Use of SEC-MALS

(Size Exclusion Chromatography - Multi Angle Light Scattering)

for protein quality and characterization

Methods for protein characterization

- Analytical SEC is a common method to characterize protein properties: size, oligomers, aggregations, and purity.

- Other methods: - light scattering (LS and DLS for mass and radius)
  - CD for secondary structure
  - SDS-PAGE: coomassie, western blot, native gels for protein identification and purity.

- Size exclusion chromatograph in line with multi angle light scattering is a useful methodology to characterize proteins size and shape in native solution conditions.

Multi Angle Light Scattering (MALS)

- When a laser light hits a macromolecule, the electric field of the light induces an oscillating dipole that re-radiates light.

- The intensity of the radiated light depends on the magnitude of the dipole and the macromolecule concentration.

- By measuring the intensity of light scattering, the mass of the molecule can be calculated.

LS- intensity of scattered light

c – concentration

K – a constant for a specific solute in a solution

$$Mw = \frac{LS}{Kc}$$

Quasi Elastic Light Scattering (QELS) / Dynamic Light Scattering (DLS)

- Measures the time-dependent fluctuations in the intensity of the scattered light caused by random motion of the macromolecules in the solution.

- The fluctuations are related to the rate of diffusion which is related to the radius of the molecule.

- <u>Stokes-Einstein equation:</u>

R- radius
k- Boltzmann constant
T-temperature
D- diffusion coefficient
η- viscosity

$$R = \frac{kT}{6\pi D\eta}$$

The instrument

- Mini DAWN TREOS Wyatt technology.
- Triple-angle MALS, 60 mW Laser, mass range: 1Da – 1000 KDa
- For continuous flow detection or for stand-alone unit in batch mode.

SEC vs. SEC-MALS

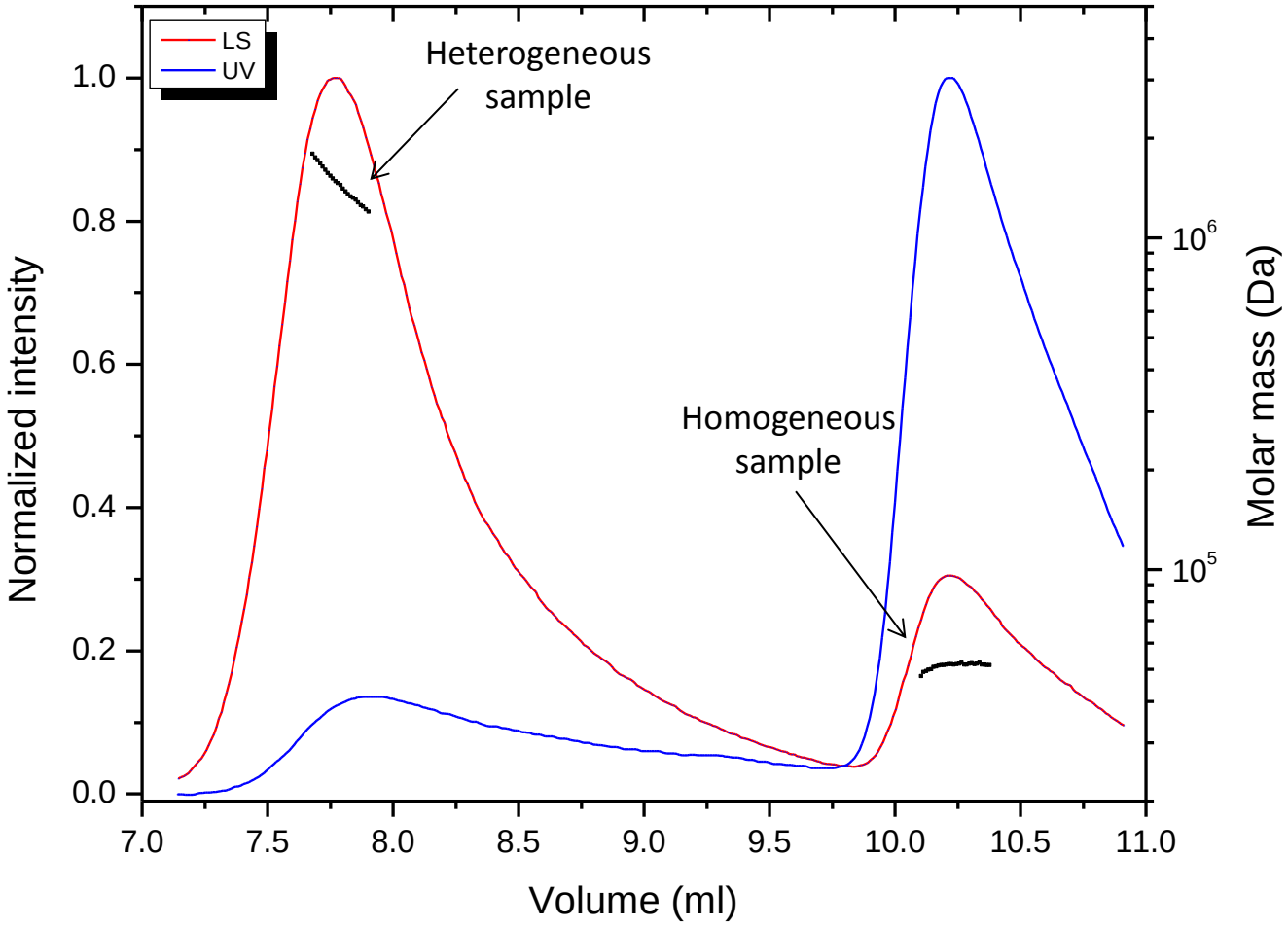
SEC

- Separation by size.
- Calculating Mw based on calibration curves of globular proteins.
- Different molecules with the same size will elute together – undetectable heterogeneous of a sample.

