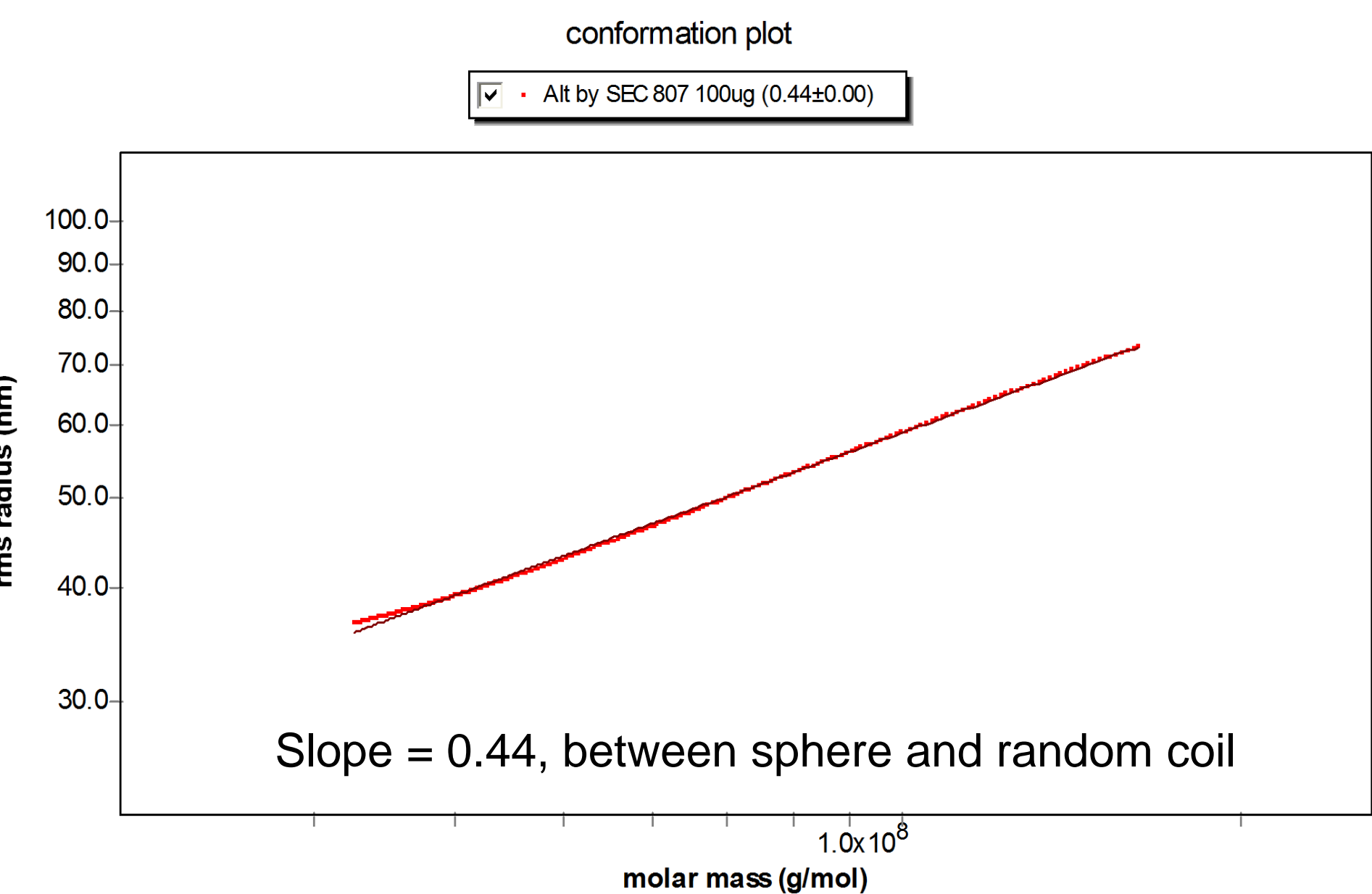
## SEC-MALS of Alternan



M<sub>w</sub> Distribution Plot

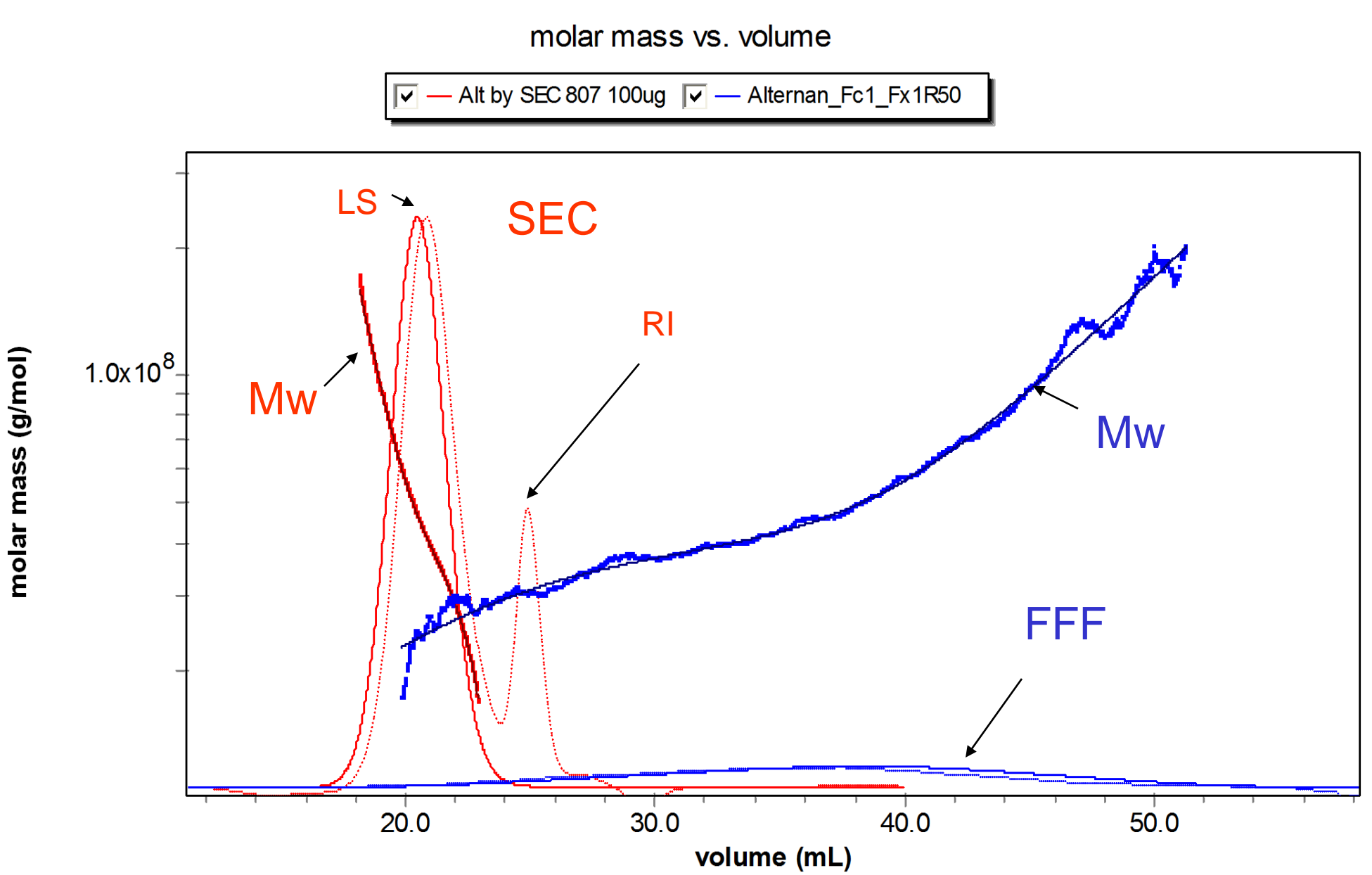


RMS Radius Distribution Plot

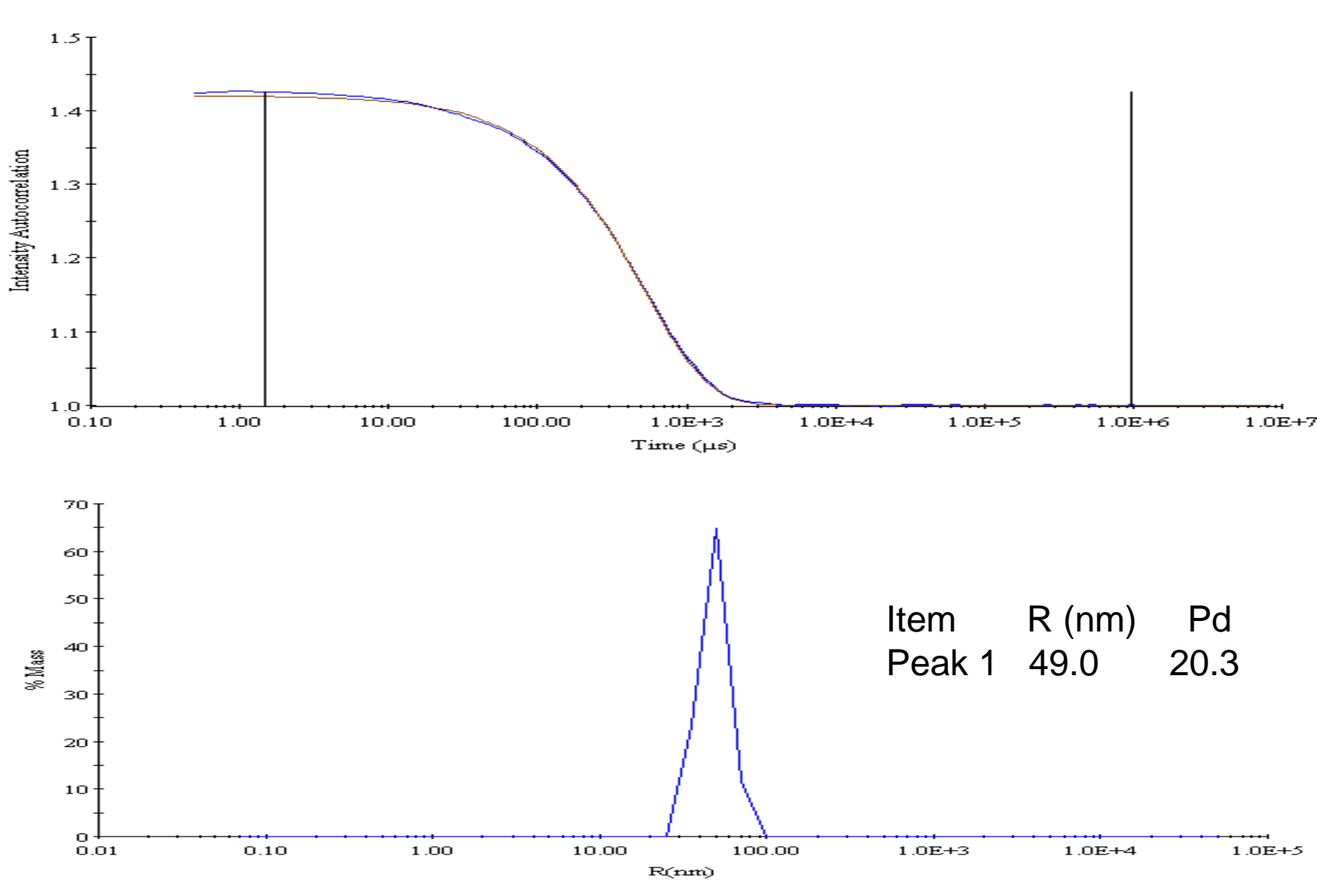


Conformation Plot

## aFFFF-MALS vs. SEC-MALS



## Batch DLS Analysis



## Conclusions:

The separation of Alternan, a highly polydisperse polysaccharide, was more effective by aFFFF than SEC on a Shodex 807 column. The FFF separation was more uniform and eliminated the small trailing peak seen by SEC separation on the Shodex SB-807 column. The conformation plot produced by the aFFFF separation resulted in a slope of 0.62, which is typical of a random coil formation. The same plot produced by the SEC separation resulted in a slope of 0.44, which suggests a more compact structure. Additional experimentation will be needed to resolve the discrepancy between these two methods.

DLS analysis showed that the  $R_h$  of the Alternan was very temperature stable at 50 nm; there was no change in the size of the molecule from 25-85°C (the highest temperature tested).