# Backbone Dynamics and Global Effects of an Activating Mutation in Minimized *Mtu* RecA Inteins

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***1 . Supplementary methods and materials***

*Analytical Ultracentrifugation (AUC)*

Equilibrium sedimentation experiments were performed using a Beckman XL-I analytical ultracentrifuge with an AN-50Ti rotor operating at 298 K. Different protein concentrations, ranging from 8 to 31 μM, in the NMR sample buffer described above, were loaded into six-sector cells and spun at three different speeds (20,000, 25,000 and 35,000 rpm). The absorbance at 280 nm, A, was recorded as a function of the radial position, r. Equilibrium was attained when scans separated by six hours were identical. The data were globally fitted using nonlinear regression software NLReg (http://www.nlreg.com/) according to a model in which a single idealspecies is present in solution:

$$A(r) = I + A_o(r_o) \cdot \exp\left[\frac{\omega^2}{2RT} M(1-\nu\rho)(r^2 - r_o^2)\right] \quad (1)$$

where A(r) is the absorbance of the protein at a radius r, $A_o(r_o)$ is the absorbance at a radius $r_o$, I is the baseline offset constant, ω is the angular velocity, R is the universal gas constant equal to $8.134 \times 10^7$ erg $mol^{-1}K^{-1}$, T is the temperature in Kelvin, M is the molecular mass, ν is the partial specific volume of the protein (0.7524 $cm^3/g$ for ΔΔIhh-V67CM and 0.7527 $cm^3/g$ for ΔΔIhh-L67CM, calculated from their amino acid composition [1] and ρ is the density of the solution (1.00 $g/cm^3$). The goodness of the fit was assessed by the magnitude and distribution of the residuals.

*Details for Modelfree analysis*

$^{15}N$ NMR relaxation was analyzed using contributions from dipole−dipole (DD) and chemical shift anisotropy (CSA) relaxation mechanisms:

$$R_1 = (d^2/4)[6J(\omega_H + \omega_N) + J(\omega_H - \omega_N) + 3J(\omega_N)] + c^2 J(\omega_N) \quad (2)$$

$$R_2 = (d^2/8)[6J(\omega_H + \omega_N) + 6J(\omega_H) + J(\omega_H - \omega_N) + 3J(\omega_N) + 4J(0)] + (c^2/6)[3J(\omega_N) + 4J(0)]$$ [2]

$$NOE = 1 + (d^2/4R_1)(\gamma_H/\gamma_N)[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)] \quad (4)$$

where $d = \mu_0 h \gamma_H \gamma_N \left\langle r_{NH}^{-3} \right\rangle / 8\pi^2$, $c = \omega_N \Delta\sigma / \sqrt{3}$, $\mu_0$ is the permeability of free space, h is Planck's constant, $\gamma_H$ and $\gamma_N$ are the magnetogyric ratios of $^1$H and $^{15}$N, respectively, $r_{NH}$ is the mean nitrogen−hydrogen bond length (taken here to be 1.02 Å), and $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$ is the CSA, taken here to be −172 ppm. This combination of $r_{NH}$ and CSA typically leads to an average order parameter of 0.85 for rigid secondary structure elements. [2] *(ω)* denotes the spectral density function of each NH bond at various frequencies; $\omega_H$ and $\omega_N$ are the Larmor frequencies of $^1$H and $^{15}$N, respectively.

We have chosen X-ray structure of ΔΔIhh-L67CM (pdb code: 2IN8) to fit our experimental NMR relaxation data. From that structure, we can see it is a globular protein of ellipsoidal shape with dimensions of 37 Å × 49 Å × 64 Å. The hydrogen atoms were added to the structure file using program InsightII (Accelrys Inc., San Diego, CA) and all of the coordinates of water molecules are removed thereafter. The inertia tensor for ΔΔIhh-L67CM has principal moments with the ratio 1.48:1.15:1.00 (program PDBINERTIA, A. G. Palmer, Columbia University), leading to the expectation of a diffusion tensor with a small degree of axially symmetric anisotropy. Therefore, to discern effects of global rotation from those of residue-specific motions, an analysis of rotational diffusion was carried out. QUADRIC (A. G. Palmer, Columbia University) was used to carry out an initial estimate of the overall tumbling ($\tau_m$) of minimized *Mtu* RecA intein and determine the diffusion tensors for spherical, axially symmetric, and fully anisotropic motional models using $R_2/R_1$ ratios and

PDB coordinates prepared above. In the analysis of ΔΔIhh-V67CM, residues for which NOE at 600 MHz ($^1$H frequency) < 0.65 (A65, E99, T100, L404, and V408) were eliminated from consideration for the initial estimate of the diffusion tensor, due to the possibility of significant internal motion.

The criterion [3]

$$\frac{\langle T_2\rangle - T_{2,i}}{\langle T_2\rangle} - \frac{\langle T_1\rangle - T_{1,i}}{\langle T_1\rangle} < 1.5 \times SD \qquad (5)$$

was applied to all residues. $<T_1>$ and $<T_2>$ are the average $T_1$ or $T_2$ values for all residues in the protein that are considered and $T_{1,i}$ and $T_{2,i}$ are the $T_1$ and $T_2$ values of individual residues in the protein that are subject to test. SD is the standard deviation of the collection of all the values in the left that are considered. Residues with high $R_2$ values ($R_2 \geq \langle R_2\rangle + \sigma R_2$) were also excluded, unless their corresponding $R_1$ values were low ($R_1 \leq \langle R_1\rangle - \sigma R_1$)[4], where $\langle R_i\rangle$ and $\sigma R_i$ are the average and standard deviation of the relaxation rate, respectively. This further eliminated G5, R7, G25, R26, V46, E426, T430, and V438, because of probable chemical exchange contributions. The $T_1^{600}/T_2^{600}$ ratios of the remaining 107 residues were used to calculate local correlation times and estimate isotropic, axially symmetric, and fully anisotropic diffusion tensors by the quadratic representation approach of Brüschweiler. [5] Similarly, in the analysis of ΔΔIhh-L67CM, residues E99, T100, L404, and V408 were eliminated because $NOE^{600}$< 0.65. Residues G25, V46, E426, L428, T430, and N440 were eliminated because of the criterion in Eq. 5. The choice between isotropic, axial, and fully anisotropic diffusion tensors calculated from the $T_1/T_2$ data and the quality of the model was characterized by the goodness of the fit based on the minimum of $\chi^2$ and F-test. The axial diffusion tensors fit the 600 MHz ΔΔIhh-V67CM data shows improvement over the best isotropic correlation time of 7.77 ± 0.01 ns. The 600 MHz data diffusion tensor was oblate

($\tau_m$ = 7.76 ± 0.01 ns, $D_{\parallel}/D_{\perp}$ = 0.89 ± 0.02; F=8.8 (axial symmetric over isotropic) vs F=2.68 for $\alpha$=0.05). The axial tensor was also compared to the fully anisotropic result ($\tau_m$ = 7.75 ± 0.01 ns, $2D_{zz}/(D_{xx} + D_{yy})$ = 1.09 ± 0.01, $D_{xx}/D_{yy}$ = 0.91 ± 0.01). The fully anisotropic tensor was neither prolate-like nor oblate like ($2D_{zz}/(D_{xx} + D_{yy}) > 1$ but $D_{xx}/D_{yy} \approx \neq 1$) and no significant improvement was observed over the axial diffusion tensors fit (F=1.0 vs F=3.1 choosing at confidence level of $\alpha$=0.05). Since the best statistical results from the diffusion tensor analysis were obtained by fitting the data with the axially symmetric model, we chose this model as the initial input to the program MofelFree to fit the global tumbling of both ΔΔIhh-V67CM and ΔΔIhh-L67CM and explore the their backbone dynamics of ΔΔIhh-V67CM.The same parameters for initial estimate of the rotational diffusion tensor were used for the model-free analysis of ΔΔIhh-L67CM, because it likely has the same structure as ΔΔIhh-V67CM. The final diffusion tensor was determined by a global fit of diffusion tensor and internal motional parameters.