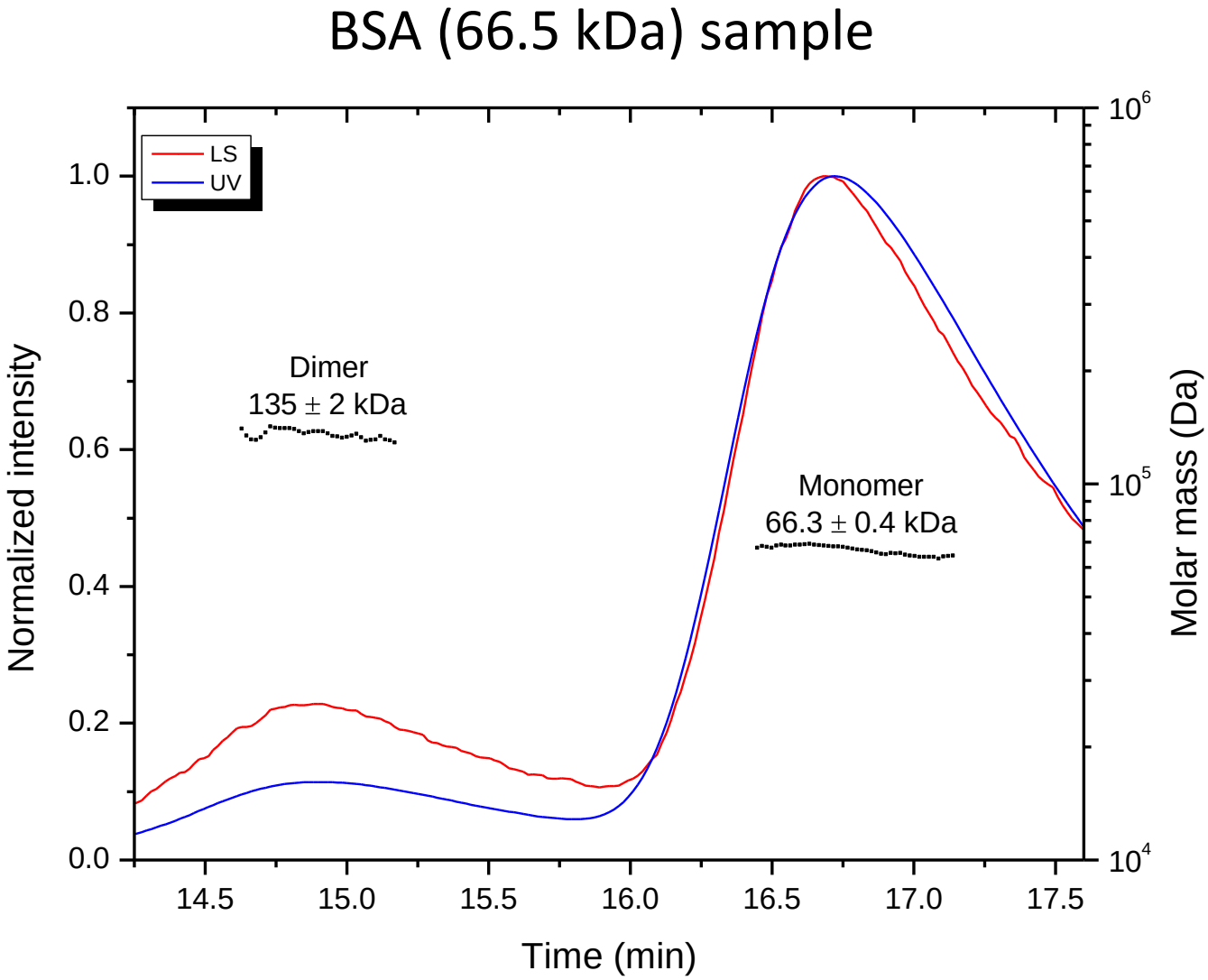
SEC-MALS

- Separation by size.
- Calculating Mw and radius from the light scattering equations – much more accurate.
- Calculate the Mw during the elution peaks- indicate homogeneous of a sample.
- Detect low amount of aggregation – large molecules amplify the intensity of LS.

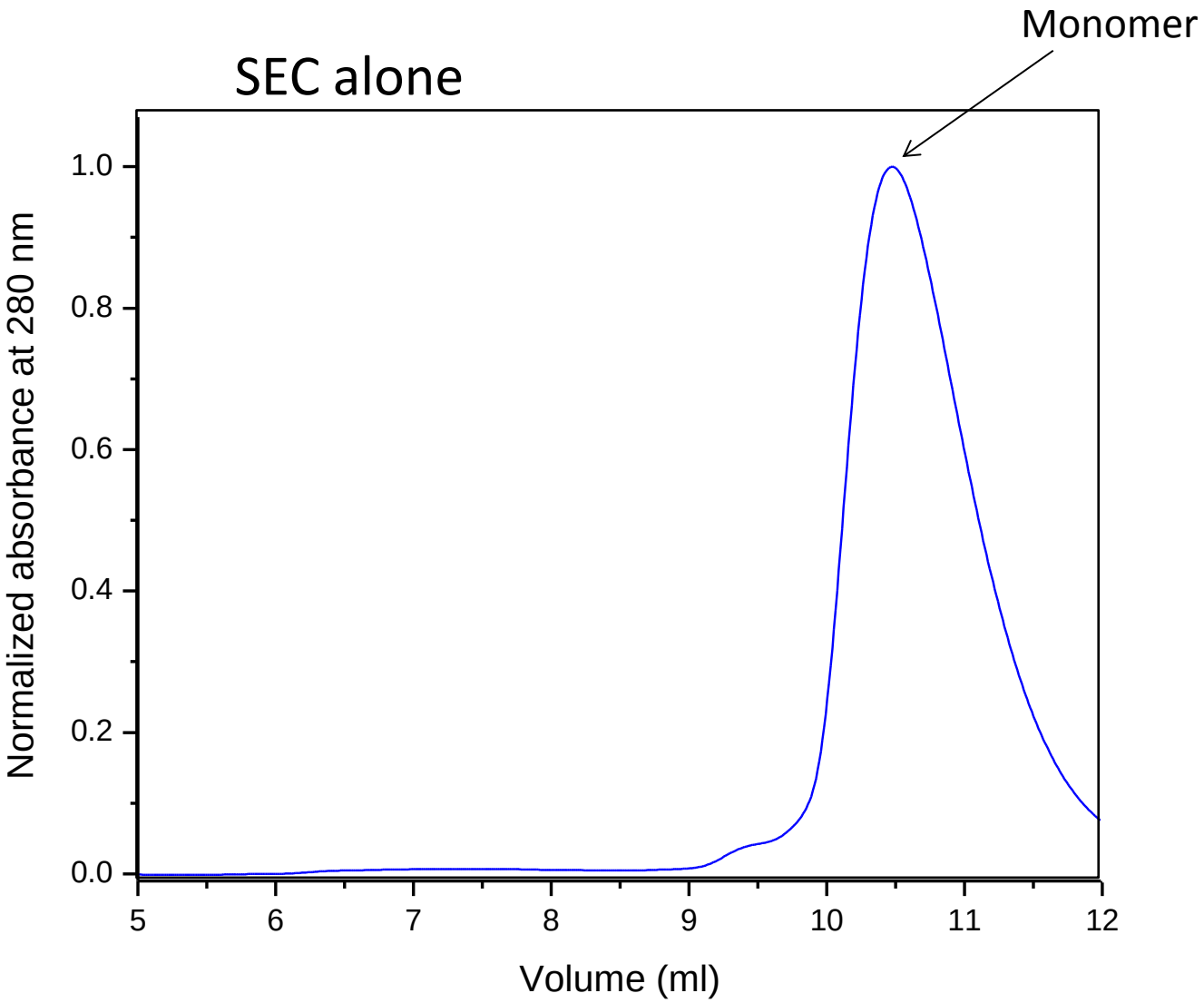
Homogeneous and heterogeneous of a sample



