^{1}H-Detected REDOR with Fast Magic-Angle Spinning of a Deuterated Protein

Manali Ghosh[1], Chad M. Rienstra[1,2,3]

[1] Department of Chemistry, [2] Center for Biophysics and Quantitative Biology, [3] Department of Biochemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801, USA

For insoluble biological systems like amyloid fibrils, solid-state NMR spectroscopy (SSNMR) has been a powerful technique for structural elucidation, as well as small molecule interactions.[1,2] Three-dimensional structure determination is enhanced by measurements of quantitative distance restraints. The rotational echo double resonance (REDOR)[3] technique is well known for measuring heteronuclear distances. Since the distance measurement range depends on the gyromagnetic ratio (γ) of the nuclei, involving ^{1}H (NMR active and stable nucleus that has the largest γ) as one of the spins increases the measurement range. It also helps in improving the sensitivity of the spectrum. Performing ^{1}H detection at fast magic-angle spinning (MAS) and deuteration strategies enhance the resolution of the spectrum by decoupling ^{1}H-^{1}H dipolar interaction. In this study, we combine ^{1}H detection and frequency selective REDOR (FSR)[4] to perform ^{1}H-detected ^{13}C-dephased studies (Fig. 1) on different spectral regions and obtain quantitative ^{1}H-^{13}C distances (up to 5 Å within 2.4 ms dephasing time) in uniformly ^{13}C,^{15}N, ^{2}H labeled alanine and uniformly ^{13}C,^{15}N, ^{2}H labeled GB1 protein, back-exchanged with 30% H_2O.[5]

We apply frequency selective soft π-pulses to the ^{13}C nuclei to select certain spectral regions like backbone, methyl, carbonyl and aromatic regions. The selective REDOR dephasing can be observed in the peak intensities in a ^{15}N-^{1}H correlation spectrum. We show that such a technique helps in spectral filtering of backbone ^{13}C atoms, and secondary and tertiary structural insights can be obtained by utilizing the methyl- and aromatic- specific dephasing versions respectively. Even the orientation of the aromatic rings can be determined with respect to the backbone amide ^{1}H. In the Cα selective version, at short dephasing times (< 360 μs), the backbone amide ^{1}H show dephasing. Only at 600 μs dephasing, the sidechain amine sites show significant dephasing. In the methyl selective version, the strongest effects (ΔS/S0 between 0.6-0.8 at 600 μs dephasing time) arise in Ala residues in helical conformations and some Thr/Val residues in favorable conformations. For aromatic selective dephasing, when the ring is in gauche- conformation, the REDOR effect is 0.4-0.6 (Fig. 1). If the ring is in trans- conformation with respect to its own backbone ^{1}H, the REDOR effects will be less than 0.4, and the (i+1) amide ^{1}H will show larger REDOR effects (Fig. 1). Longer range effects are also observed since the aromatic ring tends to be further away from intraresidue amide and closer to neighboring or long-range backbone or sidechain amides.

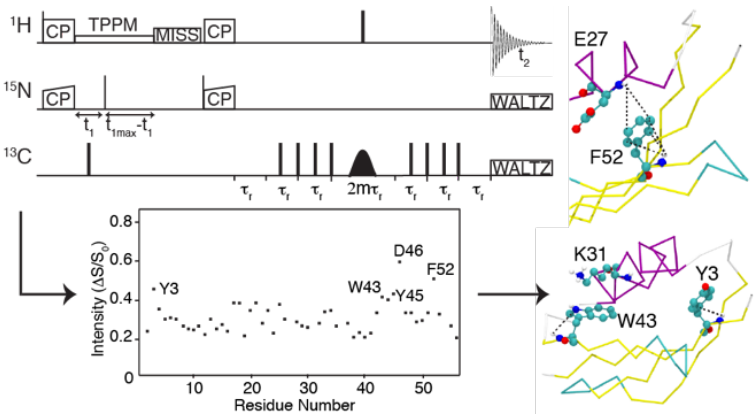

Fig. 1: Pulse sequence for ^{1}H detected frequency selective REDOR spectroscopy is shown (top left). The measured REDOR effects for the aromatic dephasing version are plotted against the residues of GB1 protein (bottom left). These REDOR effects can be validated in the GB1 structure (PDB: 2QMT) (right)

One thing to note for the aromatic region dephasing is that the aromatic ^{13}C encompasses a large chemical shift range. The shifts can vary from Tyr Cζ (at 160 ppm) to Trp Cγ (at 110 ppm). This gives us a large range of possible excitable frequencies using very selective soft pulses. Different aromatic region atoms can give different structural restraint information. This pulse sequence can also be extended to perform ^{1}H {^{19}F} or ^{1}H {^{31}P} REDOR in the case of phospholipids and fluorinated drugs bound to proteins. With $^{1}H_N$ T_2 >10 ms, the ^{1}H{^{19}F} REDOR version should be able to detect distances in excess of 15 Å. We also anticipate that this pulse sequence can be extended to 3D transferred echo double resonance (TEDOR)[6] studies on sparsely labeled protein samples.

References

1. Tuttle MD et al. (2016) *Nat. Struct. Mol. Biol.* 23:409-415
2. Bagchi DP, Yu L, Perlmutter JS, Xu J, Mach RH, Tu Z, Kotzbauer PT (2013) *PLoS One* 8:e55031
3. Gullion T, Schaefer J (1989) *J. Magn. Reson.* 81:196-200
4. Jaroniec, C.P. *et al.* (2001) *J. Am. Chem. Soc.* 123: 3507-3519
5. Ghosh, M., Rienstra, C.M. (2017) *J. Phys. Chem. B.* 121: 8503-8511
6. Jaroniec, C.P. *et al.* (2002) *J. Am. Chem. Soc.* 124: 10728-10742