The amplitudes and time scales of the intramolecular internal motions of the protein were determined from the relaxation data according to the model-free formalism pioneered by Lipari and Szabo [6-7] and extended by Clore et al. [8], by using the program Modelfree (version 4.15, A. G. Palmer, Columbia University) in combination with FastModelfree. [9] PDB coordinates of ΔΔIhh-L67CM (2IN8) [11] were accessed from www.pdb.org. The hydrogen atoms were added to the structure file using program InsightII (Accelrys Inc., San Diego, CA) and all of the coordinates of water molecules are removed thereafter. The coordinate system of the PDB entry was moved to the center of mass of the molecule using PDBINERTIA (A. G. Palmer, Columbia University). QUADRIC (A. G. Palmer, Columbia University) was used

to carry out an initial estimate of the overall tumbling ($\tau_m$) of *Mtu* RecA and determine the diffusion tensors for spherical, axially symmetric, and fully anisotropic motional models using $R_2/R_1$ ratios and PDB coordinates prepared above. Residues with NOE values of <0.65 were eliminated as well as residues with high $R_2$ values ($R_2 \geq \langle R_2 \rangle + \sigma R_2$), unless their corresponding $R_1$ values were low ($R1 \leq \langle R1 \rangle - \sigma R1$). [4]The axis of the PDB coordinate system was rotated so that they were aligned with the principal axis of the diffusion tensor after QUADRIC analysis and the F test was used to check the statistical improvement of the fitting to each model. In model-free analysis, the spectral density function, *J(ω)*, chosen to reflect an axially symmetric diffusion tensor, is modeled as [11-12]

$$J(\omega) = \frac{2}{5} S_f^2 \sum_{j=1}^{3} A_j \left[ \frac{S_s^2 \tau_j}{1+(\omega \tau_j)^2} + \frac{(1-S_s^2)\tau_j'}{1+(\omega \tau_j')^2} \right] \qquad (6)$$

where $S^2$ ($=S_f^2 S_s^2$) is the square of the generalized order parameter characterizing the amplitude of internal motions, and $S_f^2$ and $S_s^2$ are the squares of the order parameters for the internal motions on the fast ($\tau_e$<~200ps) and slow ($\tau_e$>~200ps) time scales, respectively. $\tau_j' = \tau_j\tau_e/(\tau_j + \tau_e)$, $\tau_e$is the effective correlation time for internal motions, $\tau_1^{-1} = 6D_\perp$, $\tau_2^{-1} = 5D_\perp + D_{II}$, $\tau_3^{-1} = = 2D_\perp + 4D_{II}$, $D_\perp$and $D_{II}$ are the components of an axially symmetric diffusion tensor. $A_1 = (3\cos^2\theta - 1)^2/4$, $A_2 = 3\sin^2\theta\cos^2\theta$, and $A_3 = (3/4)\sin^4\theta$, where θ is the angle between the N−H vector and the unique axis of the principal component of the diffusion tensor.

$\tau_e$ is the effective correlation time for internal motions.

The selection of the models for model-free analysis was carried out using a procedure previously proposed by Mandel et al. [13] The five models used to fit our experimental relaxation data were

Model 1: $S^2(\tau_e=0, R_{ex}=0, S_f^2=1)$

Model 2: $S^2,\tau_e$ $(R_{ex}=0, S_f^2=1)$

Model 3: $S^2$, $R_{ex}(\tau_e=0, S_f^2=1)$

Model 4: $S^2,\tau_e$, $R_{ex}$ $(S_f^2=1)$

Model 5: $S^2,\tau_e$, $S_f^2(R_{ex}=0)$.

The model-free analysis results for both ΔΔIhh-V67CM and ΔΔIhh-L67CM are summarized and compared in Table S3. The detailed results are listed in the supplementary material Table S6. The results are plotted against residue number in Figure 4 for both ΔΔIhh-V67CM and ΔΔIhh-L67CM. The majority of the residues (~67%) can be fitted to model 1, indicating that internal motions for these residues are so rapid ($\tau_e=0$) that their only effect is to reduce the order parameter from 1 to $S^2$. A significant number of residues were fitted to model 2, which means that for these residues, fast internal motions $\tau_e$ on the sub-nanosecond time scale contributes significantly to the observed relaxation. Several residues were fitted to model 3 (G5, R18, E20, R26, F49, E426, T430, A433, and V438 in ΔΔIhh-V67CM; G5, H30, V46, E424, L428, T430, and N440 in ΔΔIhh-L67CM) for which a slow (microsecond-to-millisecond) component of motion $R_{ex}$ resulting in exchange broadening was needed. Six residues (K27, I29, R92, R419, E424, and L428) in ΔΔIhh-V67CM while none in ΔΔIhh-L67CM were fitted to model 4, suggesting that for these residues both $\tau_e$ and $R_{ex}$ contributed significantly to the relaxation. Finally, model 5 were used to fit two residues in ΔΔIhh-V67CM (G64 and A65) and four residues (R54, G65, T100, and A418) in ΔΔIhh-L67CM, where there are two-time scale internal motions that contributes to the relaxation. On both cases, three residues were not fitted to any model (H30, V98, and R405 in ΔΔIhh-V67CM; V23, T77, and Y79 in ΔΔIhh-L67CM).

*2. Figure S1*

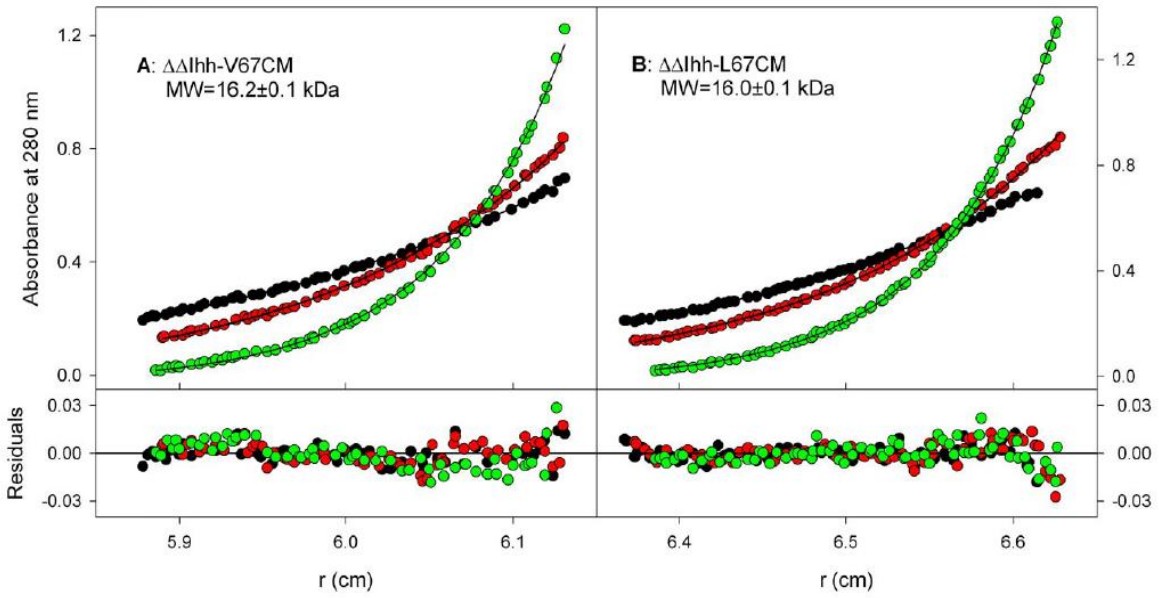


**Figure S1.** Equilibrium sedimentation analysis of ΔΔIhh-V67CM (A) and ΔΔIhh-L67CM (B) proteins at 298 K. Representative traces at 20,000 rpm, 25,000 rpm (red) and 35,000 rpm. Solid lines represent the global fit according to equation (1) in Material and Methods. The molecular weight obtained for ΔΔIhh-V67CM and ΔΔIhh-L67CM from the fits were 16.2± 0.1 kDa and 16.0±0.1 kDa, respectively, while the theoretical molecular weights are 15487.4Da and 15473.4 Da, respectively (assuming 100% $^{15}$N labeling).

