Applications of Protein NMR Spectroscopy: (i) Unambiguous Sequential Assignment of Intrinsically Disordered Proteins, and (ii) Unambiguous Determination of Protein Arginine Ionization States in Solution

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Recent advances in protein NMR spectroscopy have enabled characterization of the structure and dynamics of proteins in solution. In particular, NMR is the most powerful tool for studying intrinsically disordered proteins (IDPs). However, study of IDPs by NMR spectroscopy is often limited by poor chemical shift dispersion caused by their high flexibility. In the first part of my presentation, a strategy for backbone NMR assignment of disordered proteins will be presented. Although sequential resonance assignment strategies are typically based on matching one or two chemical shifts of adjacent residues, extended sequential connectivity strongly lifts chemical shift degeneracy of the backbone nuclei. Multiple, recurrent long-range correlations allow complete assignment of backbone ^{15}N and ^{13}C' resonances.

In the second part, a new NMR experiment for probing individual arginine residues in proteins will be presented. NMR experiments relying on ^{1}H detection for probing arginine head group are often compromised due to the presence of chemical and conformational exchange effects. Because cryogenic probes are available today for ^{13}C detection, the experiment takes advantage of the favorable relaxation properties of the ^{13}Cζ nucleus due to the absence of directly attached protons. In the experiment, arginine head group ^{15}Nη and ^{15}Nε chemical shifts are correlated with that of the directly attached ^{13}Cζ. In the resulting spectrum, the number of protons in the arginine head group can be obtained directly from the ^{15}N–^{1}H scalar coupling splitting pattern. We applied this method to unambiguously determine the ionization state of the R52 side chain in the photoactive yellow protein from *Halorhodospira halophila*. Although only three Hη atoms were previously identified by neutron crystallography, we show here that R52 is predominantly protonated in solution.

References

1) Yoshimura, Y., Kulminskaya, N. V., Mulder, F. A. A., *J. Biomol. NMR*, **61,** 109–121 (2015)
2) Yoshimura, Y., Oktaviani, N. A., Yonezawa, K., Kamikubo, H., Mulder, F. A. A., *submitted*