

Composition Gradient Static Light Scattering:

A Powerful Tool for the Detection and Characterization of Reversible Macromolecular Associations

By means of a programmable dual syringe infusion pump, we introduce a solution of time-varying composition into a combination of light scattering and absorbance flow detectors that is capable of *simultaneously* measuring the absorbance and light scattering of each element of volume as a function of time (Fig 1)^{3,4}.

From the combination of scattering and absorbance data (Fig 2) we calculate the Rayleigh ratio R scaled to an optical constant K as a function of the total concentration of a single protein or the total concentrations of each of two proteins in a mixture over a broad range of composition (Fig 3).

In a solution containing one or two macromolecular components, the measured dependence of R/K upon the total weight/volume concentration of each component may be modeled by an equilibrium association scheme specifying the concentration of each individual species as a function of the molecular weight of each monomeric species and the equilibrium association constants relating the molar concentrations of each oligomeric species to the concentrations of constituent monomers. The correct self- and/or hetero-association scheme, and the best-fit values of monomer molecular weights and equilibrium constants are determined by least-squares fitting of candidate models to the experimental data.

Validation experiments have shown that the method is capable of obtaining values of monomer molecular weights and association equilibrium constants that are precise *and* accurate. At present, approximately 0.5 - 1 mg of each protein is required for each experiment. Exclusive of the time required for sample purification and preparation, each composition gradient scattering experiment requires 5-10 minutes, and is therefore suitable for moderately high throughput screening for detection of associations in solution.

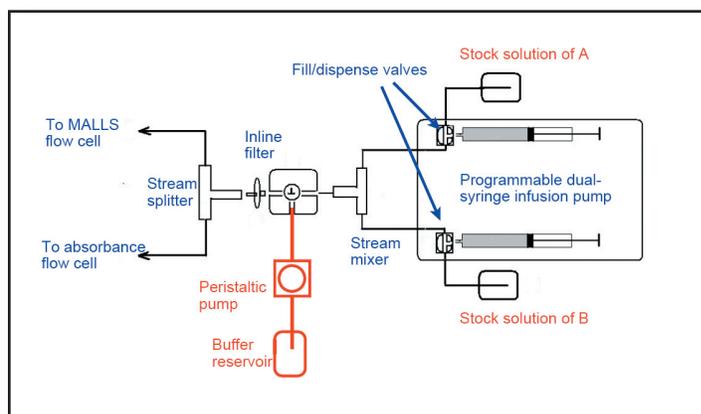


Figure 1. The experimental set-up.

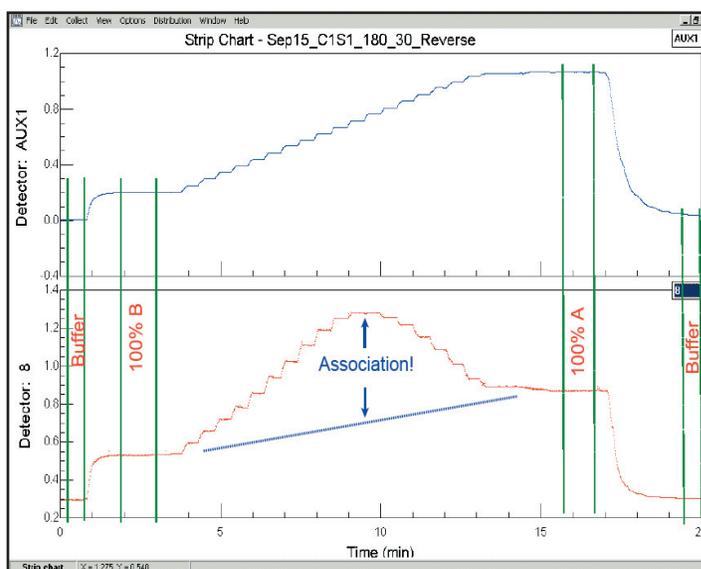


Figure 2. The ASTRA strip chart showing where association occurs.

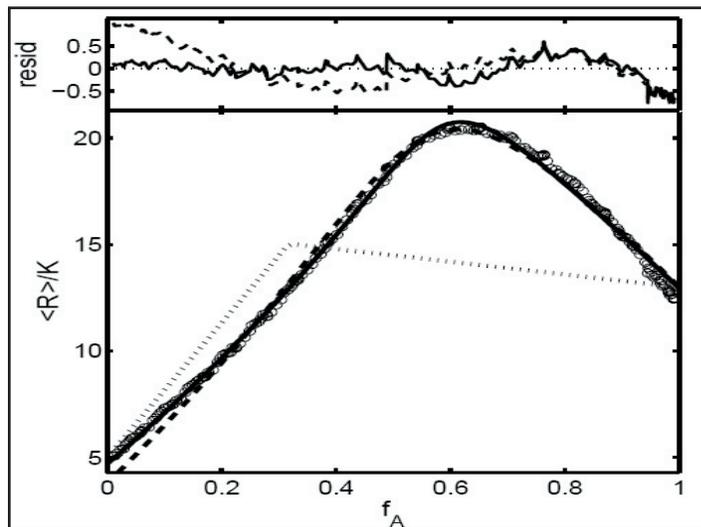


Figure 3. Normalized scattering plotted as a function of solution composition. Points: data; curves: best fits of different hetero-association models.

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