Elucidating Protein Structure and Folding Using Novel Long-Range Distance Solid-State NMR

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In this talk I will present our recent development of two classes of techniques that increase the distance reach of solid-state NMR from ~5 Å to ~2 nm, and I will show how these long-range distances restrain protein structures. The first class of techniques uses ^{19}F as the distance probe. I will show that ^{19}F-^{19}F homonuclear distances can be measured by both spin diffusion and dipolar recoupling techniques under fast MAS frequencies of 25 – 55 kHz at the medium-high magnetic field of 14.1 Tesla. Fast MAS suppresses the ^{19}F chemical shift anisotropy sidebands without compromising the dipolar coupling measurements. ^{19}F-^{19}F spin diffusion buildup curves contain semi-quantitative distance information up to ~2 nm through an analytical equation. Complementary to ^{19}F-^{19}F distances, we also demonstrate ^{13}C-^{19}F and ^{1}H-^{19}F distance techniques under fast MAS. These ^{19}F-based distance experiments are essential for determining the cholesterol-binding site of the influenza M2 protein, where fluorinated cholesterol and ^{13}C-labeled protein were used. The cholesterol-bound structure of M2 gives novel insights into the mechanism of membrane scission of the virus from the host cell. ^{13}C-^{19}F distance restraints also effectively constrain the interhelical packing of the influenza B M2 protein.

In addition to ^{19}F MAS NMR, we have developed improved ^{15}N-^{13}C and ^{13}C-^{13}C dipolar recoupling pulse sequences where up to 1 nm distances can be measured. These techniques are based on the PAR and PAIN-CP class of experiments, but replaces continuous-wave pulses with pulsed spin lock, which significantly simplifies the implementation of these experiments. These pulsed-spin-lock ^{13}C-^{13}C and ^{13}C-^{15}N distance techniques have allowed us to extract intermolecular distances in the amyloid fibril formed by the pharmaceutical peptide glucagon, yielding a novel atomic-resolution structure.