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Development of Solid-state NMR Methods using Ultra-fast Magic Angle Spinning and Their Applications for Protein Characterization

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Solid-state Nuclear Magnetic Resonance (SSNMR) spectroscopy is a powerful analytical technique in structural biology. With recent advancements, modern SSNMR has stepped into an era of ultra-fast magic angle spinning (UFMAS). Although much higher sensitivity and resolution could be achieved by UFMAS, faster spinning makes various traditional SSNMR techniques developed for slow to moderate spinning frequencies ineffective. Thus, it is crucial to develop new methodologies suitable for biomolecular SSNMR under UFMAS.

This thesis consists of three chapters. Chapter 1 presents a brief introduction of the research background. In Chapter 2, first we demonstrate the applicability of ^{1}H-detected SSNMR under UFMAS for the high-throughput screening of trace amounts of selectively ^{13}C- and ^{15}N-labeld Aβ42 fibril prepared with 0.01% of Alzheimer's disease (AD) patient-derived amyloid (~4 pmol) as a seed. Our results suggest the feasibility of assessing the fibril structure from ~1 pmol of brain amyloid seed in ~2.5 h. We also present the first structural characterization of trace amounts (~42 nmol) of synthetic fully-protonated Aβ42 fibril by a combined analysis of ^{1}H-detected 3D and 4D SSNMR experiments under UFMAS within a total experimental time of 12.7 days. With semi-automated and manual signal assignment procedures, site-specific resonance assignment was completed. The assigned resonances suggest that this Aβ42 fibril exhibits a novel polymorph structure.

In Chapter 3, we present the recent progress of high-field SSNMR for small organic molecules and biomolecules using ^{13}C cross-polarization magic angle spinning (CPMAS) under UFMAS at a spinning frequency of 100 kHz, by discussing the major differences between a modern low-power radio-frequency (RF) scheme using UFMAS in an ultra-high field, and a traditional CPMAS scheme using a moderate sample spinning in a lower field. Our results indicate 12-fold higher mass-sensitivity for the modern low-power RF scheme compared to the traditional CPMAS. We also present the superior performance of low-power WALTZ-16 ^{1}H-decoupling scheme over traditional low-power decoupling schemes designed for SSNMR. To the best of our knowledge, this was the first demonstration of efficient composite-pulse ^{1}H-decoupling for rigid solids.