*3. Figure S2*

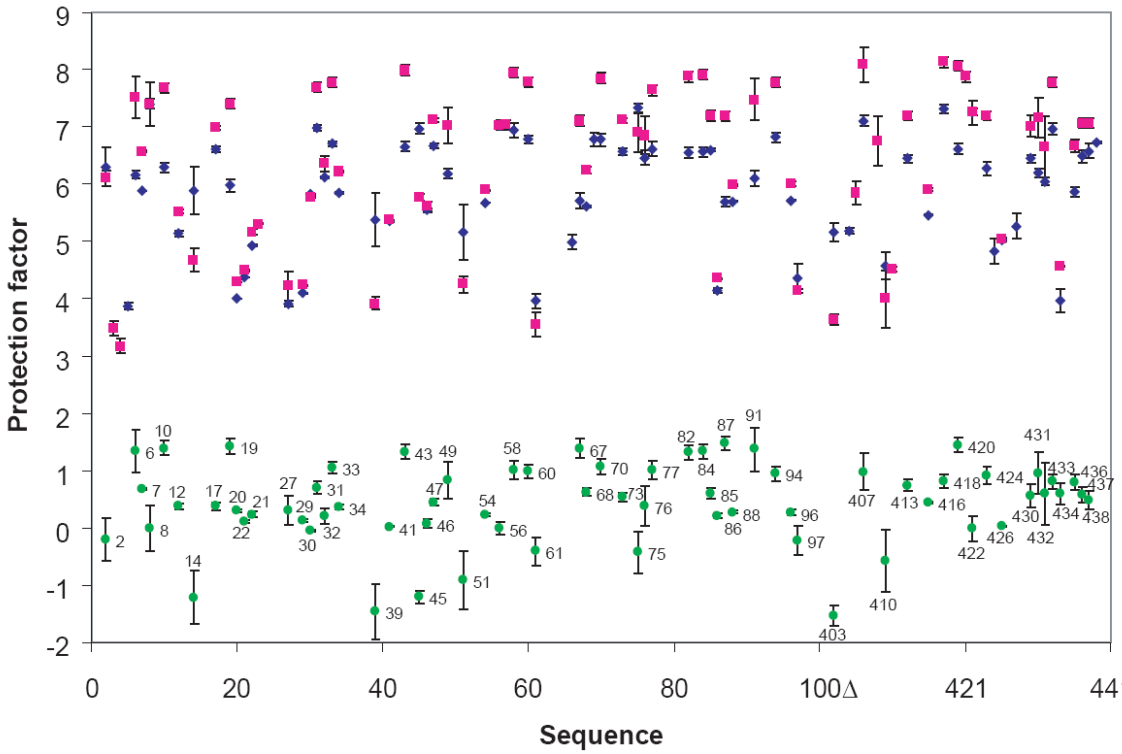


**Figure S2:** Comparison of the protection factor between ΔΔIhh-V67CM (red) and ΔΔIhh-L67CM (blue) plotted against residue number. The protection factor enhancement is obtained through equation $\Delta P = P_{\Delta\Delta Ihh\text{-}L67CM} - P_{\Delta\Delta Ihh\text{-}V67CM}$ and are colored in green.

*4. Figure S3*

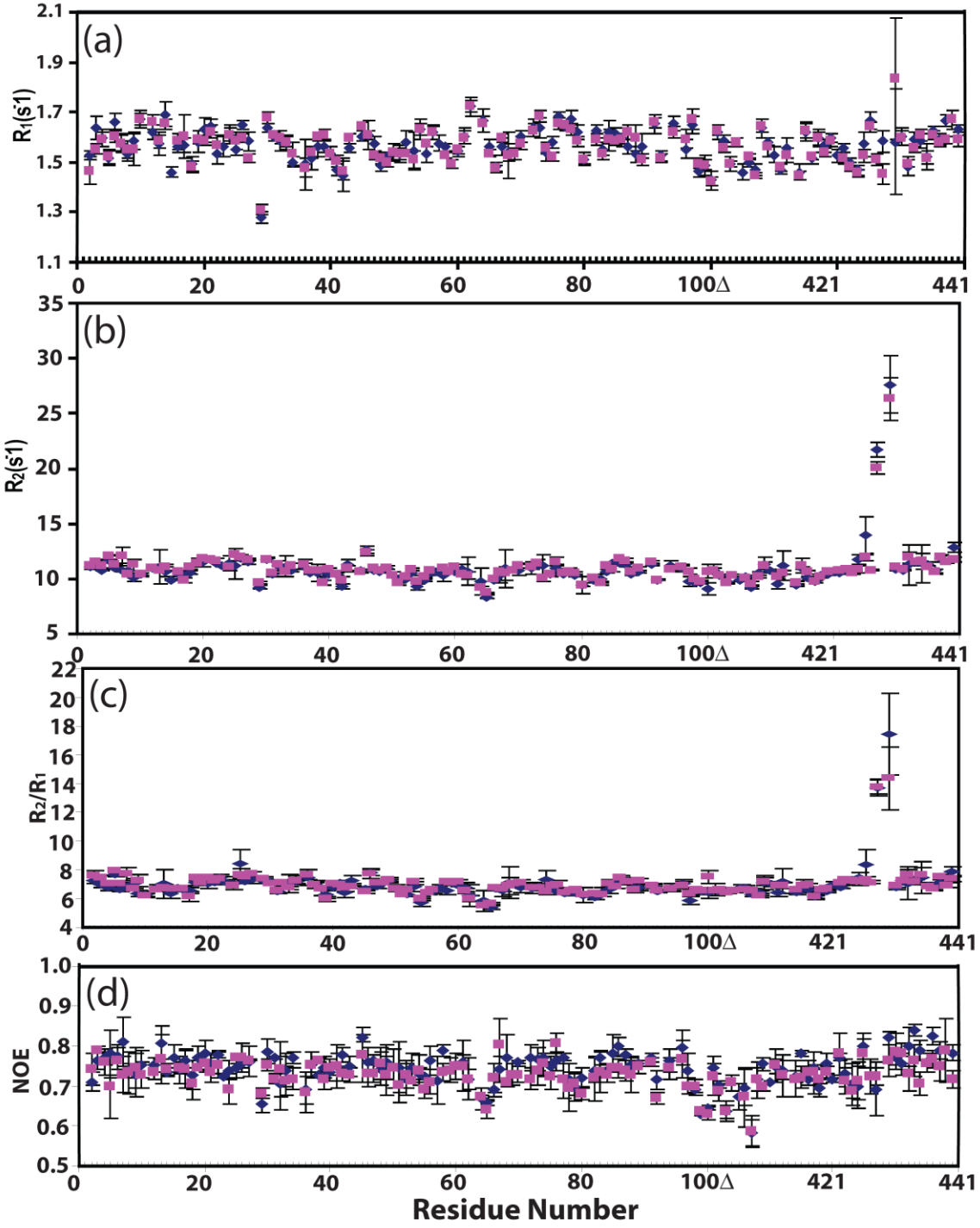


**Figure S3.** Comparison of $R_1$, $R_2$, $R_2/R_1$, and NOE measured at 600 MHz and 298 K between ΔΔIhh-V67CM (red) and ΔΔIhh-L67CM (blue).

