

A Light Scattering Toolbox for Characterizing Transport Proteins and their Interactions

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Summary

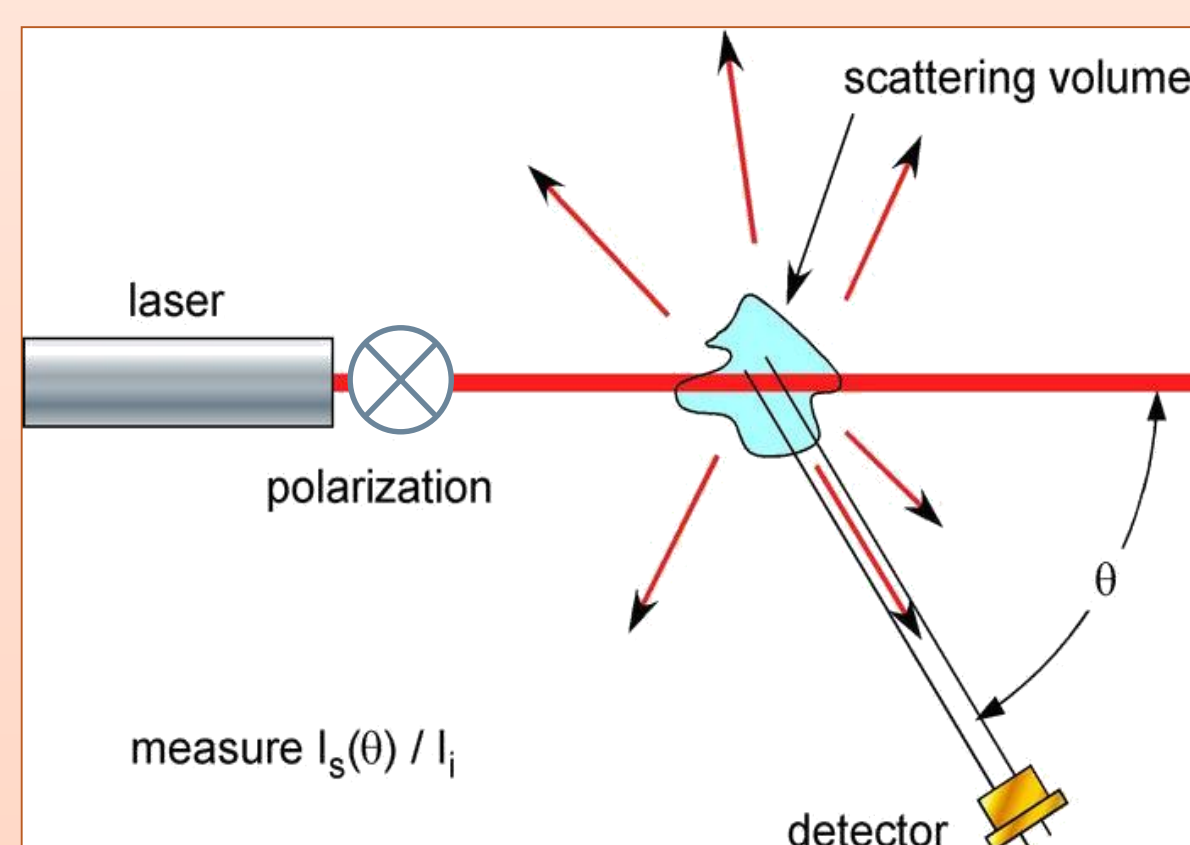
Understanding the function and behavior of transport proteins often requires detailed characterization of the protein's structure. Multi-angle light scattering (MALS) is uniquely capable of measuring the absolute molar mass of these proteins and their complexes in solution, providing direct assessment of their oligomeric state. Size exclusion chromatography (SEC) coupled with MALS, UV, and refractive index (RI) detection of a transmembrane protein solubilized in detergent reveals the mass of the protein complex and can distinguish the protein mass from the associated detergent micelle. Dynamic light scattering (DLS) performed in the same measurement volume enables quantification of the hydrodynamic radius of the protein or complex. In addition, composition-gradient MALS (CG-MALS) can be used to determine the affinity and stoichiometry of reversible interactions among soluble protein ligands, extracellular and cytosolic domains which may influence gating or transport behavior.

