Fast field cycling relaxometry of long-lived spin states to probe weak ligand-protein binding

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The survey of the interactions between biological molecules in living organisms (i.e., metabolites, proteins, nucleic acids) constitutes a crucial analytical field of interactomics, which provides a powerful tool for biology and medical diagnostics. Nuclear magnetic resonance (NMR) spectroscopy, with its ability to non-intrusively probe dynamical processes, has proved to be one of the most perspective methods to detect the interactions between biological molecules. The characterization of weak interactions between metabolites and macromolecules attracts a particular interest, as it is not always amenable to existing approaches. Such interactions can be identified with the help of high-resolution relaxometry, which is the study of the dependence of proton relaxations rates on the strength of the external magnetic field [1]. We propose the extension of this approach which utilizes the preparation of spin states, which are immune to rapid dipolar relaxation, which makes them perfect probes for the study of slow dynamical processes. The relaxation time of such long lived states (LLS) at low magnetic fields can reach more than ten-fold excess over the relaxation time of the longitudinal magnetization [2].

We perform experiments with a number of small biological molecules, namely, citric acid, dipeptide alanine-glycine, and others, acting as ligands and human serum albumin as a target protein. For the considered ligands we optimize the preparation of long-lived spin order at high magnetic field with the consecutive fast field cycling of the sample in the range from 1 mT to 16 T. The addition of even a small amount of binding protein results in a drastic decrease of the relaxation time of the long-lived state at low magnetic fields, which provides a robust tool for the detection of weak binding process. We also propose a theoretical model, which describes the field dependence of LLS for arbitrary metabolite / protein concentration ratio, which allows a detailed characterization of their interaction. The proposed approach can become a valuable contribution to the field of NMR interactomics.

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