*5. Figure S4*

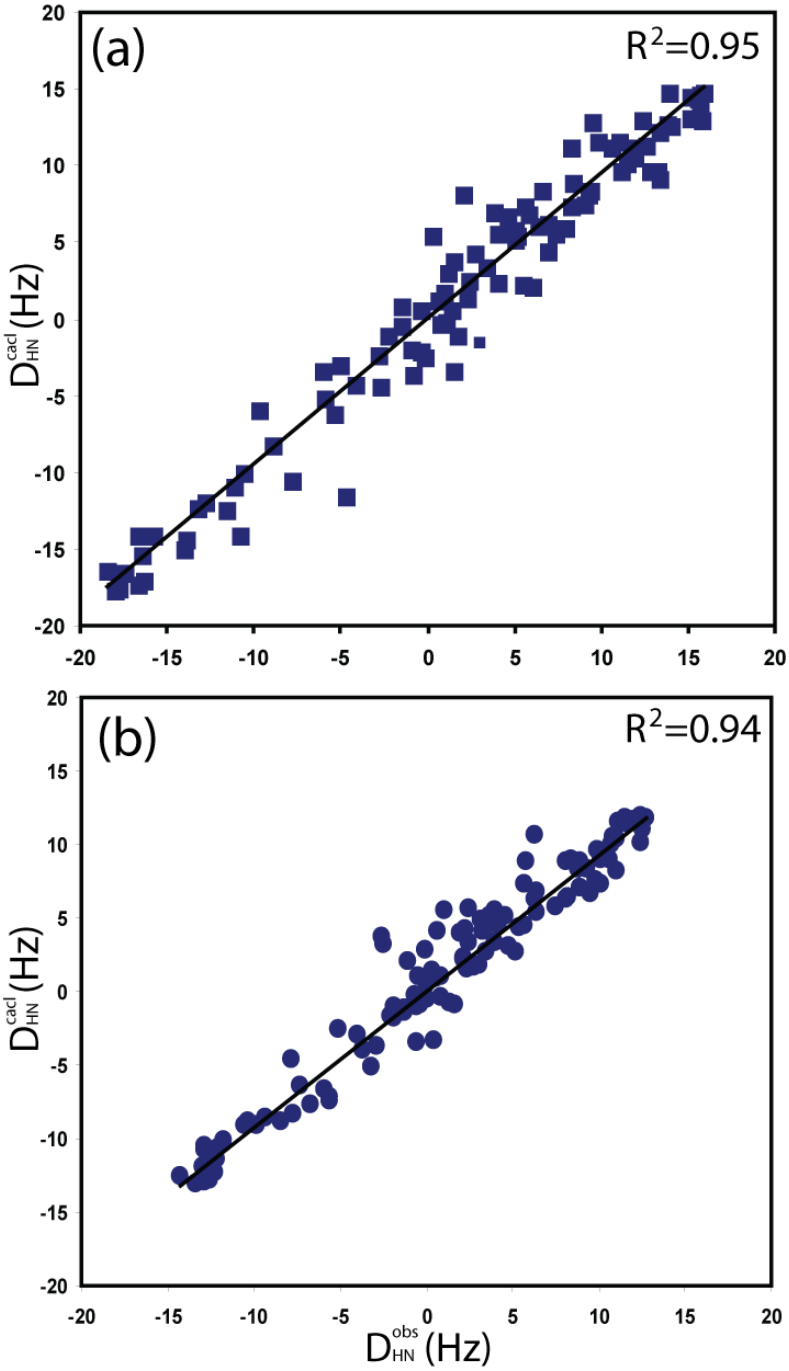


**Figure S4:** Comparison of residual dipolar coupling observed and calculated using PALES program for (a) ΔΔIhh-V67CM and (b) ΔΔIhh-L67CM.

### *6. Table S1. HD exchange results for ΔΔIhh-V67CM and ΔΔIhh-L67CM at 298 K.*

H-D exchange rates and protection factors. Fast exchange represents peaks that were isolated but were not observed in the first HSQC spectrum after exchange in $D_2O$ started.