Light Scattering Measurements

- Multi-angle static light scattering (MALS) measures absolute molar mass (M_w) in solution:

$$I_{scattered} \propto M \cdot c \cdot \left(\frac{dn}{dc}\right)^2$$

- Dynamic light scattering (DLS) measures hydrodynamic radius (R_h)



CG-MALS (+DLS)



Analysis of CG-MALS data provides

- Self- and hetero-association, affinity and stoichiometry
- Nonspecific interactions, both attractive and repulsive
- Reversible and irreversible aggregation kinetics

The Calypso system combines hardware and software for unattended CG-MALS experiments.

SEC-MALS (+DLS)

Analysis of SEC-MALS data provides

- Absolute M_w and radius in solution
- Oligomeric state and polydispersity
- Characterization and quantification of aggregates
- Protein conjugate/copolymer analysis

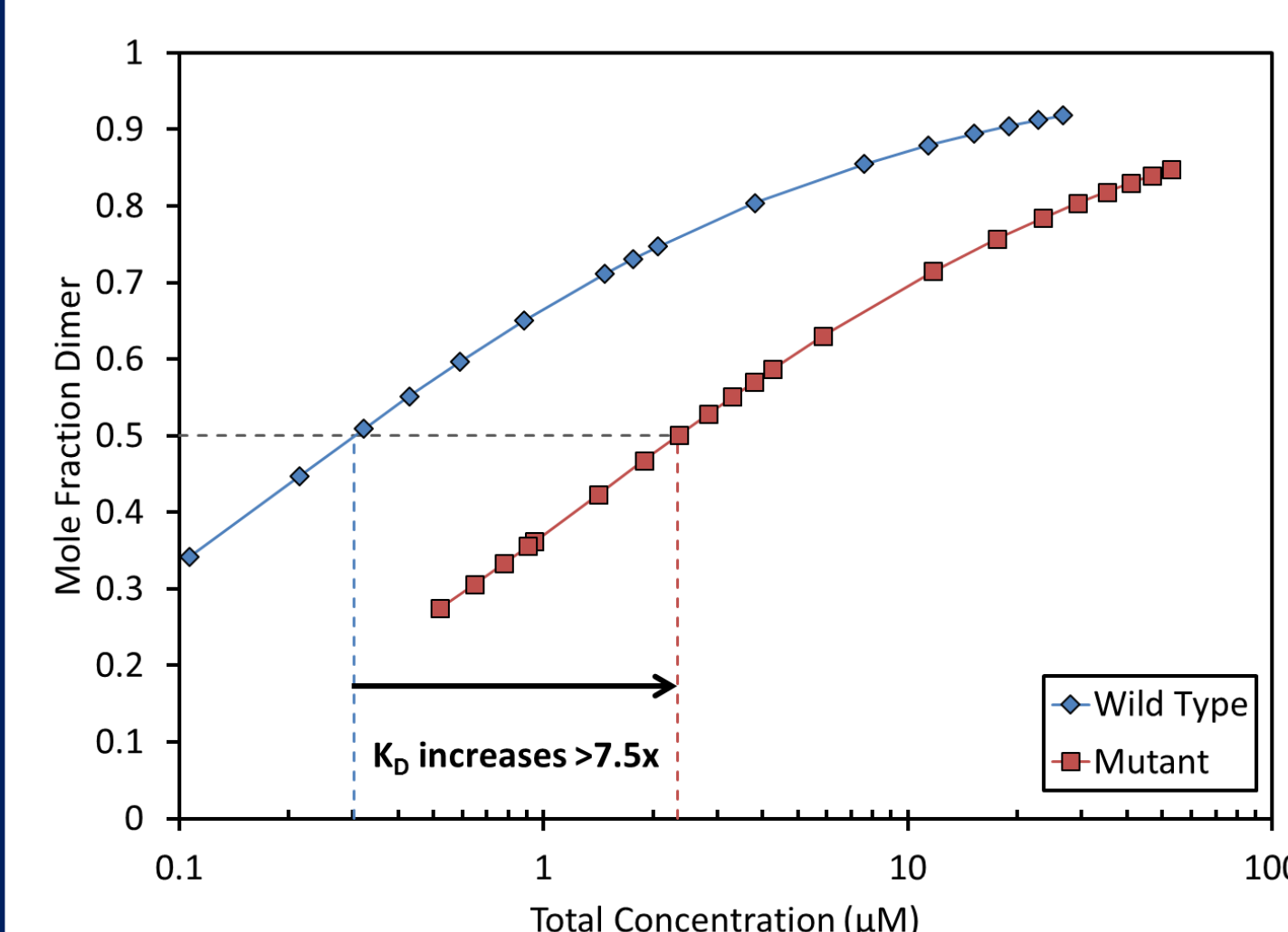
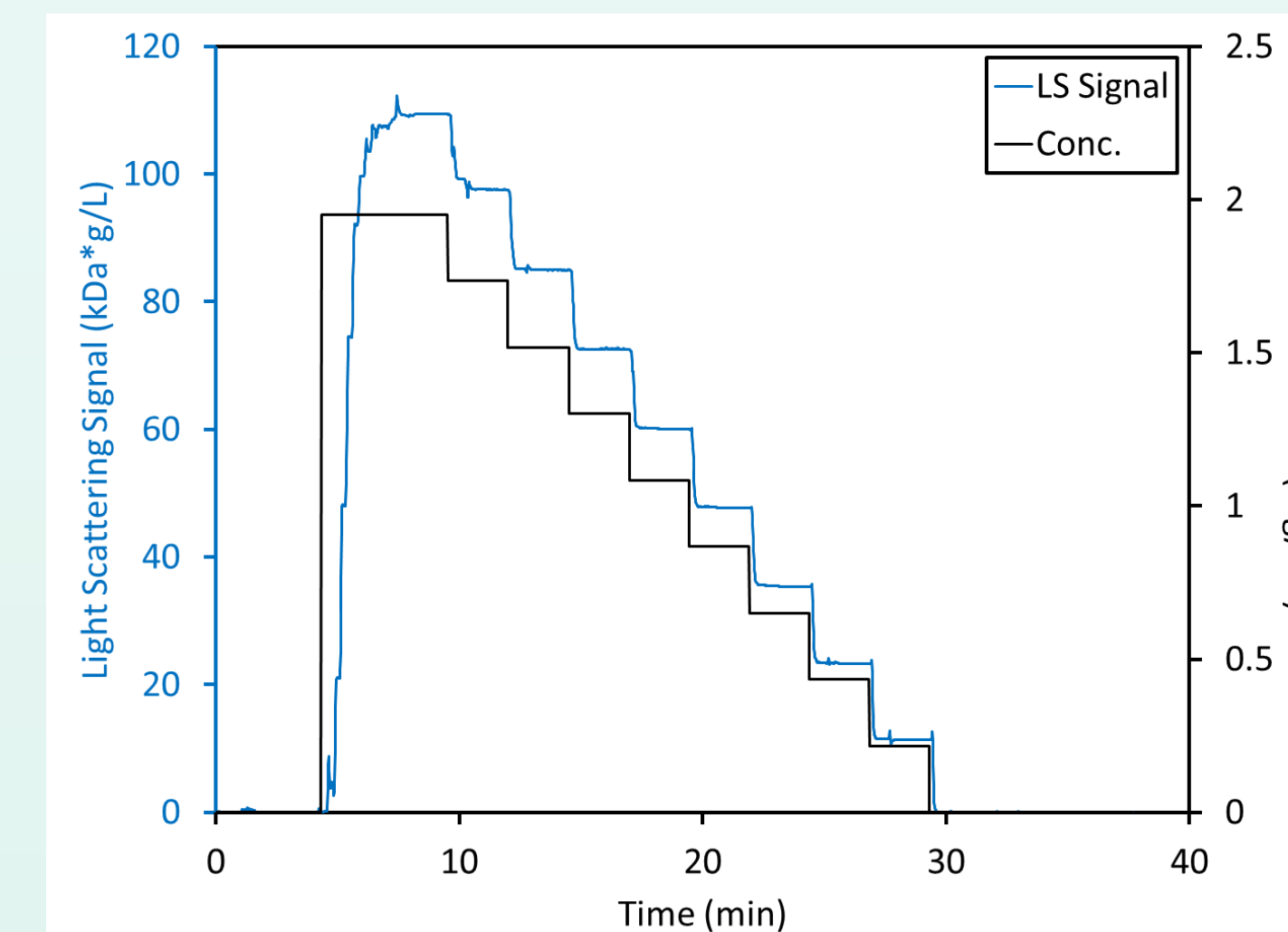


