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Quantitative Characterization of Protein Associations in Highly Concentrated Solution[1]

ALLEN MINTON, National Institutes of Health

With few exceptions, one cannot reliably predict the behavior of a protein at high concentration on the basis of knowledge obtained from experiments carried out at low concentration. Detection and quantitative characterization of protein-protein interactions in the high concentration regime (> 50 g/L) therefore presents both experimental and theoretical challenges to the investigator. Two experimental methods devised in our laboratory specifically for this purpose are described. (1) *Non-ideal tracer sedimentation equilibrium.* Instrumentation and theory for measuring and interpreting the equilibrium gradient of a labeled dilute tracer protein in a solution containing an arbitrary concentration of one or more unlabeled macromolecules are outlined. The composition dependence of the equilibrium gradient of several proteins, including ribonuclease at concentrations up to 200 g/L and immunoglobulin G at concentrations up to 125 g/L, will be presented and interpreted in the context of models taking into account both equilibrium self-association, and nonspecific repulsive steric or electrostatic repulsion. (2) *Non-ideal light scattering.* Recently developed instrumentation and theory for rapid measurement and interpretation of the light scattering of a protein solution over a broad range of concentration are outlined. The concentration-dependent light scattering of chymotrypsin A at three different pH values at concentrations up to 60 g/L, and the concentration-dependent light scattering of two monoclonal antibodies at concentrations up to over 200 g/L in solutions of varying ionic strength, are quantitatively accounted for by models that take into account both nonideal repulsion between protein molecules and specific modes of equilibrium self-association.

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