| | | ΔΔIhh-V67CM | | ΔΔIhh-L67CM | | Diff |
|---|---|---|---|---|---|---|
| Res | 2° | $k_{ex}(min^{-1})$ | P | $k_{ex}(min^{-1})$ | P | $P_{diff}$ |
| C1 | β | Fast exchange | | | | |
| L2 | β | (8.1±6.4)E-03 | 6.3±0.3 | (1.3±0.4)E-02 | 6.1±0.1 | -0.2 |
| A3 | β | Fast exchange | | (8.2±2.4)E-02 | 3.5±0.1 | |
| E4 | β | Fast exchange | | (8.7±2.6)E-02 | 3.2±0.1 | |
| G5 | | (7.3±1.1)E-02 | 3.9±0.1 | Fast exchange | | |
| T6 | β | (3.5±0.5)E-04 | 6.2±0.1 | (1.6±1.3)E-05 | 7.5±0.4 | 1.3 |
| R7 | β | (1.0±0.1)E-03 | 5.9±0.1 | (2.1±0.1)E-04 | 6.6±0.1 | 0.7 |
| I8 | β | <5.0E-06 | >7.4 | <5.00E-06 | >7.4 | |
| F9 | β | Fast exchange | | | | |
| D10 | β | (1.2±0.3)E-04 | 6.3±0.1 | <5.00E-06 | >7.7 | >1.4 |
| P11 | | Proline | | | | |
| V12 | | (3.4±0.4)E-04 | 5.1±0.1 | (1.4±0.1)E-04 | 5.5±0.1 | 0.4 |
| T13 | | Fast exchange | | | | |
| G14 | | (1.5±1.5)E-03 | 5.9±0.4 | (2.5±1.2)E-02 | 4.7±0.2 | -1.2 |
| T15 | β | Overlap | | Fast exchange | | |
| T16 | β | Fast exchange | | | | |
| H17 | β | (3.9±0.5)E-04 | 6.6±0.1 | (1.6±0.1)E-04 | 7.0±0.1 | 0.4 |
| R18 | β | Fast exchange | | | | |
| I19 | β | (1.3±0.3)E-04 | 6.0±0.1 | <5.0E-06 | >7.4 | >1.4 |
| E20 | α | (7.4±1.4)E-03 | 4.0±0.1 | (3.7±0.3)E-03 | 4.3±0.1 | 0.3 |
| D21 | α | (6.2±0.1)E-03 | 4.4±0.1 | (4.7±0.2)E-03 | 4.5±0.1 | 0.1 |
| V22 | α | (6.3±0.2)E-04 | 4.9±0.1 | (3.7±0.4)E-04 | 5.2±0.1 | 0.3 |
| V23 | α | Overlap | | (2.9±0.1)E-04 | 5.3±0.1 | |
| G24 | α | Fast exchange | | | | |
| G25 | α | Fast exchange | | | | |
| R26 | | Fast exchange | | | | |
| K27 | | (7.4±0.9)E-02 | 3.9±0.1 | (3.7±2.2)E-02 | 4.2±0.3 | 0.3 |
| P28 | | Proline | | | | |
| I29 | β | (3.5±0.1)E-03 | 4.1±0.1 | (2. 6±0.1)E-03 | 4.2±0.1 | 0.1 |
| H30 | β | (8.8±0.2)E-04 | 5.8±0.1 | (9.8±0.3)E-04 | 5.8±0.1 | |
| V31 | β | (2.5±0.3)E-05 | 7.0±0.1 | <5.0E-06 | >7.7 | >0.7 |
| V32 | β | (4.4±0.2)E-05 | 6.1±0.1 | (2.7±0.8)E-05 | 6.4±0.1 | 0.3 |
| A33 | β | (5.7±0.6)E-05 | 6.7±0.1 | <5.0E-06 | >7.8 | >1.1 |
| A34 | β | (5.7±0.3)E-04 | 5.9±0.1 | (2.5±0.1)E-04 | 6.2±0.1 | 0.3 |
| A35 | β | Overlap | | | | |
| K36 | | Fast exchange | | | | |
| D37 | | Fast exchange | | | | |
| G38 | | Fast exchange | | | | |
| T39 | β | (2.2±2.4)E-03 | 5.4±0.5 | (6.2±1.5)E-02 | 3.9±0.1 | -1.5 |
| L40 | β | Fast exchange | | | | |
| H41 | β | (2.7±0.1)E-03 | 5.4±0.1 | (2.6±0.1)E-03 | 5.4±0.1 | 0 |
| A42 | β | Fast exchange | | | | |
| R43 | β | (1.1±0.2)E-04 | 6.7±0.1 | <5.0E-06 | >8.0 | >1.3 |
| P44 | β | Proline | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| V45 | β | <5.00E-06 | 7.0±0.1 | (7.9±1.1)E-05 | 5.8±0.1 | -1.2 |
| V46 | β | (1.7±0.1)E-04 | 5.6±0.1 | (1.4±0.2)E-04 | 5.6±0.1 | 0.0 |
| S47 | β | (1.5±0.1)E-04 | 6.7±0.1 | (5.3±0.5)E-05 | 7.1±0.1 | 0.4 |
| W48 | β | Fast exchange | | | | |
| F49 | β | (1.2±0.2)E-04 | 6.2±0.1 | (1.8±1.3)E-05 | 7.0±0.3 | 0.8 |
| D50 | β | Fast exchange | | Overlap | | |
| Q51 | β | (2.2±2.4)E-03 | 5.2±0.5 | (1.7±0.6)E-02 | 4.3±0.1 | -0.9 |
| G52 | β | Fast exchange | | | | |
| T53 | β | Fast exchange | | | | |
| R54 | β | (1.7±0.1)E-03 | 5.7±0.1 | (9.8±0.3)E-04 | 5.9±0.1 | 0.2 |
| D55 | β | Fast exchange | | | | |
| V56 | β | <5.0E-06 | 7.0±0.1 | <5.0E-06 | >7.0 | >0 |
| I57 | β | Overlap | | <5.0E-06 | >7.0 | |
| G58 | β | (5.1±1.5)E-05 | 6.9±0.1 | <5.0E-06 | >8.0 | >1.0 |
| L59 | β | Fast exchange | | Overlap | | |
| R60 | β | (5.0±0.9)E-05 | 6.8±0.1 | <5.0E-06 | >7.8 | >1 |
| I61 | β | (1.4±0.4)E-02 | 4.0±0.1 | (3.5±1.7)E-02 | 3.6±0.2 | -0.4 |
| A62 | β | Fast exchange | | | | |
| G63 | | Chemical change broadening | | | | |
| G64 | | Fast exchange | | | | |
| A65 | β | Fast exchange | | | | |
| I66 | β | (7.8±2.3)E-04 | 5.0±0.1 | Fast exchange | | |
| V/L67 | β | (9.1±2.9)E-05 | 5.7±0.1 | <5.0E-06 | >7.1 | >1.4 |
| W68 | β | (2.9±0.1)E-04 | 5.6±0.1 | (5.7±0.8)E-05 | 6.2±0.1 | 0.6 |
| A69 | β | (5.1±1.5)E-05 | 6.8±0.1 | Overlap | | |
| T70 | β | (5.8±1.4)E-05 | 6.8±0.1 | <5.0E-06 | >7.9±0.1 | >1.1 |
| P71 | | Proline | | | | |
| D72 | | Fast exchange | | | | |
| H73 | β | (1.8±0.3)E-04 | 6.6±0.1 | (5.0±0.6)E-05 | 7.1±0.1 | 0.5 |
| K74 | β | Fast exchange | | | | |
| V75 | β | <5.0E-06 | 7.3±0.1 | (1.3±1.0)E-05 | 6.9±0.3 | -0.4 |
| L76 | β | (2.8±0.8)E-05 | 6.5±0.1 | (1.1±0.8)E-05 | 6.9±0.3 | 0.4 |
| T77 | β | (5.2±1.6)E-05 | 6.6±0.1 | <5.0E-06 | >7.6 | >1.0 |
| E78 | β | Fast exchange | | | | |
| Y79 | | Fast exchange | | | | |
| G80 | β | Fast exchange | | | | |
| W81 | β | Overlap | | | | |
| R82 | β | (1.0±0.2)E-04 | 6.6±0.1 | <5.0E-06 | >7.9 | >1.3 |
| A83 | β | Fast exchange | | | | |
| A84 | β | (1.1±0.2)E-04 | 6.6±0.1 | <5.0E-06 | >7.9 | >1.3 |
| G85 | | (1.9±0.1)E-04 | 6.6±0.1 | (4.8±1.0)E-05 | 7.2±0.1 | 0.6 |
| E86 | | (1.3±0.1)E-02 | 4.2±0.1 | (8.2±0.2)E-03 | 4.4±0.1 | 0.2 |
| L87 | | (1.5±0.3)E-04 | 5.7±0.1 | <5.0E-06 | >7.2 | >1.5 |
| R88 | | (6.0±0.2)E-04 | 5.7±0.1 | (3.1±0.1E-04 | 6.0±0.1 | 0.3 |
| K89 | | Fast exchange | | | | |
| G90 | | Overlap | | | | |
| D91 | β | (2.4±0.7)E-04 | 6.1±0.1 | (1.0±0.8)E-05 | 7.5±0.4 | 1.38 |
| R92 | β | Fast exchange | | | | |
| V93 | β | Overlap | | | | |
| A94 | β | (4.5±1.0)E-05 | 6.8±0.1 | <5.0E-06 | >7.8 | >1.0 |
| V95 | β | Overlap | | | | |
| R96 | β | (6.7±0.3)E-04 | 5.7±0.1 | (3.5±0.3)E-04 | 6.0±0.1 | 0.3 |
| D97 | β | (1.5±0.9)E-02 | 4.4±0.2 | (2.5±0.2)E-02 | 4.1±0.1 | -0.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| V98 | | Fast exchange | | | | |
| E99 | | Fast exchange | | | | |
| T100 | | Fast exchange | | | | |
| G101 | | Fast exchange | | | | |
| E403 | β | (1. 3±0.5)E-03 | 5.2±0.1 | (4.4±0.9E-02 | 3.6±0.1 | -1.5 |
| L404 | β | Fast exchange | | | | |
| R405 | β | (2.0±0.2)E-03 | 5.2±0.1 | Overlap | | |
| Y406 | β | Overlap | | (5.2±2.4)E-04 | 5.9±0.2 | |
| S407 | β | (8.3±1.8)E-05 | 7.1±0.1 | (8.7±6.2)E-06 | 8.1±0.3 | 1.0 |
| V408 | β | Fast exchange | | | | |
| I409 | β | Fast exchange | | (9.7±9.7)E-06 | 6.8±0.4 | |
| R410 | β | (7.8±4.2)E-03 | 4.6±0.2 | (2.90±3.4)E-02 | 4.0±0.5 | -0.6 |
| E411 | β | Overlap | | (6.5±0.5)E-03 | 4.5±0.1 | |
| V412 | β | Fast exchange | | | | |
| L413 | β | (2.8±0.5)E-05 | 6.4±0.1 | <5.0E-06 | >7.2 | >.0.8 |
| P414 | | Proline | | | | |
| T415 | | Fast exchange | | | | |
| R416 | β | (2.7±0.1)E-03 | 5.5±0.1 | (9.7±0.5)E-04 | 5.9±0.1 | 0.4 |
| R417 | β | Fast exchange | | | | |
| A418 | β | (3.3±0.6)E-05 | 7.3±0.1 | <5.0E-06 | >8.1 | >0.8 |
| R419 | β | Fast exchange | | | | |
| T420 | β | (1.4±0.3)E-04 | 6.6±0.1 | <5.0E-06 | >8.1 | >1.5 |
| F421 | β | Overlap | | <5.0E-06 | >7.9 | |
| G422 | β | (4.8±0.7)E-05 | 7.3±0.1 | (5.0±2.4)E-05 | 7.3±0.2 | 0 |
| L423 | β | Fast exchange | | | | |
| E424 | β | (4.2±1.2)E-05 | 6.3±.1 | <5.0E-06 | >7.2 | >0.9 |
| V425 | β | (8.6±4.3)E-04 | 4.8±0.2 | Fast exchange | | |
| E426 | β | (8.8±0.1)E-04 | 5.0±0.1 | (8.4±0.3)E-04 | 5.1±0.1 | 0.1 |
| E427 | | Fast exchange | | Overlap | | |
| L428 | | (4.1±2.2)E-04 | 5.3±0.2 | Fast exchange | | |
| H429 | β | Chemical change broadening | | | | |
| T430 | β | (3.7±0.5)E-04 | 6.4±0.1 | (1.0±0.4)E-04 | 7.0±0.2 | 0.6 |
| L431 | β | (1.1±0.2)E-04 | 6.2±0.1 | (1.2±1.0)E-05 | 7.2±0.3 | 1.0 |
| V432 | β | (4.5±0.7)E-05 | 6.0±0.1 | (1.1±1.4)E-05 | 6.7±0.5 | 0.7 |
| A433 | β | (3.3±0.8)E-05 | 7.0±0.1 | <5.0E-06 | >7.8 | >0.8 |
| E434 | | (1.4±0.6)E-02 | 4.0±0.2 | (3.5±0.1)E-03 | 4.6±0.1 | 0.6 |
| G435 | β | Fast exchange | | | | |
| V436 | β | (1.6±0.3)E-04 | 5.9±0.1 | (2.6±0.7)E-05 | 6.7±0.1 | 0.8 |
| V437 | β | (1.9±0.5)E-05 | 6.5±0.1 | <5.0E-06 | >7.1 | >0.6 |
| V438 | β | (1.5±0.5)E-05 | 6.6±0.1 | <5.0E-06 | >7.1 | >0.5 |
| H439 | β | (1.4±0.1)E-04 | 6.7±0.1 | Overlap | | |
| N440 | β | Fast exchange | | | | |

***7. Table S2. Chemical shifts of backbone and sidechain amide group for ΔΔIhh-V67CM and ΔΔIhh-L67CM (Table S1);***

All chemical shifts were acquired at 298 K in 50 mM sodium phosphate buffer with 100 mM NaCl, pH=7.0. Chemical shifts that are not acquired are marked with "X".