MALS and RI detectors can be added to any HPLC system.

CG-MALS of an Ion Transporter

CG-MALS Methods:

- The Calypso delivers specific compositions of sample and buffer to light scattering and concentration detectors.
- The flow is stopped after each injection to allow the mixture to come to equilibrium within the detectors.
- Three gradients were performed for each protein, measuring MALS and DLS as a function of concentration from $\sim 3 \mu\text{g/mL}$ to 3 mg/mL .

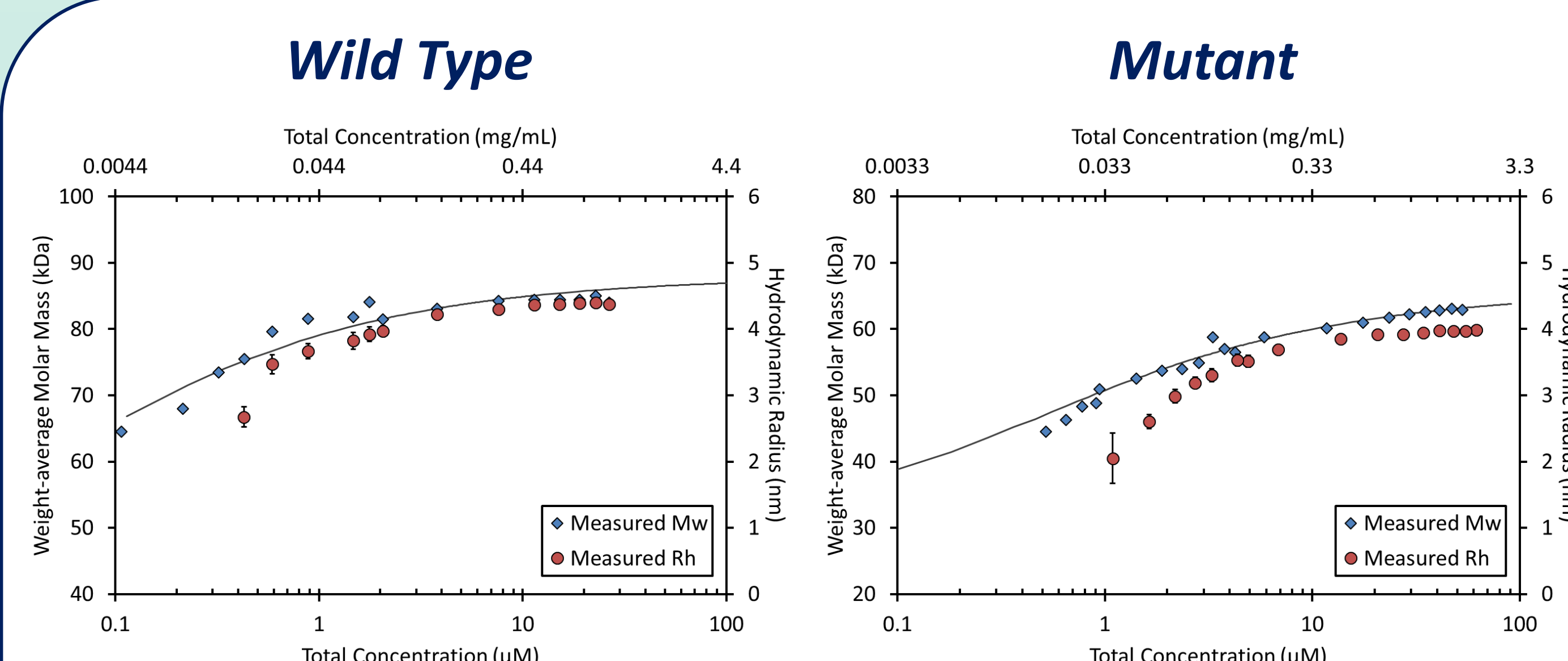


Samples:

- Soluble N-terminal domain of an ion transporter protein, which facilitates dimerization of the transporter
- Wild type monomer: 44 kDa
- Mutant monomer: 33 kDa

Results:

- Both N-terminal domains exhibit monomer-dimer equilibrium
- Deletion decreases monomer-dimer affinity from $K_D = 0.1 \mu\text{M}$ (wild type) to $K_D = 0.8 \mu\text{M}$ (mutant).
- No other higher order oligomerization was observed under these conditions for either species.

