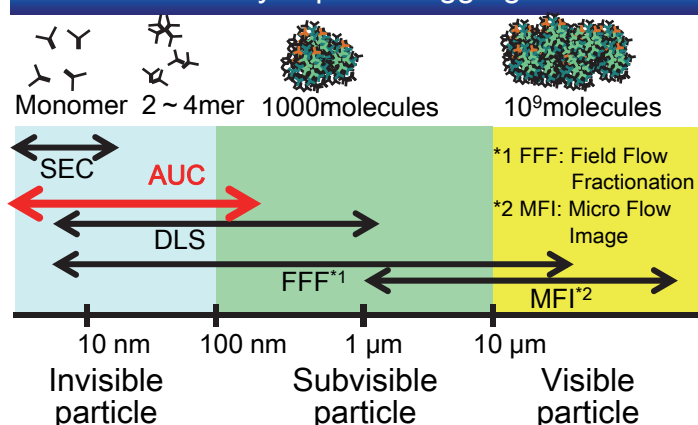


Aggregation Analysis of Biotherapeutics By Analytical Ultracentrifugation

-comparison between AUC and DLS-

Recently, the need for aggregation analysis of biotherapeutics has increased. Analytical ultracentrifugation (AUC) has attracted attention as one of the effective methods. We measured aggregates of biopharmaceuticals by AUC and Dynamic light scattering (DLS), and compared both methods.

Methods to analyze protein aggregates



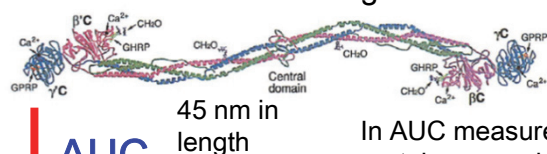
Comparison between AUC and DLS

Methods	Advantages	Disadvantages
AUC	Applicable to mixture of large-sized and small-sized particles. Enable to calculate molecular weight. Quantitative.	Relatively long measurement time.
DLS	Wide dynamic range. Sensitive to detect large-sized particles.	Signals from small-sized particles are influenced by larger ones in the mixture.

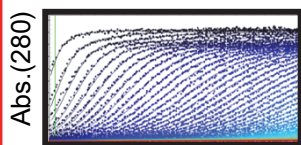
AUC measurement of fibrinogen

Fibrinogen (blood coagulation factor)
Theoretical molecular weight 340 kDa

DLS

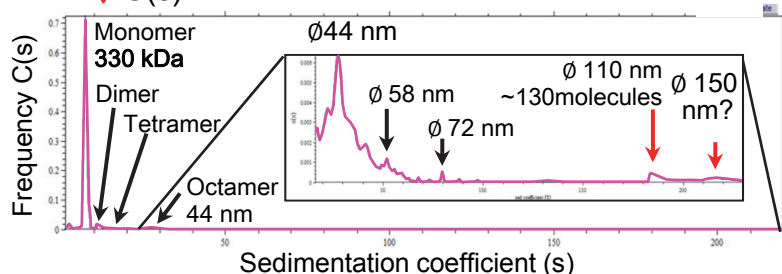


AUC



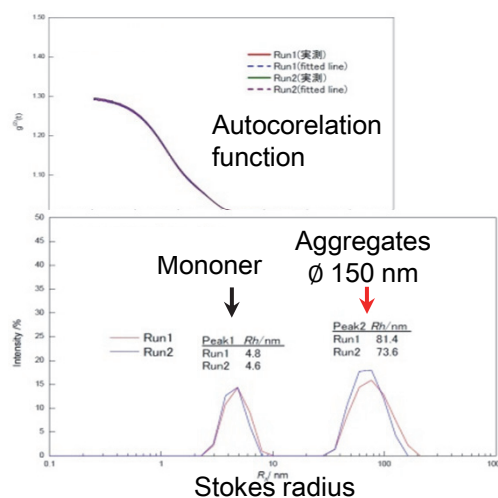
AUC sedimentation profile of fibrinogen

C(s)



- The AUC result was analyzed with reference to the DLS result.
- Aggregation up to 130 molecules was detected.
- Amount of aggregation larger than octamer was 0.1-1% of total.

DLS measurement of fibrinogen



- Aggregation of 150 nm diameter was detected.
- Large sized aggregation was well detected.
- The ratio of each component couldn't be calculated.
- Resolution was not enough.

Summary

- Large sized aggregation was well detected by DLS method.
- AUC is superior in resolution and quantitation compared with DLS method.
- It is effective to analyze aggregates by AUC with reference to the result from DLS.