



**Figure 2.5** Panel A: Size distribution of a full-length mAb determined by (a) size exclusion chromatography and (b) analytical ultracentrifugation (AUC) sedimentation velocity. Panel B: Weight average sedimentation coefficient versus protein concentration observed during AUC sedimentation velocity analysis of the mAb in PBS. Panel C: Weight average molecular weight determined by AUC sedimentation equilibrium of the mAb in PBS.

Reproduced from [Andya et al. \(2010\)](#).

### Light scattering

Light scattering is an alternative biophysical method to determine molecular weight, size, and information of protein interactions. When used as a time-averaged method, referred to as static light scattering, SLS, the main information is weight average molecular weight and shape information. Characterization of protein–protein interactions can also be done using determined second and third virial coefficients. This is obtained by measuring the scattering intensity as a function of angular dependence at a series of concentrations. When extrapolated to infinite dilution (zero concentration), the molecular weight and shape of the protein can be determined using a Zimm plot ([Zimm, 1948](#)). Since many proteins and aggregates are smaller than the wavelength of light used for the scattering measurement, usually scattering at  $90^\circ$  can provide molecular weight information. Although scattering can provide information on aggregates, even large mAbs that are not aggregating will have detectable scattering due to the size of the molecules ([Sukumar, Doyle, Combs, & Pekar,](#)