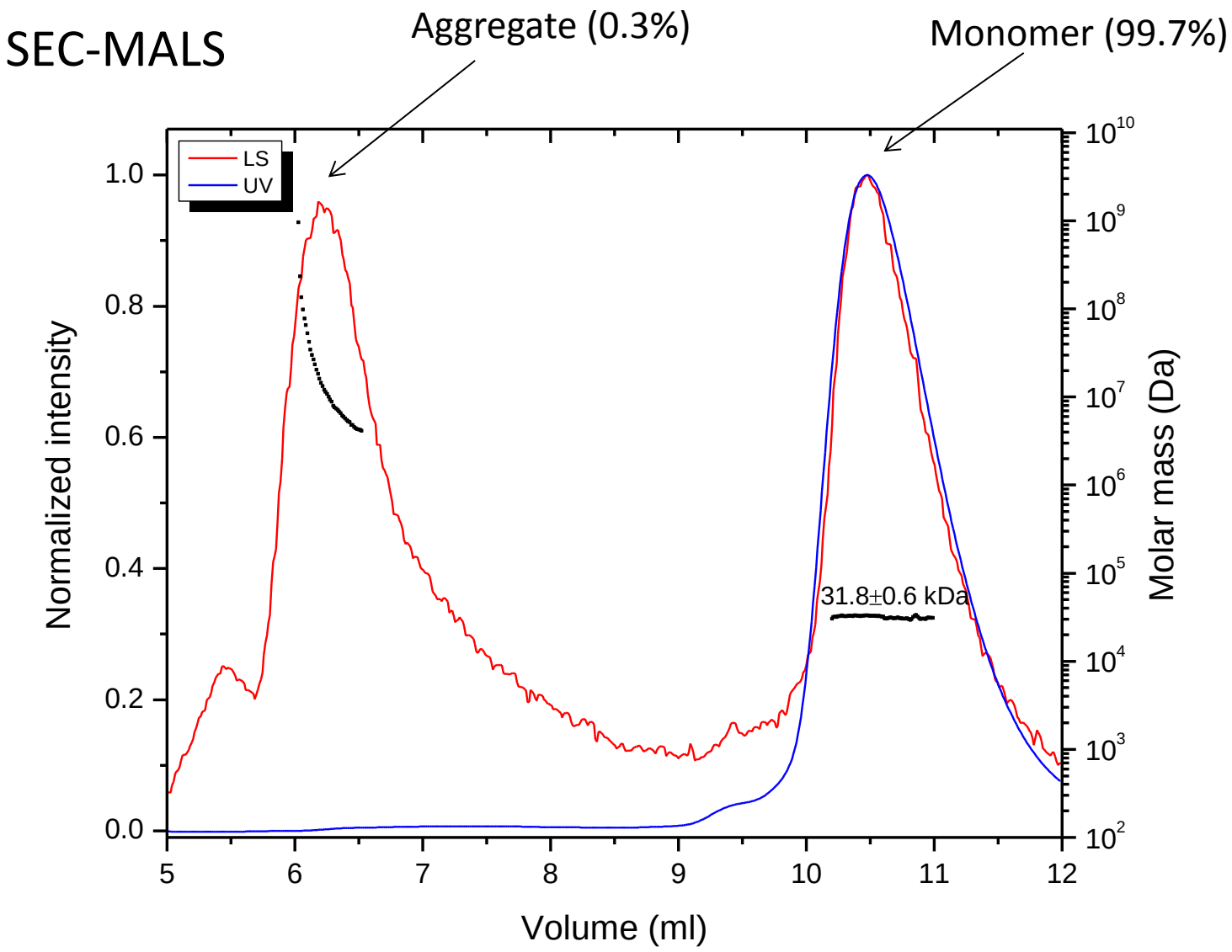
Characterizing oligomeric states



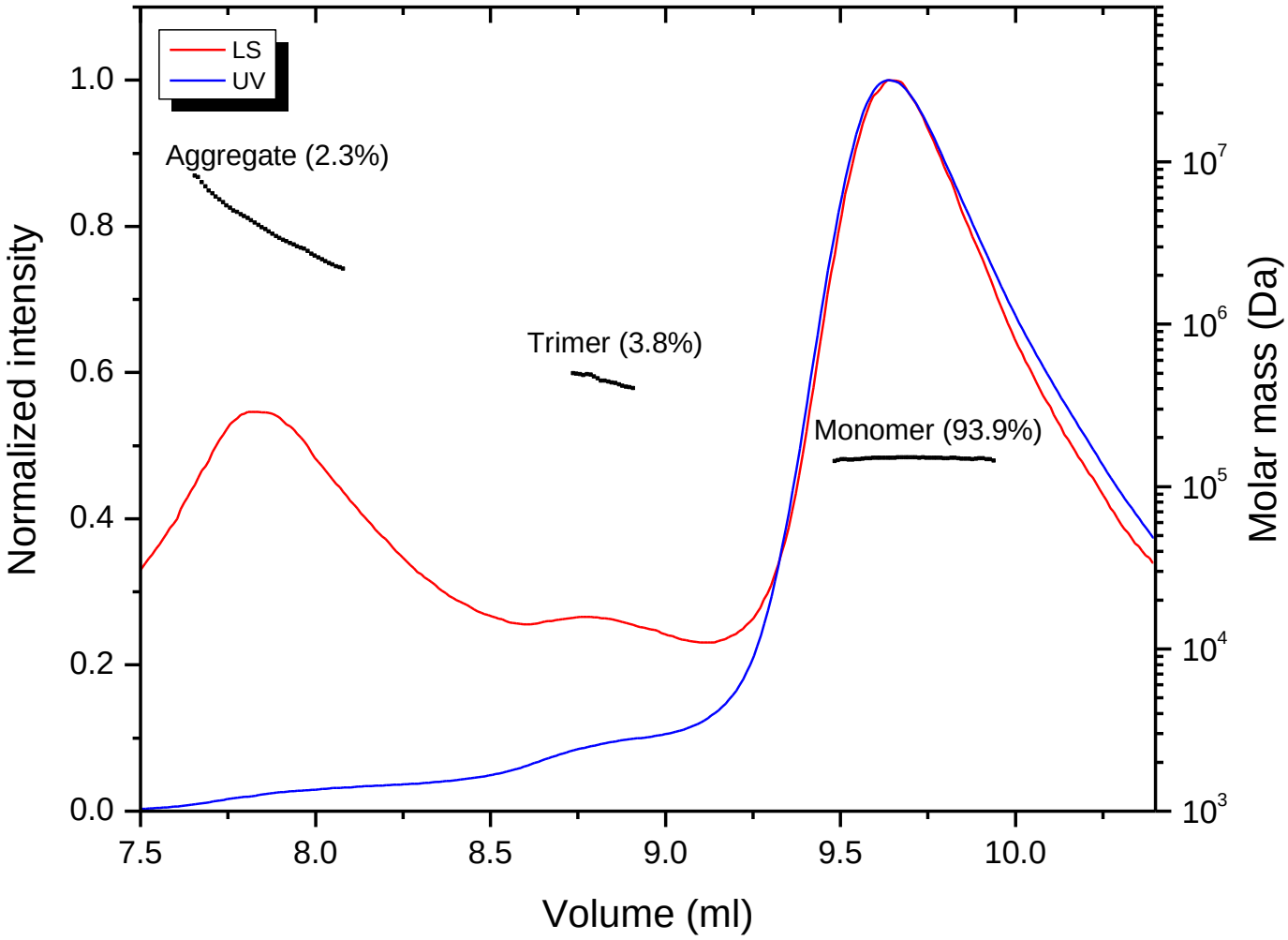
Amplification of aggregate intensity



