

Ensuring Protein Reagent Quality by SEC-MALS

Quality and consistency in reagents is critical to successful drug discovery and development. When targeting a particular protein of interest, *in vitro* experiments should be performed with proteins of biological properties similar to those for *in vivo* tests. It is important that molecularity, purity, shape and degree of heterogeneity remain the same when any alterations are made to the model protein or the formulation buffer. Multi-angle light scattering (MALS) combined with size exclusion chromatography (SEC-MALS) is a very useful technique to monitor the solution properties of the protein as changes to reagents are made.

As an example, crystallization studies typically have a higher rate of success when the proteins involved are simplified, e.g., truncated or expressed in bacteria to minimize post-translational modifications. However, these forms may not be amenable to traditional characterization such as plate-based assays and certain biophysical techniques, where native protein is preferred. Affinity tags or changes to buffer excipients may also be required. All of these differences increase the likelihood of altering solution properties.

The example in this note shows how light scattering data obtained with a **DAWN**® MALS detector and analyzed by **ASTRA**® software (both from Wyatt Technology, Santa Barbara, CA) were used to elucidate the solution properties of protein expressed from two different constructs. Construct A is the shorter of the two, designed for crystallization efforts.

Presented with data from Figure 1, combined with knowledge that the enzyme showed activity and was the predominant species in mass spectrometry, one might not question the suitability of this reagent. SEC-MALS data (Figure 2), however, show that this enzyme is in fact quite heterogeneous.

Construct B addressed this; it is the full-length enzyme. MALS data show that the protein is in its biologically active form (dimer) and is highly homogeneous. This information gave the project team confidence to move forward with this construct for crystallography, NMR and High Throughput Screening (HTS) efforts.

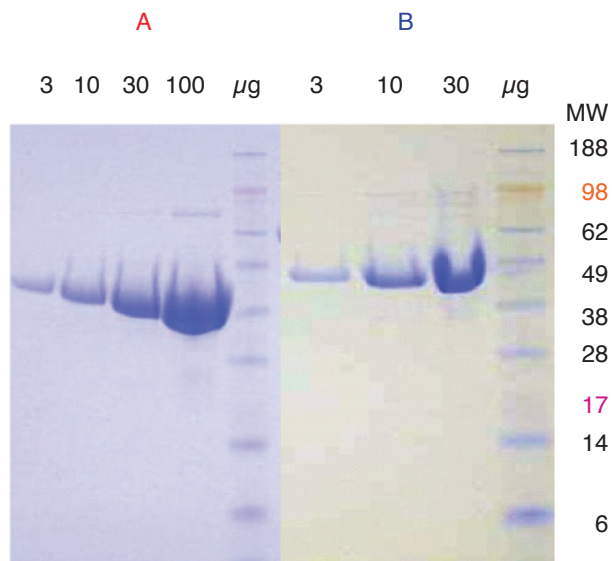


Figure 1: Two protein constructs: A (45 kDa) and B (46 kDa). SDS-PAGE shows similar purity.

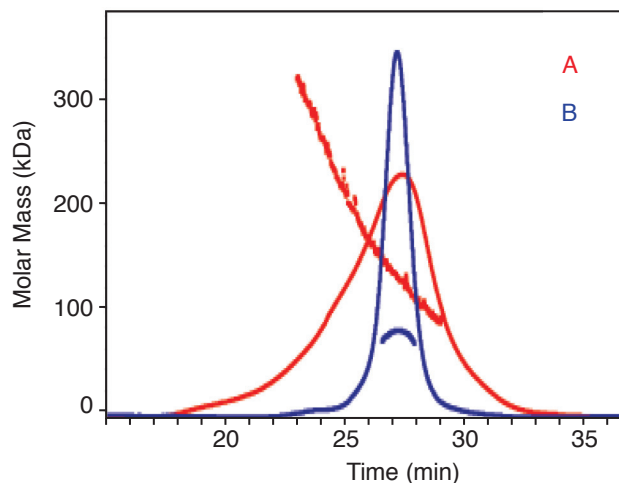


Figure 2: Standard chromatographic trace ($UV_{208\text{nm}}$) with MALS data (linear traces within the chromatograms represent the mass distribution across the peak). The enzyme from construct B is much more homogeneous, and is in its biologically active form of a dimer (89 kDa).

This note graciously submitted by Mark Tardie and George Karam; Pfizer Central Research, Groton, CT.