**Origins of Abrupt Rise in Deuteron NMR Longitudinal Relaxation Times of Protein Methyl Groups Below 90 K**

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SI1. Ribbond diagram of chicken villin headpiece protein[1] showing hydrophobic core side chains of L61, L69, and L75.
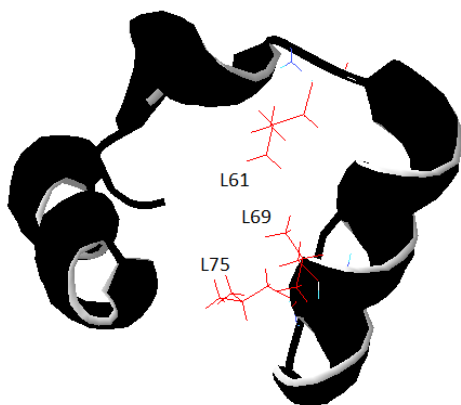
SI2 Parameters of the distribution of the activation energies for three site hops motions.[2]

residue	$\ln k_0 (\text{s}^{-1})$	$<E_a>$ (kJ/mol)	σ (kJ/mol)
L69	27.0±0.2	11.4±0.3	1.6±0.4
L61	26.7±0.1	10.7±0.2	1.3±0.4
L75	27.4±0.1	12.4±0.2	1.7±0.4

We note that for the first couple of temperatures close to 298 K we saw evidence for conformational exchange between conformers on the time scales of the longitudinal relaxation times (~50 ms) and fitted the exchange rate constants. Beyond below ~ 273 K the exchange slowed down to the regime in which it had no effect on the relaxation data.

SI3. Inclusion of deuteron tunneling improves agreement with experimental data at low temperatures.

Relaxation rates due to tunneling above 40 K are usually governed by the splitting between the ground and first excited torsional states[3] and is relatively small compared to the classical activation energy barrier for the three-site hops motions. For L69 we have conducted relaxation time measurements all the way down to 10 K, and the relaxation times level out to about 100 s starting at about 60-50 K. [4]

In the following we will demonstrate using L69 as an example that an inclusion of tunneling effect improves the agreement with the experimental data on the low temperature side. Quantitative inclusion of deuteron tunneling in the simulations is complicated and requires the knowledge of tunneling frequency and its temperature dependence.[3] In order to have a qualitative rough inclusion of deuteron tunneling in the relaxation time, we make an assumption, based on the data for L69, that the effect of tunneling leads to an additional temperature independent relaxation mechanism with T_1 of 100 s. The three-site hop rate constant corresponding to T_1 of 100 s is 4.2 10^3 s^{-1}. We include the effect of tunneling phenomenologically by adding this constant to the three-site hop rate constant of each conformer, calculated according to the model. The results with the phenomenological inclusion of the tunneling are shown by orange triangles in the following figure.
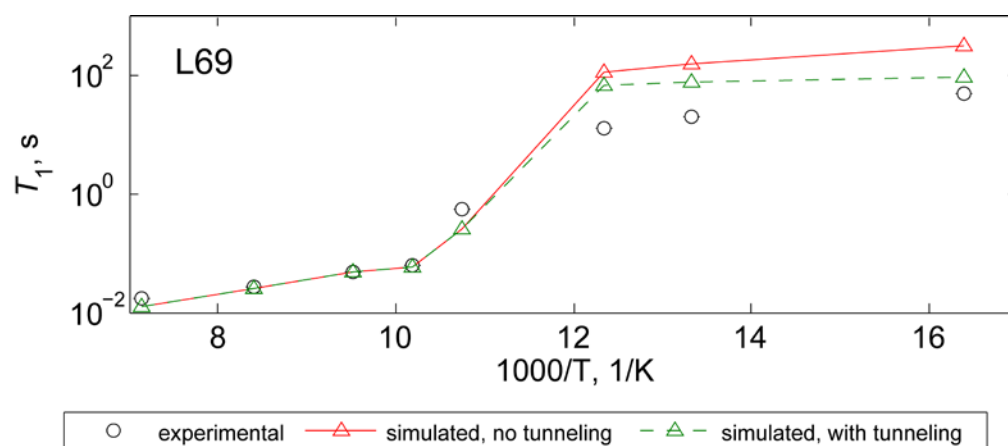

Figure SI3. Longitudinal relaxation times in 140 – 60 K temperature range for L69 residue.

