Quantifying Lipari–Szabo modelfree parameters from $^{13}$CO NMR relaxation experiments

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Abstract

It is proposed to obtain effective Lipari–Szabo order parameters and local correlation times for relaxation vectors of protein $^{13}$CO nuclei by carrying out a $^{13}$CO-R$_1$ auto relaxation experiment, a transverse $^{13}$CO CSA/$^{13}$CO – $^{13}$Ca CSA/dipolar cross correlation and a transverse $^{13}$CO CSA/$^{13}$CO/$^{15}$N CSA/dipolar cross correlation experiment. Given the global rotational correlation time from $^{15}$N relaxation experiments, a new program COMFORD (CO-Modelfree Fitting Of Relaxation Data) is presented to fit the $^{13}$CO data to an effective order parameter $S^2_{CO}$, an effective local correlation time and the orientation of the CSA tensor with respect to the molecular frame. It is shown that the effective $S^2_{CO}$ is least sensitive to rotational fluctuations about an imaginary $C_a/C_0$ axis and most sensitive to rotational fluctuations about an imaginary axis parallel to the NH bond direction. As such, the $S^2_{CO}$ information is fully complementary to the $^{15}$N relaxation order parameter, which is least sensitive to fluctuations about the NH axis and most sensitive to fluctuations about the Ca/Ca axis. The new paradigm is applied on data of Ca$^{2+}$ saturated Calmodulin, and on available literature data for Ubiquitin. Our data indicate that the $S^2_{CO}$ order parameters rapport on slower, and sometimes different, motions than the $^{15}$N relaxation order parameters. The CO local correlation times correlate well with the calmodulin’s secondary structure.

Abbreviations: COMFORD – CO modelfree fitting of relaxation data

The Lipari and Szabo spectral density function allows the dissection of NMR relaxation in terms of overall and local motion, characterized by modelfree order parameters and local correlation times in the nano-pico second time regime (Lipari and Szabo, 1982a, b). While not valid for all motional regimes, the description is intuitively clear and has as an advantage that theoretical motional models can be expressed in the same terms, allowing comparisons between experiment and theory (Lipari and Szabo, 1982a). The Lipari Szabo theory was first applied to NMR relaxation to extract dynamical information from a large number of $^1$Hz-$^{13}$C$_a$ R$_1$ and NOE relaxation vectors of cyclosporin A (Dellwo and Wand, 1989). Simultaneously, the protein NH relaxation protocol was introduced (Kay et al., 1989). Their protocol is almost synonymous with NMR protein
backbone dynamics, and analyzes the three experiments $^{15}$N R$_1$, R$_2$ and $^{1}$H$^{15}$N NOE. An extended Lipari–Szabo protocol was introduced subsequently (Clore et al., 1990). Software packages such as NMRView for extraction of relaxation curves (Johnson and Blevins, 1994), and for obtaining the spectral density parameters of these data are available Modelfree (Mandel et al., 1995), Dasha (Orekhov et al., 1994) and TENSOR2 (Cordier et al., 1998). Wagner and co-workers showed that NH relaxation maybe interpreted in terms of spectral densities alone (Peng and Wagner, 1992a, b). The NH relaxation protocol has been extended to measurements of conformational changes at the milli–micro second time domain using relaxation dispersion in the rotating frame (Orekhov et al., 1994; Loria et al., 1999).

It is because of the many $^{15}$N relaxation studies that one has obtained the general insight that entire proteins may be extremely rigid, completely floppy, or anywhere in between; it has changed the perspective on the rigidity of proteins in general. However, while the NH dynamics studies have been and will remain to be invaluable, it is obvious that complete dynamical characterization of a biomolecule can not be obtained from NH backbone dynamics alone. To extend the scope of experimental protein dynamics, Kay and co-workers developed $^2$H methyl group relaxation methodology (Yang et al., 1998). These experiments showed that the dynamics of the methyl groups in SH2 domains are not quenched upon phospho-peptide binding, conceivably Nature’s way to allow promiscuity in high-affinity ligand binding (Kay et al., 1998). In contrast, Wand and co-workers showed with these methods that an entropically costly quenching of methyl dynamics occurs upon peptide binding to calmodulin (Lee et al., 2000; Wang et al., 2005). It is thus well established that it is worthwhile to obtain $^{13}$CO relaxation data for a more complete description of the protein backbone dynamics, and for the associated entropic properties (Akke et al., 1993; Yang et al., 1997).

Recently, many open questions pertaining to the $^{13}$CO CSA tensor in proteins have been answered. With high-level DFT calculations, it was shown that the $^{13}$CO CSA tensor principal values $\sigma_{11}$ and $\sigma_{33}$ are constant within 7%, but that the $\sigma_{22}$ value and tensor orientation is highly variable from site to site in proteins (Markwick and Sattler, 2004). These computational predictions confirmed earlier solid-state NMR data that related $\sigma_{22}$ value variability to hydrogen bonding (Gu et al., 1994). The solid state NMR and theoretical findings were confirmed experimentally in solution by Bodenhausen and co-workers (Cisnetti et al., 2004; Loth et al., 2005). To do so, a set of 14 different auto- and cross-correlated relaxation experiments, involving both $^{15}$N and $^{13}$CO, was recorded and analyzed. The experimental and computational findings revealed that the variable $\sigma_{22}$ can be obtained from

$$\sigma_{22} = 3\sigma_{\text{iso}} - \sigma_{11} - \sigma_{33} \quad (1)$$

with good precision.

Both theoretical and experimental work also showed that the $\sigma_{11}$ and $\sigma_{22}$ axes remain in the peptide plane for all residues, but that the orientation of $\sigma_{11}$ can vary with an rms of 3.7 degrees with respect to the covalent bonds. The orientation