The application of 19F NMR spectroscopy in biomolecular studies as a potent technique for structural analysis of the proteins and protein-protein complexes in solution.

Lesia Kolomiiets, and Igor Zhukov

Laboratory of Biologcal NMR, Institute of Biochemistry and Biophysics, Polish Academy of Sciences

The application of 19F NMR spectroscopy in structural studies of proteins has emerged as a potent tool within the other methods of structural biology. While the classical spectroscopic methods, such as 1H, 13C, and 15N NMR, routinely used to structural analysis proteins in solution, the utilization of 19F NMR gain couple of new possibilities to contribute in structural studies. Specifically, the 100% naturally abundant of the 19F nucleus, making it an ideal candidate for NMR analysis. With a spin of 1/2 and a high gyromagnetic ratio, 19F isotope exhibits exceptional sensitivity, approximately 83% that of 1H. The shielding of the 19F nucleus is predominantly governed by a substantial chemical shift anisotropy (CSA). Consequently, fluorine chemical shifts are exquisitely responsive to variations in the local molecular environment, boasting a chemical shift range nearly 100 times larger than that of 1H. Another advantage of employing 19F as an NMR probe lies in its virtual absence from the majority of naturally occurring biomolecules. This characteristic allows for the investigation of fluorinated proteins in a wide range of routinely used buffer systems and environments, without interference from background signals. Furthermore, the van der Waals radius of the 19F atom, measuring 1.47 Å, positions it between the VdW radii of hydrogen (1.2 Å) and oxygen (1.52 Å) suggesting that incorporating 19F instead of 1H nuclei have minimal perturbing effects and often exerts little influence on a protein's biological activity.

In the Laboratory of Biological NMR, we perform the synthesis and purification of the protein, containing fluorinated versions of aromatic residues - tryptophan, phenylalanine, and tyrosine. Based on these achievements, we perform several research projects focused on the structural analysis of the proteins and protein-protein complexes utilizing 19F NMR spectroscopy. Our recent experimental findings affirm that the incorporation of fluorine atoms into aromatic residues makes it possible to obtain valuable experimental data about localization structural modifications, ligand binding, conformational dynamics, and protein interactions at the atomic scale. At the moment, the 19F NMR spectroscopy seems to be a promising and cost-effective technique, which substantially increases our knowledge about complex biomolecular systems.