

Protein-Protein Interactions

Over-expression of recombinant proteins is commonly used for the production of protein reagents in industry and academia. Problems often occur relating to the stress put on the cells to deal with this huge increase in synthesis. Cellular proteins that are part of the protein synthesis machinery are often up-regulated under such conditions. Large quantities of the recombinant protein can be bound to these cellular proteins, making purification difficult.

Scale-up purification of a 61kDa recombinant protein was found to contain a significant contaminant (Figure 1), not found in the smaller scale trials. The 28kDa protein was identified by Edman degradation as rotamase, an enzyme that assists in protein folding. Co-purification of the rotamase suggests it is bound to the protein of interest, thus preventing further purification with techniques such as size exclusion chromatography (SEC).

Analytical size exclusion chromatography, followed by on-line direct mass detection by multi-angle light scattering (MALS), showed that a significant amount of the recombinant protein was *not* bound to the rotamase.

The sample was separated with a YMC-pack Diol-200 column, and detected by Wyatt Technology's 18-angle DAWN photometer and the Optilab-DSP Interferometric Refractometer.

Three peaks were isolated which contained 93kDa, 68kDa and 28kDa species (Figure 2). The DAWN was able to identify the peaks in the following way: a non-covalent complex of one rotamase molecule and one recombinant protein molecule (peak 1), the free recombinant protein (peak 2) and the free rotamase (peak 3).

Estimating the molar masses of the proteins using columns calibrated to a set of protein standards (BioRad) resulted in significantly *different* values for the three peaks: 146kDa, 98kDa, and 46kDa, respectively. Thus, the information provided by MALS system—an absolute system because it is not calibrated with standards—contributed to the correct interpretation of the chromatogram, provided invaluable insight for development of the scale-up purification protocol and into the protein-protein interactions.

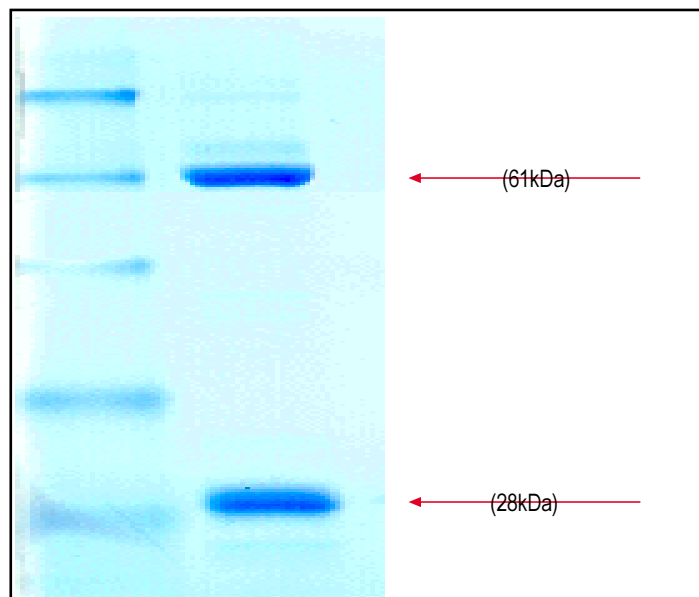


Figure 1. SDS-PAGE of a partially purified recombinant protein ($M_w = 61\text{kDa}$) containing a significant contaminant of the enzyme rotamase ($M_w = 28\text{kDa}$).

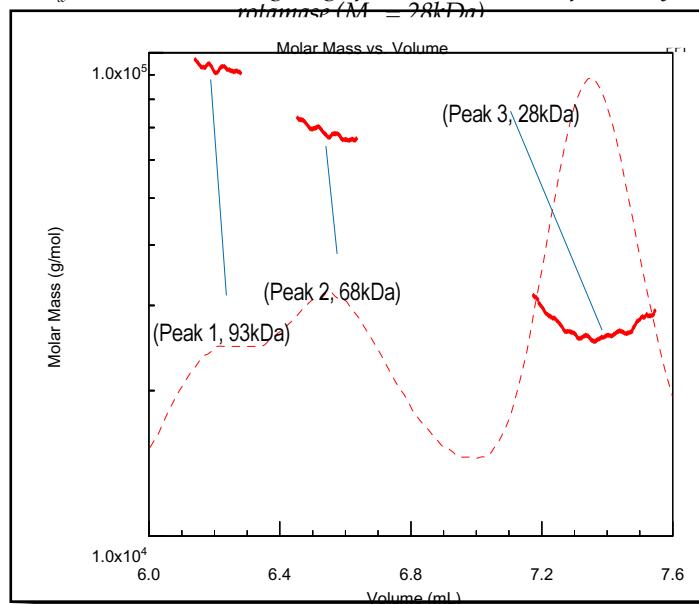
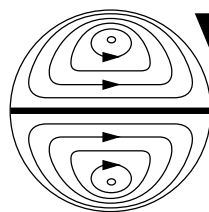


Figure 2. Peak 1, with a molar mass of 93kDa is one recombinant protein and one rotamase; Peak 2, with a molar mass of 68kDa, is a free recombinant protein; Peak 3 is a 27kDa free rotamase.

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