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NMR spectroscopy as a tool to investigate structural dynamics of proteins in solution

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Recent advances in nuclear magnetic resonance (NMR) spectroscopy have enabled characterization of the structure and dynamics of proteins in solution. My presentation will focus on some of my research projects during my postdoc period under the supervision of Professor Frans A. A. Mulder at Aarhus University, Denmark.

First, a new NMR experiment for probing individual arginine head groups in proteins will be presented. This method was applied for determination of the ionization state of the Arg52 side chain in photoactive yellow protein from *Halorhodospira halophila*. Although only three H^{η} atoms were previously identified by neutron crystallography, Arg52 is predominantly protonated in solution.[1]

NMR is especially well-suited for studying intrinsically disordered proteins (IDPs), which lack a well-defined secondary and/or tertiary structure under physiological conditions and exist as dynamic ensemble of interconverting structures. However, unambiguous resonance assignments for IDPs are often challenging due to chemical shift degeneracy, as local anisotropic interactions are averaged out by conformational fluctuation. In order to make the sequential resonance assignment of IDPs easy and unambiguous, an NMR pulse sequence which establishes the connectivity of multiple contiguous residues was implemented.[2]

Lastly, NMR contributions to the study of structural dynamics of IDPs will be presented. α-Synuclein (αSyn) is known as an IDP, and aggregation of αSyn is responsible for Parkinson's disease. Our NMR experiments suggest that the presence of long-range transient intra-molecular interactions originating form an electrostatic attraction between the N- and C-termini protects αSyn against aggregation.[3]

[1] Yoshimura *et al*. (2017) Unambiguous Determination of Protein Arginine Ionization States in Solution by NMR Spectroscopy. *Angew*. *Chem. Int*. *Ed*., **56,** 239–242.

[2] Yoshimura *et al*. (2015) Easy and Unambiguous Sequential Assignments of Intrinsically Disordered Proteins by Correlating the Backbone ^{15}N or $^{13}C'$ Chemical Shifts of Multiple Contiguous Residues in Highly Resolved 3D Spectra. *J*. *Biomol*. *NMR*, **61,** 109–121.

[3] Yoshimura *et al*. (2017) MOAG-4 Promotes the Aggregation of α-Synuclein by Competing with Self-Protective Electrostatic Interactions. *J*. *Biol*. *Chem*., **292,** 8269–8278.