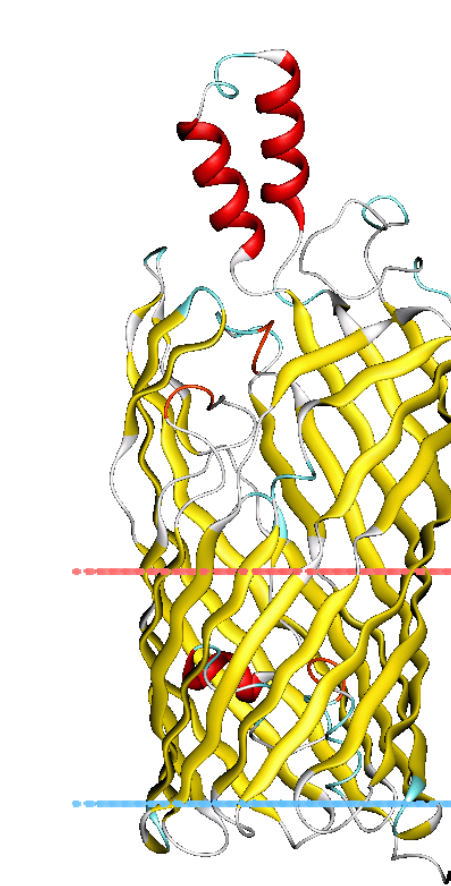
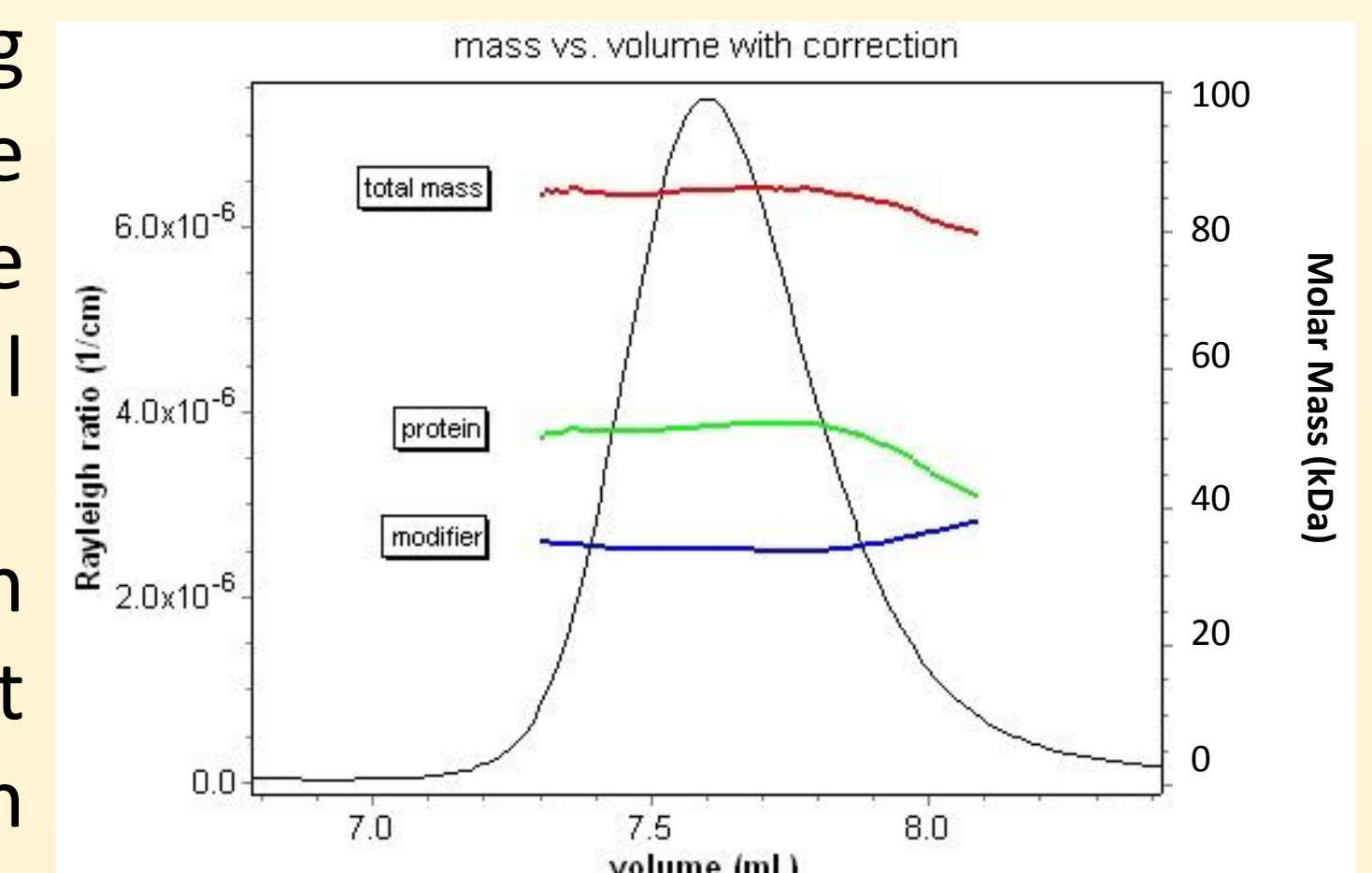


The mutant M_w increases $\sim 40\%$ across the entire concentration range tested, whereas the wild type M_w is nearly constant for concentrations $>1 \mu\text{M}$, indicating most of the solution has already dimerized. The gray line shows the best fit of the CG-MALS data for the monomer-dimer equilibrium.

SEC-MALS of a Lipid Transporter

Protein Conjugate Analysis:

- By combining light scattering with UV and RI detection, the total molar mass can be separated into its individual protein and modifier parts.
- Since the total concentration measured by UV and RI must be equal, the protein fraction of a modified protein can be determined.

