Tata Institute of Fundamental Research
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad – 500 046

Students' Annual Seminar

Increasing the accuracy of exchange parameters reporting on slow dynamics by performing CEST experiments with high B_1 fields

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Over the last decade chemical exchange saturation transfer (CEST) NMR methods have emerged as powerful tools to characterise conformational dynamics occurring between a visible major state and 'invisible' minor states. The ability of the CEST experiment to detect these minor states, and provide precise exchange parameters, hinges on using appropriate B_1 field strengths during the saturation period. Typically, a pair of B_1 fields with ω_1 (= $2\pi B_1$) values around the exchange rate k_{ex} are chosen. Here we show that the transverse relaxation rate of the minor state resonance $R_{2,B}$ also plays a crucial role in determining the B_1 fields that lead to the most informative datasets. Using $\mathrm{K} = \left[k_{ex}\left(k_{ex} + R_{2,B}\right)\right]^{\frac{1}{2}} \geq k_{\mathrm{ex}}$, to guide the choice of B_1, instead of k_{ex}, leads to data wherefrom substantially more accurate exchange parameters can be derived. The need for higher B_1 fields, guided by K, is demonstrated by studying the conformational exchange in two mutants of the 71 residue FF domain with k_{ex}~11 s^{-1} and ~72 s^{-1}, respectively. In both the cases, analysis of CEST datasets recorded using B_1 field values guided by k_{ex} lead to imprecise exchange parameters, whereas using B_1 values guided by K resulted in precise site-specific exchange parameters. The conclusions presented here will be valuable when using CEST to study slow processes at sites with large intrinsic relaxation rates, including carbonyl sites in small to medium sized proteins, amide ^{15}N sites in large proteins and when the minor state dips are broadened due to exchange among the minor states.

Friday, Apr 12th 2024

11:30 Hrs (Tea / Coffee 11:15 Hrs)

CR-1, TIFR-H