| Res. | Atom Type | ΔΔIhh-V67CM | ΔΔIhh-L67CM |
|---|---|---|---|
| Cys 1 | HN | X | X |
| | $^{15}$N | X | X |
| Leu 2 | HN | 8.99 | 9.08 |
| | $^{15}$N | 122.93 | 122.57 |
| Ala 3 | HN | 8.20 | 8.21 |
| | $^{15}$N | 120.95 | 121.27 |
| Glu 4 | HN | 8.77 | 8.77 |
| | $^{15}$N | 121.27 | 121.26 |
| Gly 5 | HN | 11.31 | 11.32 |
| | $^{15}$N | 120.12 | 120.06 |
| Thr 6 | HN | 7.89 | 7.94 |
| | $^{15}$N | 116.93 | 117.12 |
| Arg 7 | HN | 9.63 | 9.57 |
| | $^{15}$N | 130.46 | 130.22 |
| Ile 8 | HN | 9.55 | 9.60 |
| | $^{15}$N | 123.15 | 123.84 |
| Phe 9 | HN | 8.26 | 8.19 |
| | $^{15}$N | 129.25 | 129.24 |
| Asp 10 | HN | 8.31 | 8.29 |
| | $^{15}$N | 128.20 | 128.17 |
| Pro 11 | HN | X | X |
| | $^{15}$N | X | X |
| Val 12 | HN | 8.98 | 8.96 |
| | $^{15}$N | 124.20 | 124.14 |
| Thr 13 | HN | 7.16 | 7.13 |
| | $^{15}$N | 107.23 | 107.14 |
| Gly 14 | HN | 8.40 | 8.39 |
| | $^{15}$N | 113.00 | 112.90 |
| Thr 15 | HN | 7.67 | 7.66 |
| | $^{15}$N | 117.23 | 117.10 |
| Thr 16 | HN | 8.52 | 8.52 |
| | $^{15}$N | 120.43 | 120.30 |
| His 17 | HN | 9.03 | 9.01 |
| | $^{15}$N | 126.87 | 126.89 |
| Arg 18 | HN | 9.39 | 9.38 |
| | $^{15}$N | 120.88 | 120.92 |
| Ile 19 | HN | 9.08 | 9.02 |
| | $^{15}$N | 125.49 | 125.32 |

| | | | |
|---|---|---|---|
| Glu 20 | HN | 10.85 | 10.88 |
| | $^{15}$N | 123.73 | 123.84 |
| Asp 21 | HN | 7.31 | 7.31 |
| | $^{15}$N | 124.53 | 124.47 |
| Val 22 | HN | 7.47 | 7.44 |
| | $^{15}$N | 122.03 | 121.86 |
| Val 23 | HN | 8.35 | 8.35 |
| | $^{15}$N | 116.68 | 116.73 |
| Gly 24 | HN | 8.33 | 8.33 |
| | $^{15}$N | 108.24 | 108.17 |
| Gly 25 | HN | 7.36 | 7.34 |
| | $^{15}$N | 103.80 | 103.76 |
| Arg 26 | HN | 7.28 | 7.29 |
| | $^{15}$N | 122.15 | 122.13 |
| Lys 27 | HN | 8.05 | 8.04 |
| | $^{15}$N | 115.88 | 115.80 |
| Pro 28 | HN | X | X |
| | $^{15}$N | X | X |
| Ile 29 | HN | 7.54 | 7.56 |
| | $^{15}$N | 116.71 | 116.63 |
| His 30 | HN | 8.28 | 8.28 |
| | $^{15}$N | 118.39 | 117.97 |
| Val 31 | HN | 8.61 | 8.63 |
| | $^{15}$N | 107.33 | 107.44 |
| Val 32 | HN | 8.88 | 8.83 |
| | $^{15}$N | 120.02 | 119.98 |
| Ala 33 | HN | 9.30 | 9.26 |
| | $^{15}$N | 129.10 | 129.01 |
| Ala 34 | HN | 8.12 | 8.15 |
| | $^{15}$N | 121.30 | 121.27 |
| Ala 35 | HN | 9.11 | 9.11 |
| | $^{15}$N | 125.98 | 125.93 |
| Lys 36 | HN | 9.00 | 8.99 |
| | $^{15}$N | 121.19 | 121.16 |
| Asp 37 | HN | 7.78 | 7.77 |
| | $^{15}$N | 115.85 | 115.88 |
| Gly 38 | HN | 8.16 | 8.15 |
| | $^{15}$N | 107.75 | 107.71 |
| Thr 39 | HN | 7.70 | 7.70 |
| | $^{15}$N | 110.45 | 110.42 |
| Leu 40 | HN | 8.26 | 8.24 |
| | $^{15}$N | 120.88 | 120.86 |
| His 41 | HN | 9.26 | 9.26 |

| | | | |
|---|---|---|---|
| | $^{15}N$ | 120.29 | 120.16 |
| Ala 42 | HN | 9.19 | 9.12 |
| | $^{15}N$ | 125.37 | 125.21 |
| Arg 43 | HN | 9.02 | 9.01 |
| | $^{15}N$ | 124.69 | 124.40 |
| Pro 44 | HN | X | X |
| | $^{15}N$ | X | X |
| Val 45 | HN | 7.86 | 7.78 |
| | $^{15}N$ | 119.25 | 119.10 |
| Val 46 | HN | 8.24 | 8.21 |
| | $^{15}N$ | 114.74 | 114.68 |
| Ser 47 | HN | 6.48 | 6.48 |
| | $^{15}N$ | 113.42 | 113.39 |
| Trp 48 | HN | 7.65 | 7.64 |
| | $^{15}N$ | 120.81 | 120.89 |
| | $H_{\varepsilon}$ | 9.25 | 9.25 |
| | $^{15}N_{\varepsilon}$ | 128.75 | 128.67 |
| Phe 49 | HN | 8.90 | 8.90 |
| | $^{15}N$ | 120.48 | 120.40 |
| Asp 50 | HN | 9.10 | 9.09 |
| | $^{15}N$ | 127.61 | 127.48 |
| Gln 51 | HN | 8.01 | 8.03 |
| | $^{15}N$ | 123.41 | 123.54 |
| | $H_{\varepsilon 1}$ | 7.08 | 7.04 |
| | $H_{\varepsilon 2}$ | 6.45 | 6.42 |
| | $^{15}N_{\varepsilon}$ | 110.52 | 110.32 |
| Gly 52 | HN | 8.06 | 8.04 |
| | $^{15}N$ | 108.53 | 108.53 |
| Thr 53 | HN | 8.65 | 8.62 |
| | $^{15}N$ | 119.30 | 119.10 |
| Arg 54 | HN | 8.87 | 8.88 |
| | $^{15}N$ | 126.12 | 126.07 |
| Asp 55 | HN | 8.03 | 8.02 |
| | $^{15}N$ | 119.74 | 119.74 |
| Val 56 | HN | 9.49 | 9.49 |
| | $^{15}N$ | 114.61 | 114.58 |
| Ile 57 | HN | 8.98 | 8.95 |
| | $^{15}N$ | 114.80 | 115.07 |
| Gly 58 | HN | 6.71 | 6.75 |
| | $^{15}N$ | 107.67 | 107.52 |
| Leu 59 | HN | 8.72 | 8.85 |
| | $^{15}N$ | 123.52 | 124.22 |
| Arg 60 | HN | 8.62 | 8.65 |

| | | | |
|---|---|---|---|
| | $^{15}N$ | 123.88 | 124.21 |
| Ile 61 | HN | 9.13 | 8.93 |
| | $^{15}N$ | 128.53 | 128.29 |
| Ala 62 | HN | 8.62 | 8.53 |
| | $^{15}N$ | 128.60 | 128.05 |
| Gly 63 | HN | X | X |
| | $^{15}N$ | X | X |
| Gly 64 | HN | 8.21 | 8.21 |
| | $^{15}N$ | 107.92 | 107.99 |
| Ala 65 | HN | 7.98 | 8.00 |
| | $^{15}N$ | 125.50 | 125.80 |
| Ile 66 | HN | 8.09 | 8.00 |
| | $^{15}N$ | 118.66 | 118.15 |
| Val /Leu 67 | HN | 8.14 | 8.24 |
| | $^{15}N$ | 125.29 | 127.80 |
| Trp 68 | HN | 9.72 | 9.99 |
| | $^{15}N$ | 129.00 | 129.40 |
| | $H_{\varepsilon}$ | 10.40 | 10.38 |
| | $^{15}N_{\varepsilon}$ | 131.63 | 131.48 |
| Ala 69 | HN | 8.92 | 8.83 |
| | $^{15}N$ | 124.45 | 124.37 |
| Thr 70 | HN | 8.74 | 8.74 |
| | $^{15}N$ | 112.26 | 111.60 |
| Pro 71 | HN | X | X |
| | $^{15}N$ | X | X |
| Asp 72 | HN | 8.74 | 8.73 |
| | $^{15}N$ | 112.26 | 112.39 |
| His 73 | HN | 8.34 | 8.34 |
| | $^{15}N$ | 125.14 | 125.23 |
| Lys 74 | HN | 8.42 | 8.41 |
| | $^{15}N$ | 127.66 | 127.60 |
| Val 75 | HN | 9.46 | 9.46 |
| | $^{15}N$ | 125.63 | 125.73 |
| Leu 76 | HN | 8.20 | 8.31 |
| | $^{15}N$ | 129.93 | 129.88 |
| Thr 77 | HN | 7.40 | 7.40 |
| | $^{15}N$ | 116.34 | 116.25 |
| Glu 78 | HN | 8.18 | 8.24 |
| | $^{15}N$ | 120.13 | 120.07 |
| Tyr 79 | HN | 7.73 | 7.75 |
| | $^{15}N$ | 117.29 | 117.32 |
| Gly 80 | HN | 7.36 | 7.36 |

