
Preface

This volume is focused on methods for the characterization of aggregation processes that lead to the formation of amyloid fibrils and amyloid oligomers that feature in the etiology of a variety of human disorders collectively known as amyloidoses. The focus includes techniques for visualizing early steps on the amyloid formation pathway, methods for capturing and characterizing oligomeric, potentially toxic, intermediates, strategies for preparing and characterizing mature amyloid fibrils, and approaches for understanding templating and transmission of amyloid aggregates. The target audience includes biochemists and biophysicists with an interest in elucidating the mechanisms of protein amyloid formation, as well as chemists, pharmacologists, and clinicians with an interest in leveraging an understanding of such mechanisms for the purpose of therapeutic development.

Chapter 1 treats methods to prepare posttranslationally modified amyloid proteins with a focus on the production of phosphorylated forms of the Parkinson's disease-associated protein alpha-synuclein. Posttranslational modifications of synuclein and other amyloid proteins are often associated with disease pathology yet their role in disease etiology has remained unclear, in part because of the difficulty of producing homogeneously modified proteins for in vitro studies. Chapter 2 describes both chemical synthesis and native chemical ligation strategies for the production of isotopically labeled amyloid proteins for characterization by spectroscopic techniques such as two-dimensional infrared spectroscopy or NMR spectroscopy. Detailed procedures are provided for the production of the diabetes-linked amyloidogenic peptide amylin as well as for the amyloid-forming protein alpha-beta crystallin. Chapter 3 describes the use of paramagnetic relaxation enhancement NMR spectroscopy to detect and describe the earliest interactions between amyloid monomers, using alpha-synuclein as an example, while Chapter 4 describes the use of circular dichroism spectroscopy to detect the presence of helical intermediates during the formation of amyloid fibrils, in this case using amylin as an example. The formation of helical intermediates has been implicated as a potentially critical step in the formation of fibrillar aggregates by a number of amyloid proteins. Chapter 5 describes innovative applications of fluorescence correlation spectroscopy to measure oligomer formation both for purified amyloid proteins in vitro and also for fluorescently labeled amyloid proteins in intact cells, providing a unique approach to observing the amyloid formation process in vivo using huntingtin exon 1 polypeptides as an example. Chapter 6 describes the application of advanced Raman Spectroscopy methods for the characterization of the process by which amyloid fibrils form, allowing for the characterization of different fibril regions, such as the core or the surface, as well as for determining the order in which secondary structure is formed during fibril assembly. The method is illustrated using amyloid formation by lysozyme. Chapter 7 describes an innovative use of quantitative electron microscopy to determine the parameters that govern the fibrillization kinetics of the Alzheimer's protein tau.

Chapters 8, 9, and 10 focus on the characterization of oligomeric species formed on the pathway of amyloid fibril assembly. Chapter 8 describes the use of a powerful combination of mass spectrometry techniques: ion mobility spectrometry and electrospray ionization, in order to characterize the gas phase collision cross sections of different oligomeric species

that are present simultaneously in samples undergoing amyloid fibril assembly. The heterogeneity of species in such samples has long been a major hurdle to characterizing the fibril formation process, and this technique is one of very few that is able to provide a simultaneous analysis and resolution of different species. An application to the aggregation of beta-2-microglobulin is described. Chapter 9 describes techniques to produce stable homogeneous preparations of oligomers formed by alpha-synuclein as well as a variety of methods to characterize these oligomers, including SDS-PAGE, circular dichroism, electron microscopy, atomic force microscopy, Fourier-transform infrared spectroscopy, and fluorescence assays of phospholipid vesicle permeabilization. Chapter 10 also treats the characterization of oligomers formed from the protein alpha-synuclein but describes the application of a novel single-molecule fluorescence photobleaching approach to characterizing the number density of the oligomers. Despite intense efforts, reliably determining the distributions of the number of molecules in amyloid oligomers has remained a frustrating challenge, and this method provides a reliable solution to this long-standing issue.

