# Probing Site-Specific Slow Motions of Side Chains in Proteins

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The activity of proteins is intimately linked to molecular dynamics. Several different motions are simultaneously present at ambient temperatures, in different parts of the protein and occuring on different time scales (from picosseconds to seconds). Untangling these motions and assigning their functional roles is a major challenge. Solid state NMR is emerging as a unique powerful tool to simultaneously observe different protein motions either in a site-specific or group specific manner.

Here, we introduce an approach to measure slow motions, occurring on the ms-ms time scale, site-specifically in protein sidechains. We use methyl group nuclear spin relaxation as the motional probe, especially as they play an important role in the formation of hydrophobic cores (which are of critical importance for protein formation and function).[1-2] We present measurements on a sample of microcrystalline GB1, synthesized using specifically labelled precursors that lead to selective labelling of $^{13}CHD_2$ in isoleucine, leucine and valine side chains. With this approach, all other carbon and proton sites in the protein are $^{12}C$ and $^{2}H$.

We measure both longitudinal and transverse relaxation parameters of methyl $^{13}C$ and $^{1}H$ in a site-specific manner. In order to remove potential interference from the other interactions we perform experiments at MAS spin rates of 105 kHz on deuterated protein. $^{1}H$-$^{13}C$ distances are extracted by using the VCCP method. Moreover, we also measure backbone $^{15}N$ relaxation parameters. We analyse the data with model free (SMF), extended model free (EMF) and Gaussian axial fluctuation models (GAF), and discuss the resulting dynamical picture for the sidechains.

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2. Agarwal, V.; Xue, Y.; Reif, B.; Skrynnikov, N. R., Protein Side-Chain Dynamics As Observed by Solution- and Solid-State NMR Spectroscopy: A Similarity Revealed. *J. Am. Chem. Soc.* **2008,** *130* (49), 16611-16621.