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SI4 Detailed analysis of low temperature spectra indicates deviations from tetrahedral geometry

In the intermediate regime some deviations from the simulated spectra with C_q of 160 kHz and the 3-site jump mechanism are already observed (Figure 2 of the main text), but it is hard to analyze these deviations systematically, as the intermediate regime spectra are extremely sensitive to exact values of the distribution parameters. When we move to the slow regime, the deviations become more apparent. In particular, we see that the relative intensities of the spikelets at ±40 and ± 60 kHz for L61 and L75, and ±50 and ±60 kHz for L69 do not match the experimental spectra. The experimental ratios of intensities of spikelets at ±60/±50 kHz (L69) and ±60/±40 kHz (L61&L75) yield the value of 2.1 for L69 and the value of 1.6 for L61 and L75. However, the maximum value for these ratios that can be attained with the C_q of 160 kHz is about 1.05, as shown in Figure SI4-A (see next page). One additional discrepancy in the spectra is highlighted in Figure SI4-B for the most illustrative spectrum of L69 at 31K. The simulated spectrum with C_q of 160 does not give appreciable peak intensities at ±70 kHz, unlike the experimental spectra. If the value of C_q is taken as 175 kHz, these discrepancies are no longer present. We also note that C_q of 175 was observed directly in the quadrupolar echo spectrum of FMOC-leucine at 50 K.[5]

Thus, we conclude that C_q of 160 kHz is inadequate for the full temperature range. How do we reconcile different quadrupolar constant values at the two motional regimes? It has been suggested that deviations from tetrahedral geometry are common for methyl groups in proteins and amino acids.[6] An angle of 68.85° between the methyl axis and the C−D bond, compared to 70.5° for the tetrahedral geometry, will average C_q of 175 kHz to the effective value of 53.3 kHz in the fast limit. We note that it is usually assumed that the principal axis of the quadrupolar tensor is aligned with the C-D bond.

Thus, non-tetrahedral geometry reconciles the spectra across the entire temperature range. We note that deviations from tetrahedral geometry based on the NMR data have been previously observed in a number of works, such as a study by Wann et al.[7], Batchelder et al[8], and Hoatson et al.[9] using deuteron NMR , as well as from residual dipolar couplins measurements on ubiquitin protein in liquid crystals by Ottinget et al.[6]
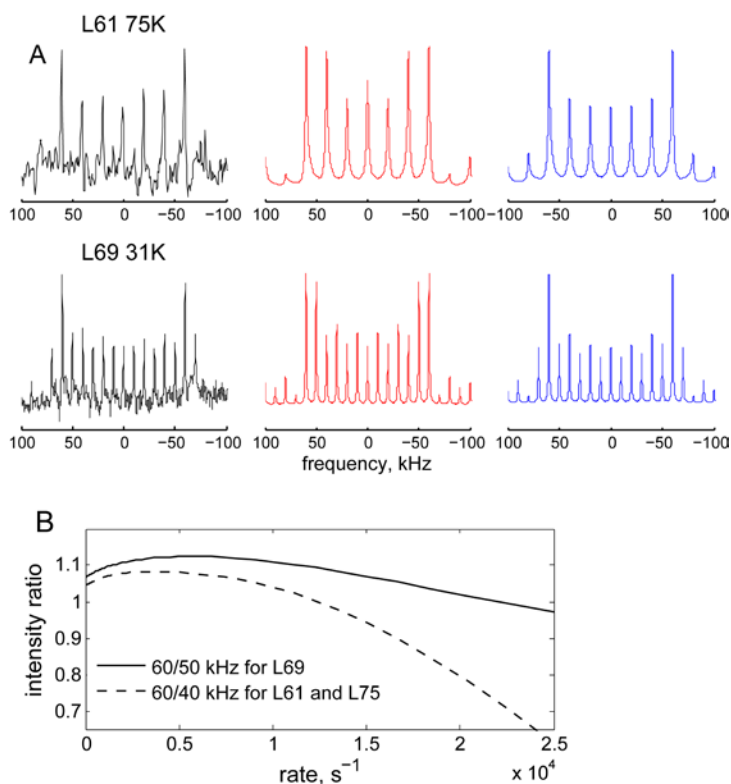
Figure SI4. A) Low temperature spectra: experimental (black), simulated with C_q=160 kHz and tetrahedral geometry (red), and simulated with C_q=175 kHz and non-tetrahedral geometry(blue). The spacing between the spikelet was 10 kHz for L69 and 20 kHz for L61. B) Simulated ratios of spikelet intensity as a function of three-site hop rate constant.

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