THE STUDY OF PROTEIN NMR RELAXATION - DURATION AND PRECISION CONSTRAINTS

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Nuclear magnetic resonance (NMR) relaxation measurements have become a powerful tool for probing protein dynamics, enabling the characterization of conformational exchange processes and kinetic behavior across a wide timescale ranging from picoseconds to milliseconds [1]. Such systems due to their size and spectral overcrowding are rarely analyzed using direct one-dimensional NMR spectroscopy. The use of higher-dimensional acquisition allows convenient spectral fingerprinting but inevitably leads to extended measurement time. Recently, researchers have found that overall time can be reduced by using sampling data points randomly. This so-called Non-Uniform-Sampling (NUS) in some cases can lead to even a 4-fold experimental time reduction but is a system-dependent parameter [2]. In this work we studied the experimental duration and precision by implementing NUS on a ^{15}N-labeled Carbonic Anhydrase II (CAII) protein. It is a well studied protein with determined backbone structure needed for peak selection and accessible 3D structure allowing it to be used as a benchmark system for method evaluation. CAII is one of the 14 isoenzymes of CAs that catalyze the reversible hydration of carbon dioxide to bicarbonate. Mutations in CAII in humans lead to osteopetrosis with renal tubular acidosis and cerebral calcifications, a disorder often associated with mental retardation [3].

The experiments involved measuring relaxation rate R_2 using Carr-Purcell-Meiboom-Gill (CPMG) method coupled with Heteronuclear Single Quantum Coherence (HSQC) pulse sequence. Measurements were performed on Bruker Avance Neo 600 MHz spectrometer equipped with a 5 mm high-resolution probe. The experimental duration was optimized by varying the level of NUS, number of scans and the number of data-points. The size of direct dimension for ^{1}H was fixed at 2 k. Acquired data were analyzed using CCPN software [4] for residuals peak position corrections and peak height calculation, and python, notably ”lmfit” and various other libraries for R_2 calculations and further results analysis. NMR peaks were assigned to different confidence limits, depending on spectral shape, positions and overlap. Peaks assigned to high confidence were compared between experiments.

We find that the errors of a single R_2 measurement do not highly depend on the level of NUS and number of scans used but the standard deviation of these experiments can vary. As these measurements are rarely repeated, the experimental precision can be easily overlooked and thus should be carefully noted. The presentation will focus and discuss the main factors contributing to precision and will inform our future studies.

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