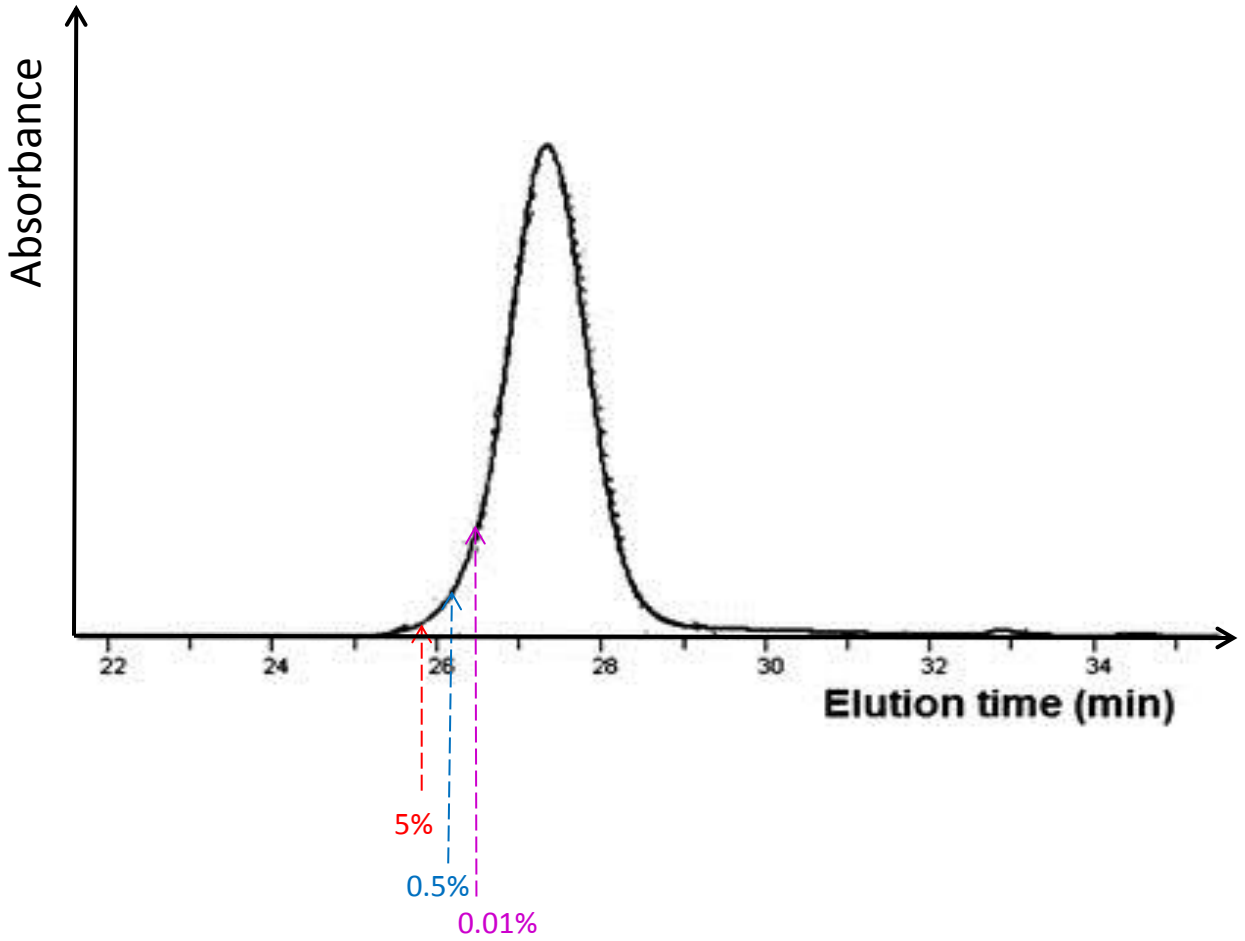
Amplification of aggregate intensity



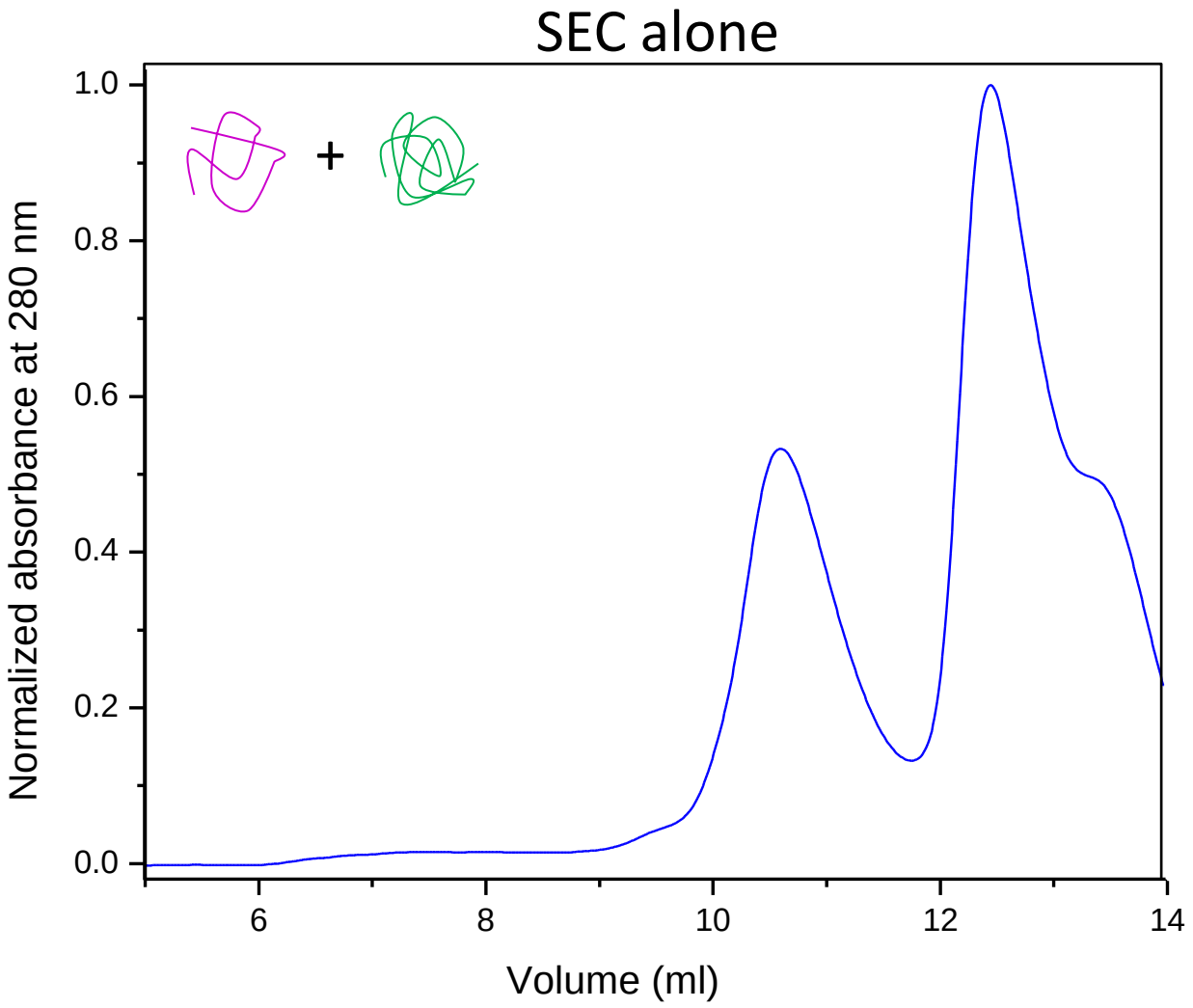
Detecting presence of aggregates – protein quality