Chapters 11 through 14 describe methods for the characterization of mature amyloid fibrils. Chapter 11 describes protocols for the preparation of alpha-synuclein amyloid fibrils for characterization by solid-state NMR spectroscopy. Solid-state NMR has provided some of our most detailed insights into the structures of amyloid fibrils of a number of proteins and continues to be at the forefront of amyloid fibril structure determination. Key to the success of this method, however, is the production of high quality samples of fibrils, and this chapter provides an avenue for achieving this. Chapter 12 describes the characterization of amyloid fibrils formed from the protein tau using electron paramagnetic resonance spectroscopy, which can produce information on local environments within fibrils, provide powerful distance constraints on fibril conformation, and also distinguish different fibril populations. Chapter 13 describes the preparation of amyloid fibrils for structure determination by x-ray crystallography. This approach has provided the highest resolution structural views of the central spines of a variety of amyloid-forming sequences. In addition, this method has recently succeeded in resolving the atomic resolution structures of amyloid oligomers, and the preparation of such samples is also described. Chapter 14 describes methods for the analysis of amyloid fibril structure using amide proton hydrogen exchange monitored by NMR spectroscopy. This approach can provide information, at the single residue level, on solvent accessible regions and hydrogen bonding patterns within fibrils.

Chapters 15, 16, and 17 describe computational approaches towards understanding the structure and assembly of amyloid aggregates. This is a rapidly growing area that provides novel insights that are difficult or impossible to obtain via experimental methods. Chapter 15 describes a protocol for executing replica exchange molecular dynamics simulations of amyloid proteins, using a fragment of the protein tau as an example. Chapter 16 describes the use of molecular dynamics simulations to model the structures of amyloid ion channels, as well as to calculate their ion permeation properties, using the Alzheimer's amyloid-beta (A-beta) peptide as an example. Ion channel formation by amyloid oligomers is an important potential mechanism for the toxic effects of such species. Chapter 17 describes a protocol for a method that employs Bayesian statistics to leverage experimental data on amyloid proteins for the identification of ensembles of model structures that "best" represent the experimental observables, including statistical parameters to evaluate the significance of various properties of the resulting ensembles.

Chapters 18, 19, and 20 describe methods for evaluating processes that may influence the toxicity and pathology of amyloid proteins *in vivo*. Chapter 18 describes experimental methods for evaluating the ability of amyloid species to permeabilize phospholipid bilayers

using amylin as an example. Indiscriminant membrane permeabilization by amyloid species has been proposed as a potentially general mechanism for their toxicity. Chapter 19 describes protocols to study the transmission of amyloid species between cells, using the example of alpha-synuclein. Cell-to-cell spread of amyloid species, potentially via a prion-like mechanism, has emerged as an area of tremendous interest and may explain observations of how amyloid pathology spreads through the body and brain in neurodegenerative disease. Chapter 20 describes the preparation of amyloid fibrils seeded using material obtained from diseased human or mouse brain tissues in a way that preserves the ultrastructure of the material in the original tissues, using Alzheimer's brain derived A-beta fibrils as an example. Such samples can then be characterized structurally, in this example using solid-state NMR, in order to delineate the structural basis for different disease-associated fibrillar states and to characterize amyloid strains. The possibility that different amyloid strains, corresponding to different molecular structures of amyloid fibrils, may be associated with different disease presentation and phenotype goes hand-in-hand with the idea that a single or a small number of nucleating aggregation events in the brain or body can lead, via cell-to-cell transmission, to a single or a few dominant fibril forms.

In summary, this volume presents modern methods and protocols for characterizing amyloid aggregation, amyloid aggregates, and amyloid spread and toxicity from the very earliest manifestations in the form of nucleating conformers or transiently interacting monomers, through the formation of helical intermediates, oligomeric species, membrane-bound oligomers or channels, and finally arriving at mature amyloid fibrils, which can be spread from cell to cell, and the molecular details of which may underlie the specific features of human disease presentation.

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