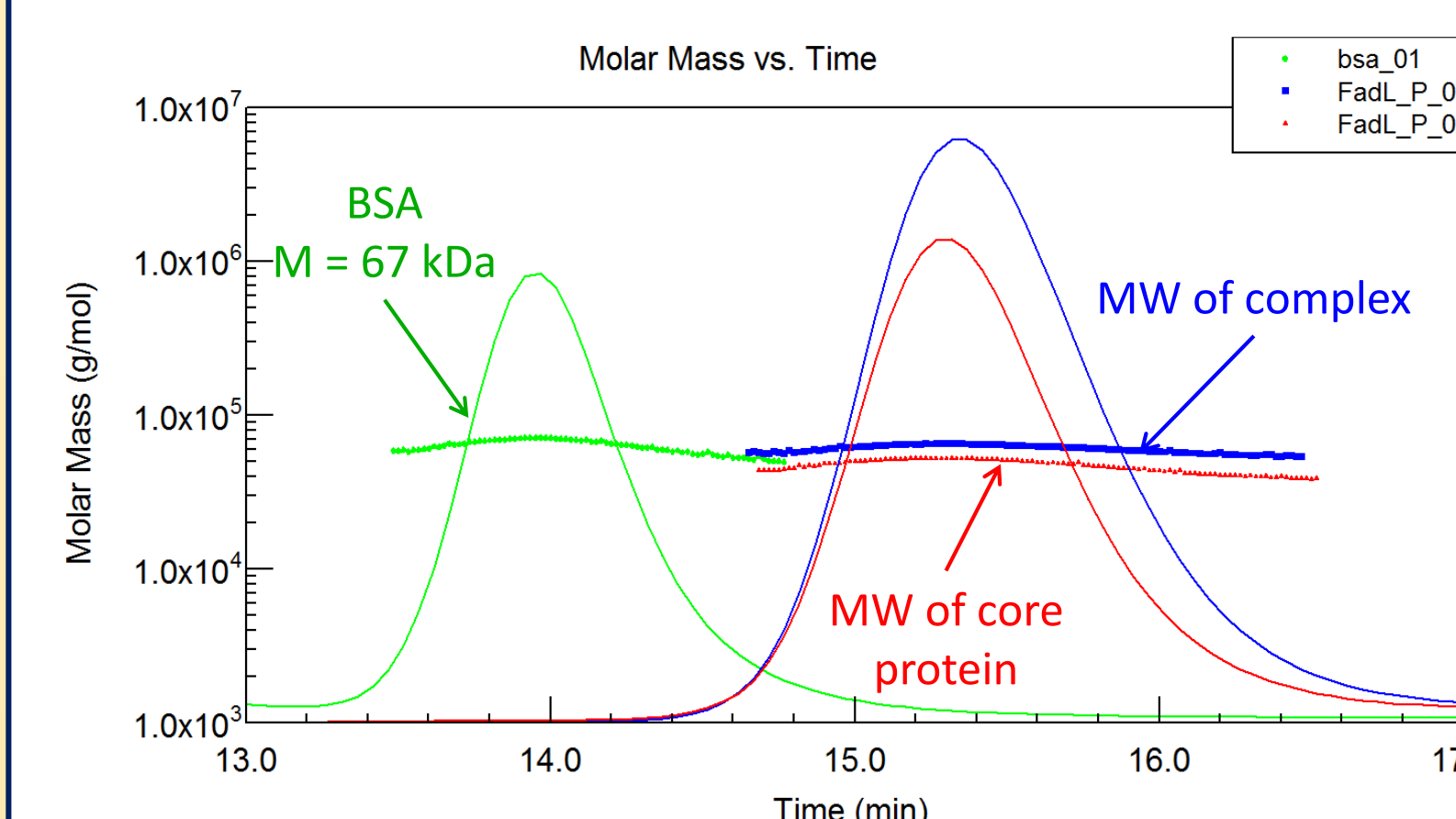


Samples:

- Outer membrane protein FadL
 - Expected protein monomer: 48 kDa
 - Mobile phase detergent: 0.08% LDAO
- Yeast protein YEB
 - Expected protein monomer: 62 kDa
 - Mobile phase detergent: 0.1% DHPC