| | | | |
|---|---|---|---|
| | $^{15}N$ | 106.92 | 106.96 |
| Trp 81 | HN | 9.13 | 9.07 |
| | $^{15}N$ | 125.82 | 125.75 |
| | $H_\varepsilon$ | 10.49 | 10.45 |
| | $^{15}N_\varepsilon$ | 130.50 | 130.31 |
| Arg 82 | HN | 9.41 | 9.45 |
| | $^{15}N$ | 124.63 | 124.68 |
| Ala 83 | HN | 8.84 | 8.81 |
| | $^{15}N$ | 126.02 | 125.97 |
| Ala 84 | HN | 9.34 | 9.35 |
| | $^{15}N$ | 125.84 | 125.85 |
| Gly 85 | HN | 9.75 | 9.73 |
| | $^{15}N$ | 102.34 | 102.28 |
| Glu 86 | HN | 8.15 | 8.16 |
| | $^{15}N$ | 117.62 | 117.65 |
| Leu 87 | HN | 7.21 | 7.21 |
| | $^{15}N$ | 119.83 | 119.80 |
| Arg 88 | HN | 9.11 | 9.13 |
| | $^{15}N$ | 120.80 | 120.92 |
| Lys 89 | HN | 8.45 | 8.43 |
| | $^{15}N$ | 119.88 | 119.88 |
| Gly 90 | HN | 8.86 | 8.91 |
| | $^{15}N$ | 115.05 | 115.15 |
| Asp 91 | HN | 8.18 | 8.20 |
| | $^{15}N$ | 121.76 | 121.74 |
| Arg 92 | HN | 8.82 | 8.78 |
| | $^{15}N$ | 118.10 | 117.88 |
| Val 93 | HN | 8.86 | 8.86 |
| | $^{15}N$ | 115.39 | 115.14 |
| Ala 94 | HN | 8.54 | 8.59 |
| | $^{15}N$ | 126.20 | 126.31 |
| Val 95 | HN | 8.87 | 8.88 |
| | $^{15}N$ | 115.63 | 115.24 |
| Arg 96 | HN | 8.15 | 8.08 |
| | $^{15}N$ | 119.79 | 119.44 |
| Asp 97 | HN | 8.89 | 8.90 |
| | $^{15}N$ | 131.32 | 131.24 |
| Val 98 | HN | 8.46 | 8.43 |
| | $^{15}N$ | 126.17 | 126.18 |
| Glu 99 | HN | 8.22 | 8.19 |
| | $^{15}N$ | 118.98 | 118.94 |
| Thr 100 | HN | 8.05 | 8.03 |
| | $^{15}N$ | 106.43 | 106.36 |

| | | | |
|---|---|---|---|
| Gly 101 | HN | 8.20 | 8.19 |
| | $^{15}$N | 111.27 | 111.26 |
| Glu 403 | HN | 7.54 | 7.53 |
| | $^{15}$N | 118.54 | 118.50 |
| Leu 404 | HN | 8.39 | 8.38 |
| | $^{15}$N | 121.66 | 121.82 |
| Arg 405 | HN | 9.09 | 9.08 |
| | $^{15}$N | 126.87 | 127.17 |
| Tyr 406 | HN | 8.35 | 8.33 |
| | $^{15}$N | 116.45 | 116.25 |
| Ser 407 | HN | 9.28 | 9.28 |
| | $^{15}$N | 118.45 | 118.30 |
| Val 408 | HN | 8.35 | 8.31 |
| | $^{15}$N | 122.19 | 122.30 |
| Ile 409 | HN | 8.76 | 8.72 |
| | $^{15}$N | 123.42 | 123.54 |
| Arg 410 | HN | 9.53 | 9.45 |
| | $^{15}$N | 130.35 | 130.33 |
| Glu 411 | HN | 7.66 | 7.61 |
| | $^{15}$N | 117.05 | 116.80 |
| Val 412 | HN | 8.72 | 8.74 |
| | $^{15}$N | 125.74 | 125.76 |
| Leu 413 | HN | 9.77 | 9.85 |
| | $^{15}$N | 132.54 | 132.73 |
| Pro 414 | HN | X | X |
| | $^{15}$N | X | X |
| Thr 415 | HN | 7.75 | 7.73 |
| | $^{15}$N | 112.94 | 112.70 |
| Arg 416 | HN | 9.16 | 9.15 |
| | $^{15}$N | 123.40 | 123.27 |
| Arg 417 | HN | 8.40 | 8.39 |
| | $^{15}$N | 119.22 | 119.24 |
| Ala 418 | HN | 8.75 | 8.75 |
| | $^{15}$N | 125.06 | 125.01 |
| Arg 419 | HN | 8.74 | 8.74 |
| | $^{15}$N | 120.97 | 120.91 |
| Thr 420 | HN | 7.76 | 7.76 |
| | $^{15}$N | 111.55 | 111.53 |
| Phe 421 | HN | 8.98 | 9.00 |
| | $^{15}$N | 114.80 | 114.50 |
| Gly 422 | HN | 9.53 | 9.50 |
| | $^{15}$N | 107.77 | 107.62 |
| Leu 423 | HN | 8.43 | 8.46 |

| | | | |
|---|---|---|---|
| | $^{15}N$ | 118.87 | 118.87 |
| Glu 424 | HN | 7.09 | 7.13 |
| | $^{15}N$ | 118.58 | 118.62 |
| Val 425 | HN | 8.86 | 8.81 |
| | $^{15}N$ | 129.45 | 129.26 |
| Glu 426 | HN | 8.15 | 8.08 |
| | $^{15}N$ | 128.75 | 128.57 |
| Glu 427 | HN | 8.54 | 8.50 |
| | $^{15}N$ | 121.11 | 121.03 |
| Leu 428 | HN | 9.42 | 9.45 |
| | $^{15}N$ | 120.83 | 120.98 |
| His 429 | HN | 7.10 | 7.06 |
| | $^{15}N$ | 107.70 | 107.57 |
| Thr 430 | HN | 6.93 | 6.95 |
| | $^{15}N$ | 105.49 | 105.75 |
| Leu 431 | HN | 9.13 | 9.12 |
| | $^{15}N$ | 111.69 | 111.71 |
| Val 432 | HN | 7.58 | 7.51 |
| | $^{15}N$ | 119.63 | 119.67 |
| Ala 433 | HN | 9.73 | 9.73 |
| | $^{15}N$ | 135.06 | 134.74 |
| Glu 434 | HN | 8.99 | 9.12 |
| | $^{15}N$ | 118.07 | 118.80 |
| Gly 435 | HN | 7.23 | 7.29 |
| | $^{15}N$ | 100.07 | 100.09 |
| Val 436 | HN | 8.06 | 8.11 |
| | $^{15}N$ | 122.51 | 122.46 |
| Val 437 | HN | 8.28 | 8.32 |
| | $^{15}N$ | 126.47 | 126.48 |
| Val 438 | HN | 9.37 | 9.40 |
| | $^{15}N$ | 118.76 | 118.74 |
| His 439 | HN | 8.66 | 8.57 |
| | $^{15}N$ | 120.69 | 121.30 |
| Asn 440 | HN | 7.82 | 7.93 |
| | $^{15}N$ | 126.26 | 126.83 |
| | $H_{\delta 1}$ | 7.34 | 7.39 |
| | $H_{\delta 2}$ | 8.18 | 8.19 |
| | $^{15}N_{\delta}$ | 114.20 | 114.17 |

***8. Table S3. 10% trimmed mean values of $^{15}N$ relaxation parameters acquired for ΔΔIhh-V67CM and ΔΔIhh-L67CM at 600 Mz an 298 K.***

| 600 MHz | ΔΔIhh-V67CM (120 entries) | ΔΔIhh-L67CM (120 entries) |
|---|---|---|
| $R_1(s^{-1})$ | 1.57±0.03 | 1.57±0.03 |
| $R_2(s^{-1})$ | 10.82±0.21 | 10.70±0.33 |
| NOE | 0.73±0.03 | 0.75±0.03 |
| $R_2/R_1$ | 6.92±0.18 | 6.83±0.27 |

9. ***$^{15}$N relaxation data and residual dipolar coupling (RDC) data for ΔΔIhh-V67CM and ΔΔIhh-L67CM at 298 K, pH=7.0. (Table S4);***

$^{15}$N relaxation data were acquired at 600 MHz ($^{1}$H frequency), 14.1 Telsa. Residual dipolar coupling (RDC) data were acquired at 800 MHz ($^{1}$H frequency), 18.8 Telsa. RDC data were acquired using 7% polyacrylamide gels with a stretch ratio $d_o/d_N$ of 1.29. The RDC was measured using IPAP-HSQC pulse sequence at 298 K.