Downstream application in industry – measure aggregation percent



Proteins with the same mass elute differently in SEC due to their shape



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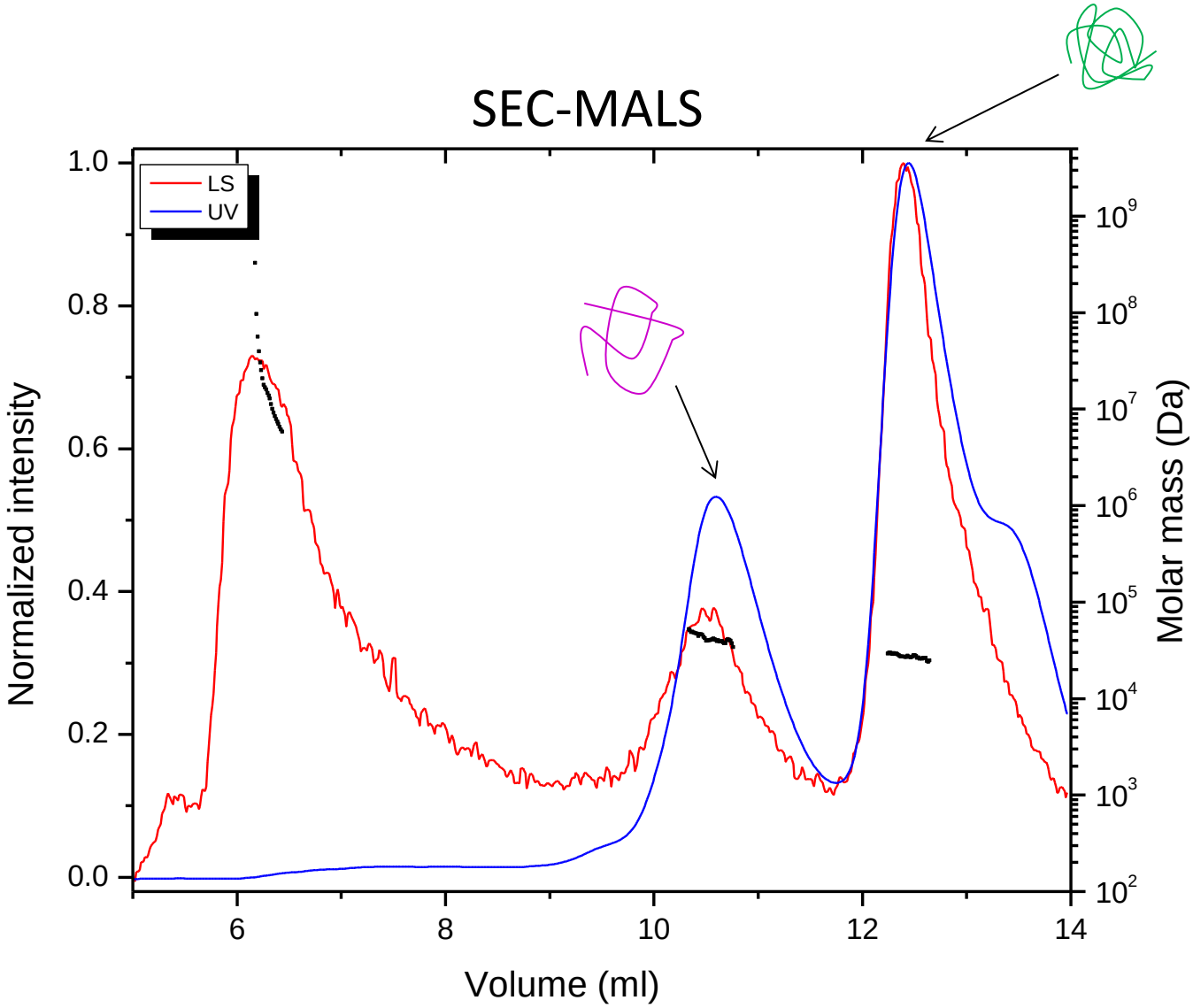
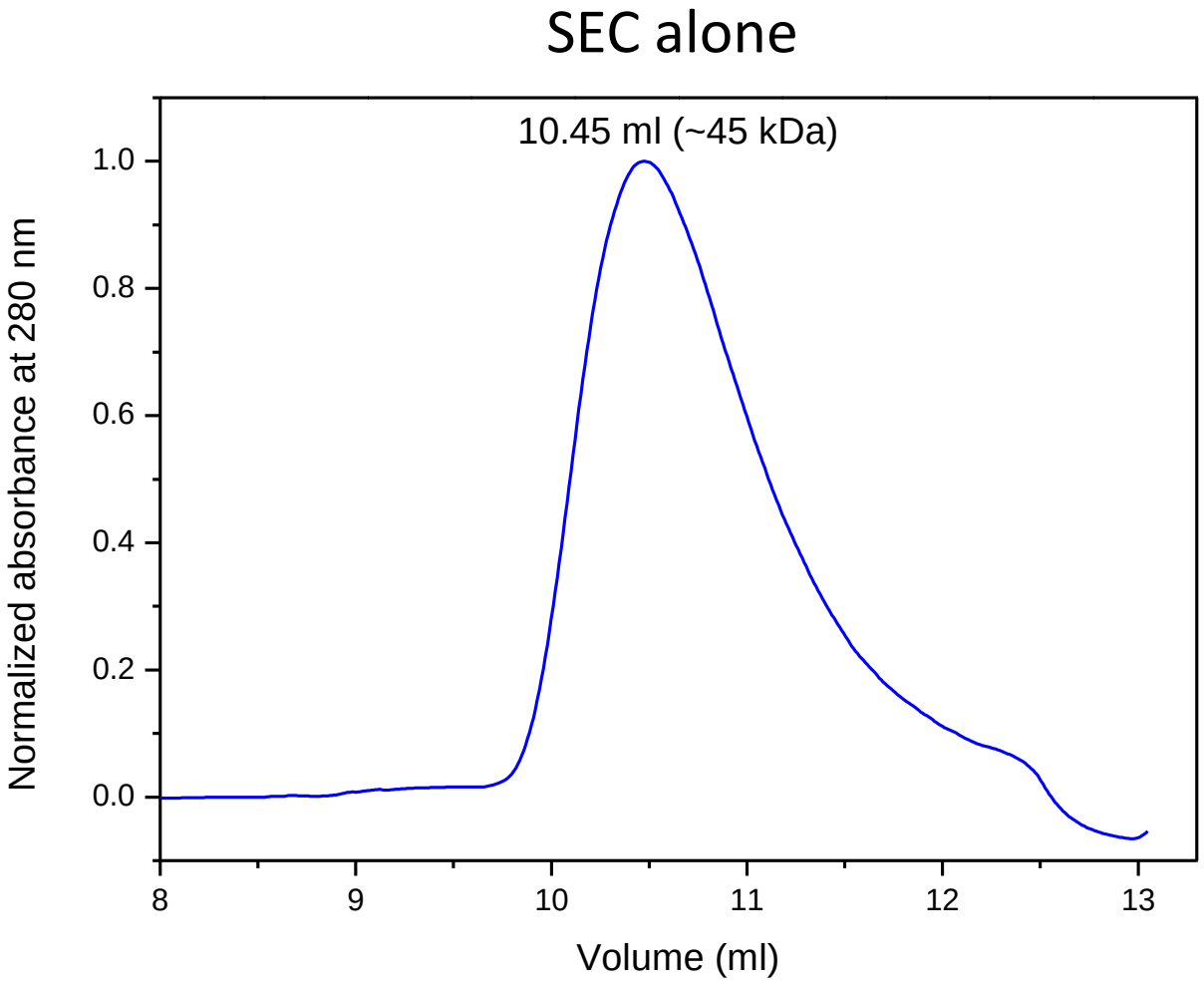
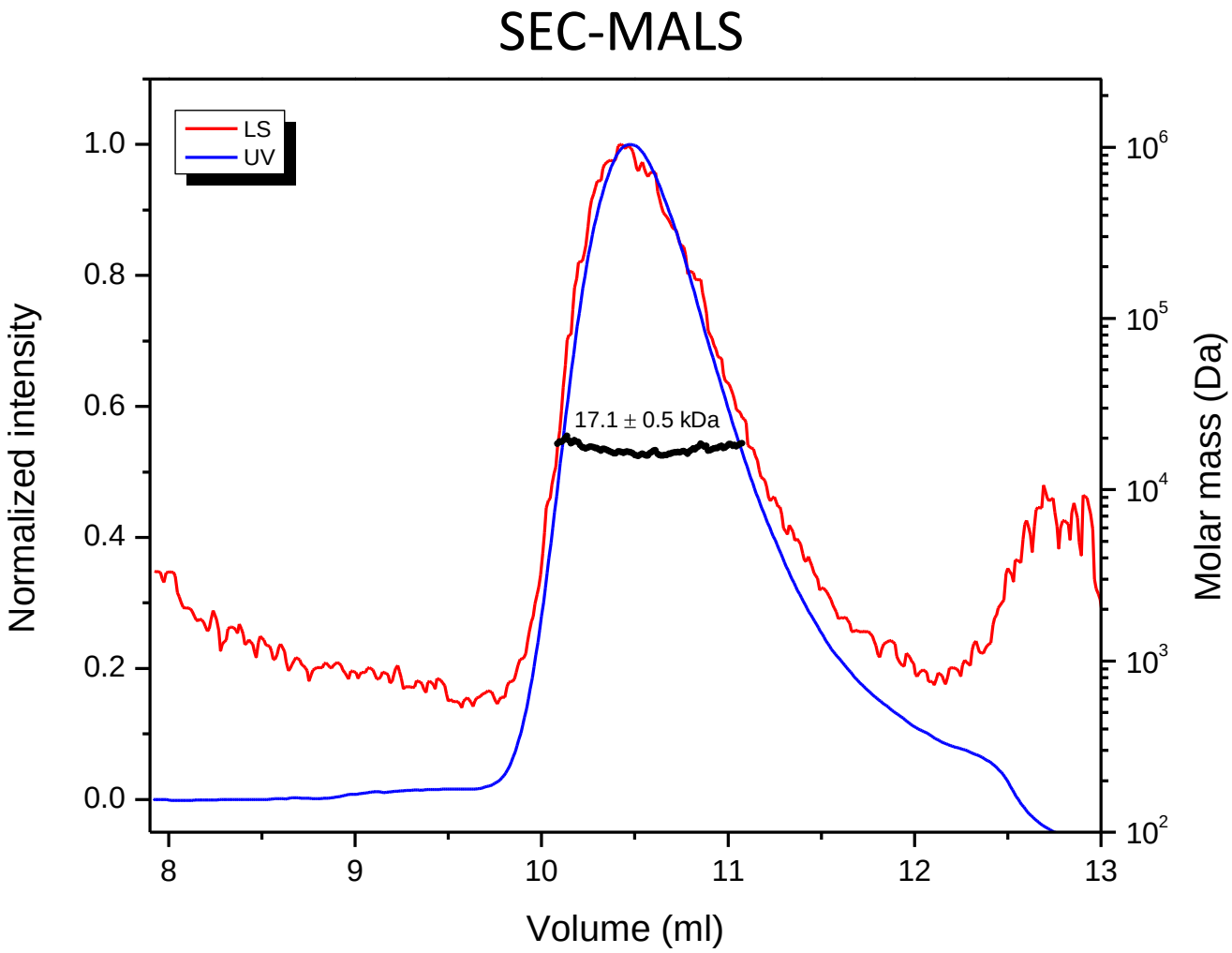


