PREFACE

Analytical ultracentrifugation (AUC) is a powerful method to characterize the size, shape, interactions, and homogeneity of macromolecules in free solution. AUC is widely used to characterize biomacromolecules, including proteins, nucleic acids, and carbohydrates. It can be applied over a broad range of molecular sizes, from short peptides and oligonucleotides to large assemblies such as viruses, chromatin assemblies, and nanoparticles.

It is worth noting that AUC has a long history, beginning with Svedberg's investigations of colloids and hemoglobin in the early 1920s. With the development of commercial instrumentation after World War II, the use of AUC exploded in subsequent decades. However, by 1980 interest in AUC waned as new methods to determine molecular weights became available. During the ensuing "dark ages," only a few laboratories maintained active research programs in AUC using aging instruments. The development of a new generation of analytical ultracentrifuges in the 1990s combined with powerful data analysis algorithms running on fast computers has catalyzed a renaissance in AUC research that continues to this day. The goal of this volume of *Methods in Enzymology* is to provide a broad overview of this rapidly evolving field, with chapters devoted to developments in instrumentation, theory, data analysis, and applications in basic biology and biopharmaceutical research and development.

Instrumentation and Analysis. The development of a multiwavelength absorption detector (Chapter 1) and associated data analysis methods (Chapter 2) adds an additional dimension of spectral characterization to complement hydrodynamic information. Chapter 3 provides the theoretical basis for analysis of sedimentation velocity. Sedimentation velocity experiments provide shape information that can be precisely interpreted in the context of high-resolution structures with hydrodynamic modeling (Chapter 4). Organization of large AUC data sets has become cumbersome and Chapter 5 describes software that simplifies the processing and presentation of AUC data.

Proteins. A major application of AUC is the analysis of proteins and protein association reactions. The self-association behavior of proteins is affected by crowding, which can be studied by sedimentation equilibrium and complementary light-scattering measurements (Chapter 6). Self-association is often thermodynamically linked to small-molecule binding

(Chapter 7). The resolution of complex heterointeractions by sedimentation velocity experiments is improved by generating two-dimensional size-and-shape distributions from sedimentation velocity data (Chapter 8). AUC, complemented by structural studies and molecular dynamics simulations, can provide insight into the regulation of enzymes by changes in quaternary structure (Chapter 9). AUC has important applications in the analysis of proteins containing intrinsically disordered regions (Chapter 10), amyloid oligomers and fibrils (Chapter 11), and membrane proteins (Chapter 12).

Nucleic Acids. DNAs, RNAs, and their interactions with proteins are also amenable to investigation by AUC. The hydrodynamic properties of G-quadruplex DNAs determined from sedimentation velocity provide valuable structural information (Chapter 13). AUC has been applied to the characterization of nonspecific protein–nucleic acid interactions (Chapter 14), the cooperative assembly of protein clusters on DNAs (Chapter 15), and large protein–DNA complexes formed during chromatin compaction (Chapter 16). In the case of steroid receptors, protein self-association is linked to sequence-specific, cooperative assembly at DNA promoters (Chapter 17).

Other Biomacromolecules. Chapter 18 provides an example of the strength of AUC in the analysis of complex biomacromolecules. Unlike proteins, carbohydrates are highly polydisperse and exhibit significant nonideality.

Biopharmaceuticals. AUC is widely used in the biotechnology industry. Chapter 19 provides a broad overview of the application of AUC in formulation development and research on protein therapeutics. A key application of AUC is detection of potentially harmful protein aggregates (Chapter 20). *In vivo*, biopharmaceuticals function in complex, crowded solutions. Using fluorescence detection, AUC is one of the few biophysical techniques capable of probing the association state and interactions of protein therapeutics in complex media such as serum (Chapter 21).

Finally, I would like to thank the authors for their excellent chapters and the staff at Elsevier for their assistance in assembling this volume. I hope that it will serve as a useful resource to both new and experienced researchers.

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