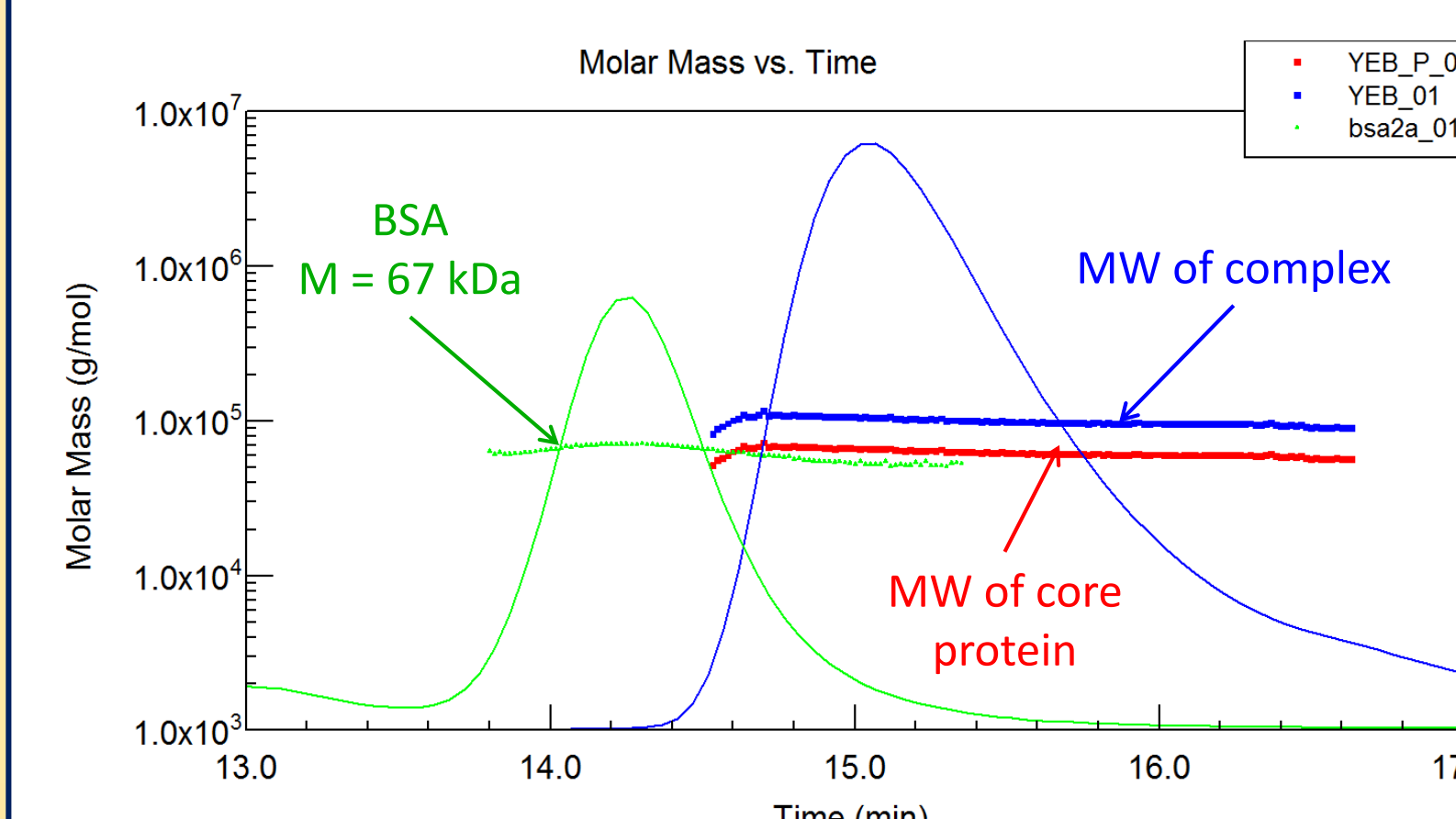
Results:

The analysis based on data from LS, UV, and RI detectors revealed the the MWs for core protein and protein-detergent complex for each sample. The results from BSA are also shown in green to demonstrate that the SEC properties of these two samples are very different.



FadL:

- Core protein MW: **48 kDa**
- Complex MW: **81 kDa**
- Weight fraction protein: 60%
- Detergent molecules per complex: 144



YEB:

- Core protein MW: **62 kDa**
- Complex MW: **97 kDa**
- Weight fraction protein: 64%
- Detergent molecules per complex: 72