Calculating mass of disordered protein – using a calibration curve



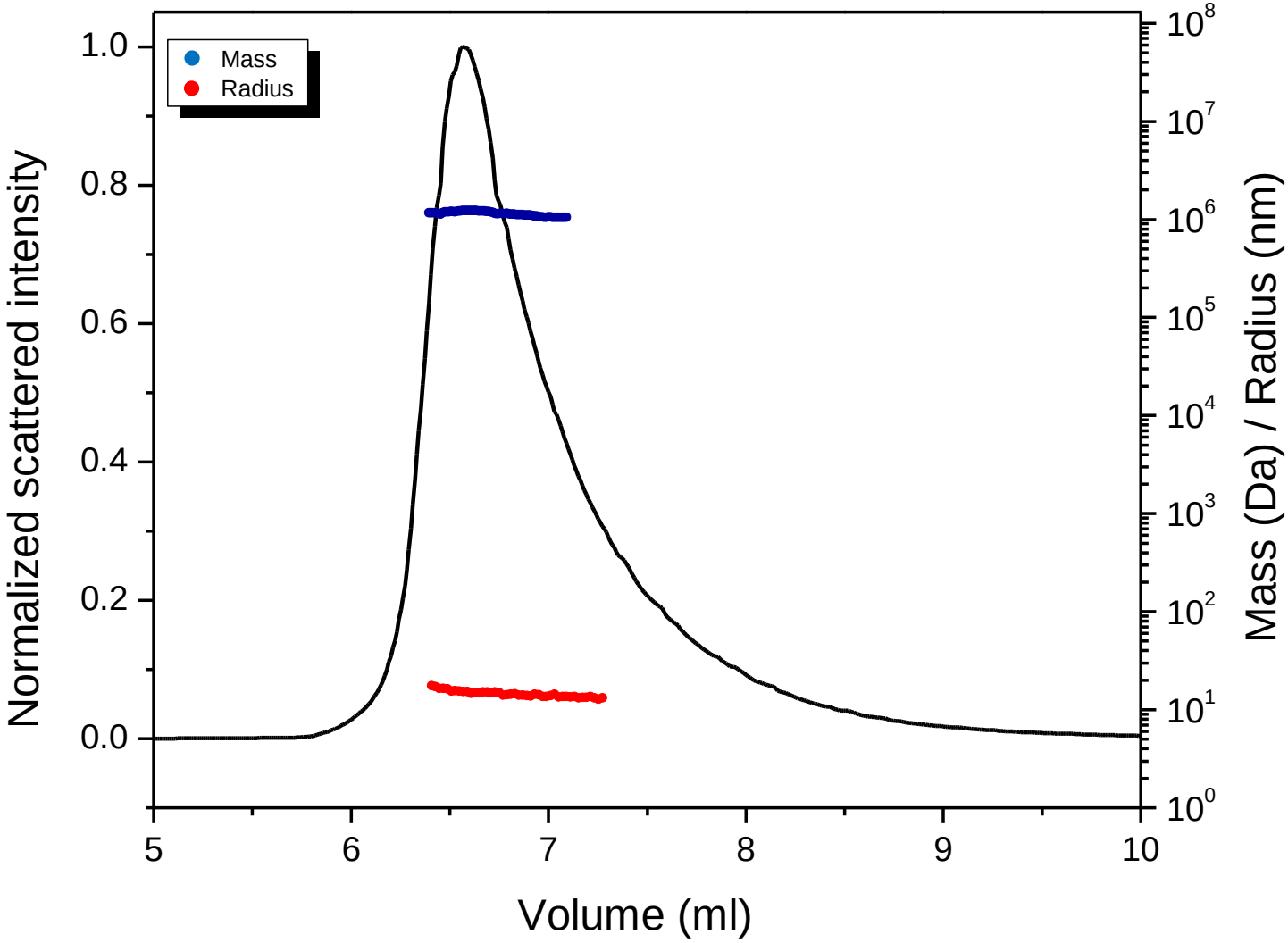
Protein Mw = 17 kDa

Calculating mass of disordered protein – using MALS

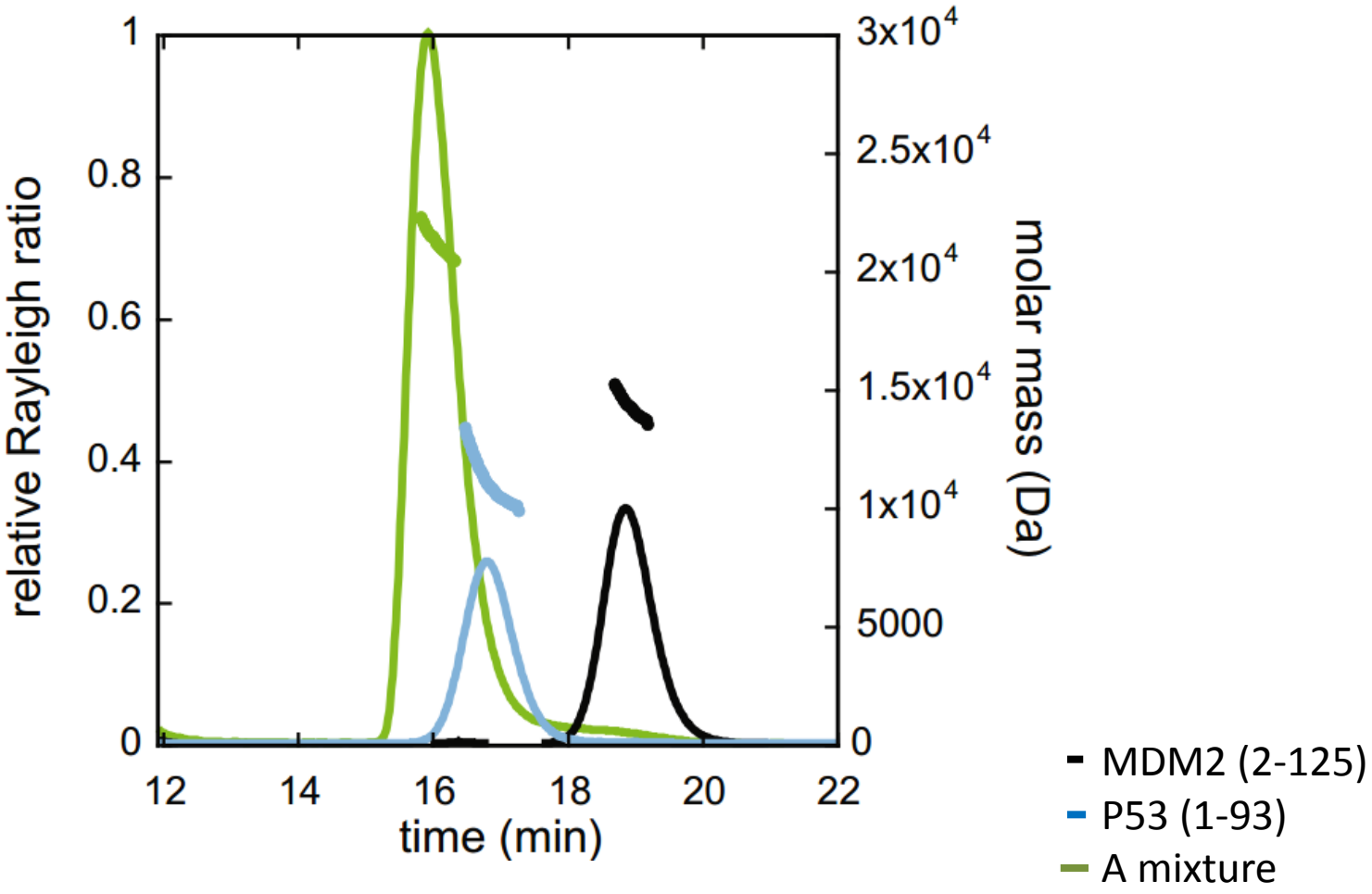


Protein Mw = 17 kDa

Calculation of mass and radius



Studying protein-protein interactions



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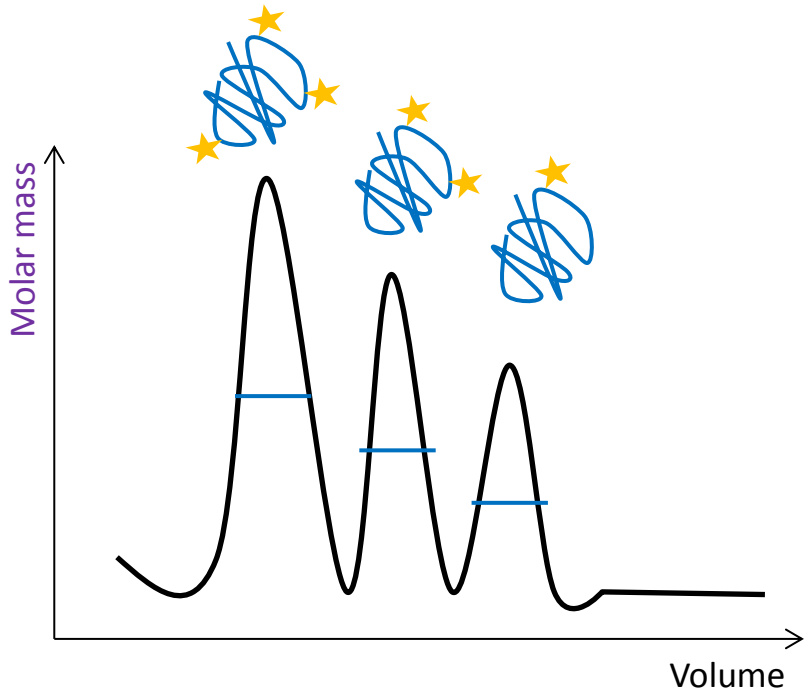
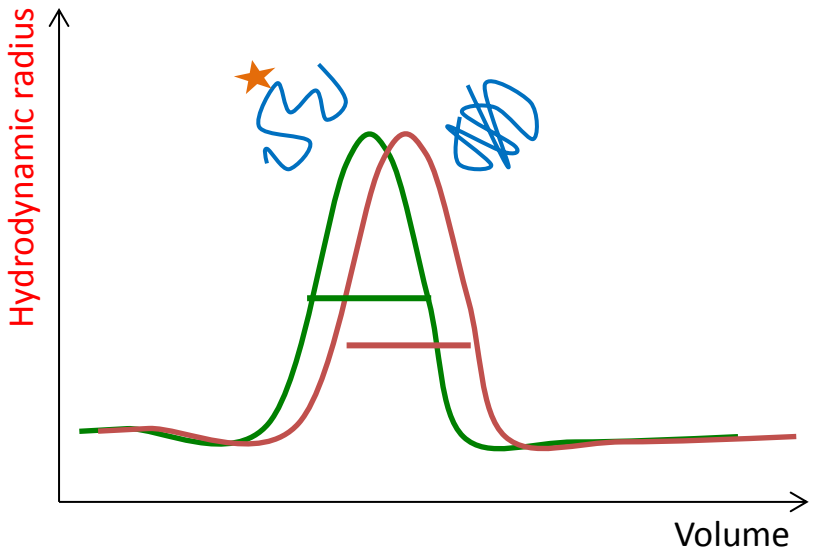
Studying protein modifications

- Use to study protein modification such as glycosylation and pegylation.
- Can be used to characterize number of modifications.
- Can be used to study structural changes in modified proteins.





Summary

- SEC-MALS is a powerful tool to study protein structure – shape and mass.
- Used to characterize protein oligomerization/aggregation, protein shape, changes in protein mass or shape caused by protein/ligand interaction or by modification or by environmental conditions.
- Limitation: can detect low amount of large macromolecules but needs high concentration of small macromolecules.