NMR SPECTROSCOPY REVEALING CHALLENGES IN MONITORING INTERACTION OF SMALL MOLECULES WITH PROTEINS

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Comprehending the interactions between small molecules and proteins is crutial for numerous domains of biomedical science. Nuclear magnetic resonance spectroscopy (NMR) is highly effective tool for examining intermolecular interactions, providing apparent advantages over alternative analytical methods. The observation of small molecule interactions with proteins by ligand-based NMR spectroscopy entails monitoring alterations in the NMR signals of the ligand (small molecule) when it binds transiently to a protein. [1] This technique is valuable because it primarily focuses on the ligand's resonances, facilitating detection of binding events without requiring isotopic labeling of the protein, and it is suitable for large protein targets. The technique can also facilitate structure-based drug design by providing atomic-level insights into interactions without requiring full protein structure or resonance assignments.

The *in situ* kinetics NMR experiment constitutes a pseudo 2D NMR technique that enables real-time observation of reaction rates and processes directly within the reaction environment, without disturbing or isolating reaction components. This method allows detailed observation of the temporal evolution of substrate, intermediate, and product concentration changes with great chemical specificity, eliminating the necessity for sample extraction or quenching. Additionally, provide the simultaneous tracking of numerous species by their unique NMR signals, including transient intermediates that may be challenging to detect using alternative approaches. [2] The *In situ kinetics* study using NMR spectroscopy is a potent method for elucidating reaction pathways and rates with chemical accuracy, providing substantial benefits in understanding the dynamic of molecular processes at the atomic level.

Our study demonstrates that the newly developed ^{1}H NOE pumping experiment enables the observation of interactions within a single experiment. This ^{1}H *in situ* NMR experiment has proven to be an excellent method for monitoring both enzyme inhibition and reactivation. Both experimental methodologies constitute a valuable approach and are suitable for the comprehensive evaluation of a wide array of cholinesterase inhibitors. This is especially important in the examination of neurodegenerative diseases, such as Alzheimer's disease.

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