

## Stoichiometry of Lysin Holoenzyme

Lysins are enzymes produced by bacteriophages that lyse the host's cell wall to allow the release of newly formed phage progeny. The streptococcal C1 bacteriophage lysin, PlyC, is composed from two subunits: a big, 50 kDa, "catalytic" subunit and a small, 8 kDa, "binding" subunit. Neither the cross-linking experiments, nor labeling the enzyme with  $^{35}\text{S}$ -methionine provided results that would unambiguously define the stoichiometry of the PlyC holoenzyme. We have used size exclusion chromatography coupled with MALS, refractive index and absorbance detection (SEC-MALS/RI/UV) to define the subunit organization of PlyC.

During SEC-MALS/RI/UV analysis PlyC eluted as a single peak (Fig. 1) with molar mass of  $114 \pm 0.4$  kDa, which suggested a complex composed of *one* large subunit of 50 kDa and *eight* small subunits of 8 kDa each. Alternatively, a complex of two big subunits and two small subunits yields a mass of 116 kDa.

To discriminate between these two possible models we measured the extinction coefficient "on-line" for the eluting complex utilizing signals from the refractive index detector and the absorbance detector. The large subunit of 50 kDa has a very high extinction coefficient because it contains 13 Tryptophans, 28 Tyrosines and 6 Cysteines while the small, 8 kDa peptide contains only 2 Tyrosines, thus producing very low absorbance at 280 nm. Hence, the two possible complexes with either one or two large subunits in a holoenzyme, would differ *significantly* in their extinction coefficient at 280 nm. The measured extinction coefficient for PlyC protein matched well with the extinction coefficient computed for the complex that contains one "catalytic" subunit and eight "binding" subunits.

These SEC-MALS/RI/UV results provide a coherent model for subunit organization for PlyC protein. Information provided from all three detectors—light scattering, refractive index and absorbance detector—were utilized to define the stoichiometry for the protein complex that constitutes PlyC holoenzyme.

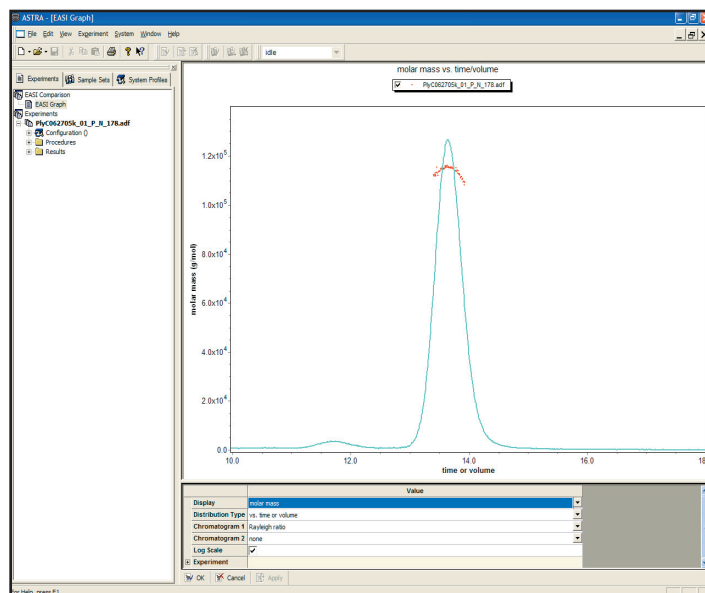
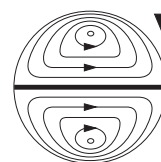


Figure 1. Molar Mass Distribution Plot from SEC-LS/RI/UV data for PlyC holoenzyme. Column Superdex 200, buffer: 20 mM HEPES, pH=7.4, 150 mM NaCl, 1 mM EDTA; UV trace—solid line, Molar Mass—filled circles (for clarity only every 5th measurement of molar mass across the eluting peak is plotted).

This note graciously submitted by Dr. Ewa Foltá-Stogniew, Yale University, Biophysics Resource of Keck Facility, New Haven; protein provided by Dr. Daniel Nelson, Rockefeller University, New York



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