## Table S4a : ΔΔIhh-V67CM:

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | |
| 2 | 1.467 | 0.057 | 11.145 | 0.243 | 0.742 | 0.040 | 7.596 | 0.339 | 13.804 |
| 3 | 1.553 | 0.020 | 11.565 | 0.192 | 0.790 | 0.009 | 7.445 | 0.157 | |
| 4 | 1.591 | 0.031 | 11.252 | 0.240 | 0.759 | 0.033 | 7.074 | 0.206 | |
| 5 | 1.522 | 0.029 | 12.137 | 0.079 | 0.699 | 0.080 | 7.977 | 0.160 | 12.405 |
| 6 | 1.605 | 0.024 | 11.260 | 0.089 | 0.763 | 0.034 | 7.015 | 0.119 | -8.939 |
| 7 | 1.576 | 0.032 | 12.140 | 0.673 | 0.729 | 0.045 | 7.704 | 0.455 | 10.581 |
| 8 | 1.552 | 0.035 | 10.405 | 0.101 | 0.733 | 0.052 | 6.705 | 0.165 | -1.459 |
| 9 | 1.554 | 0.065 | 11.282 | 0.479 | 0.745 | 0.050 | 7.259 | 0.433 | 1.033 |
| 10 | 1.673 | 0.023 | 10.442 | 0.136 | 0.728 | 0.033 | 6.241 | 0.119 | -15.750 |
| 11 | | | | | | | | | |
| 12 | 1.661 | 0.029 | 11.033 | 0.226 | 0.734 | 0.018 | 6.643 | 0.179 | 1.521 |
| 13 | 1.585 | 0.026 | 10.769 | 0.205 | 0.767 | 0.060 | 6.793 | 0.169 | -4.196 |
| 14 | 1.658 | 0.015 | 11.065 | 0.255 | 0.741 | 0.016 | 6.672 | 0.164 | -18.000 |
| 15 | | | | | | | | | |
| 16 | 1.584 | 0.031 | 10.630 | 0.174 | 0.745 | 0.015 | 6.710 | 0.172 | -18.425 |
| 17 | 1.604 | 0.085 | 9.880 | 0.189 | 0.742 | 0.047 | 6.158 | 0.346 | |
| 18 | 1.483 | 0.022 | 11.034 | 0.134 | 0.705 | 0.039 | 7.439 | 0.144 | |
| 19 | 1.591 | 0.018 | 11.367 | 0.317 | 0.745 | 0.003 | 7.147 | 0.216 | 8.209 |
| 20 | 1.584 | 0.017 | 11.824 | 0.232 | 0.758 | 0.024 | 7.465 | 0.166 | 6.020 |
| 21 | 1.623 | 0.025 | 11.777 | 0.238 | 0.734 | 0.026 | 7.257 | 0.186 | 3.405 |
| 22 | 1.571 | 0.037 | 11.644 | 0.230 | 0.754 | 0.033 | 7.414 | 0.229 | 11.736 |
| 23 | | | | | | | | | |
| 24 | 1.611 | 0.027 | 11.089 | 0.315 | 0.693 | 0.039 | 6.885 | 0.228 | 4.987 |
| 25 | 1.584 | 0.034 | 12.197 | 0.595 | 0.770 | 0.013 | 7.702 | 0.409 | 5.048 |
| 26 | 1.598 | 0.015 | 12.003 | 0.221 | 0.771 | 0.037 | 7.511 | 0.156 | 15.142 |
| 27 | 1.515 | 0.017 | 11.717 | 0.113 | 0.765 | 0.002 | 7.732 | 0.112 | 15.628 |
| 28 | | | | | | | | | |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 29 | 1.310 | 0.021 | 9.690 | 0.122 | 0.682 | 0.013 | 7.395 | 0.151 | 2.068 |
| 30 | 1.676 | 0.023 | 11.790 | 0.137 | 0.752 | 0.014 | 7.035 | 0.125 | |
| 31 | 1.607 | 0.021 | 10.559 | 0.146 | 0.718 | 0.014 | 6.569 | 0.124 | -5.898 |
| 32 | 1.600 | 0.028 | 11.348 | 0.291 | 0.741 | 0.069 | 7.092 | 0.221 | 9.243 |
| 33 | 1.583 | 0.022 | 10.675 | 0.136 | 0.712 | 0.036 | 6.744 | 0.127 | 12.527 |
| 34 | 1.534 | 0.046 | 11.156 | 0.301 | 0.718 | 0.026 | 7.275 | 0.294 | |
| 35 | | | | | | | | | |
| 36 | 1.478 | 0.036 | 11.328 | 0.393 | 0.684 | 0.052 | 7.664 | 0.324 | |
| 37 | 1.541 | 0.024 | 10.749 | 0.123 | 0.754 | 0.032 | 6.976 | 0.135 | 9.486 |
| 38 | 1.603 | 0.018 | 10.731 | 0.187 | 0.764 | 0.005 | 6.695 | 0.137 | -0.851 |
| 39 | 1.607 | 0.023 | 9.626 | 0.227 | 0.715 | 0.022 | 5.989 | 0.165 | -13.196 |
| 40 | 1.536 | 0.024 | 10.830 | 0.190 | 0.747 | 0.021 | 7.052 | 0.164 | -2.676 |
| 41 | 1.504 | 0.025 | 10.467 | 0.114 | 0.748 | 0.054 | 6.958 | 0.140 | 3.831 |
| 42 | 1.467 | 0.037 | 9.884 | 0.322 | 0.731 | 0.018 | 6.739 | 0.277 | 12.831 |
| 43 | 1.597 | 0.020 | 10.955 | 0.255 | 0.727 | 0.019 | 6.861 | 0.182 | |
| 44 | | | | | | | | | |
| 45 | 1.646 | 0.017 | 10.699 | 0.206 | 0.777 | 0.068 | 6.502 | 0.142 | -17.452 |
| 46 | 1.608 | 0.058 | 12.397 | 0.282 | 0.731 | 0.033 | 7.708 | 0.329 | 5.473 |
| 47 | 1.529 | 0.017 | 10.928 | 0.109 | 0.732 | 0.021 | 7.147 | 0.107 | 0.304 |
| 48 | 1.517 | 0.023 | 10.770 | 0.168 | 0.756 | 0.040 | 7.100 | 0.153 | 0.608 |
| 49 | 1.499 | 0.015 | 10.986 | 0.174 | 0.727 | 0.049 | 7.329 | 0.136 | 0.668 |
| 50 | 1.538 | 0.034 | 10.318 | 0.277 | 0.744 | 0.048 | 6.711 | 0.232 | |
| 51 | 1.533 | 0.024 | 9.660 | 0.212 | 0.702 | 0.043 | 6.303 | 0.171 | -10.581 |
| 52 | 1.536 | 0.013 | 10.173 | 0.143 | 0.729 | 0.034 | 6.621 | 0.108 | -1.521 |
| 53 | 1.508 | 0.039 | 10.870 | 0.145 | 0.712 | 0.003 | 7.209 | 0.212 | -0.426 |
| 54 | 1.632 | 0.025 | 9.789 | 0.204 | 0.740 | 0.037 | 6.000 | 0.155 | -14.048 |
| 55 | 1.577 | 0.015 | 10.162 | 0.085 | 0.690 | 0.020 | 6.445 | 0.081 | -17.756 |
| 56 | 1.619 | 0.027 | 10.744 | 0.130 | 0.711 | 0.032 | 6.638 | 0.137 | -0.913 |
| 57 | | | | | | | | | |
| 58 | 1.527 | 0.010 | 10.938 | 0.088 | 0.734 | 0.021 | 7.165 | 0.075 | 15.689 |
| 59 | 1.494 | 0.017 | 10.645 | 0.108 | 0.740 | 0.031 | 7.127 | 0.108 | 9.790 |
| 60 | 1.549 | 0.013 | 11.079 | 0.192 | 0.748 | 0.033 | 7.154 | 0.138 | 6.628 |
| 61 | 1.600 | 0.026 | 10.475 | 0.196 | 0.750 | 0.064 | 6.546 | 0.161 | 0.912 |
| 62 | 1.724 | 0.024 | 10.350 | 0.325 | 0.717 | 0.052 | 6.003 | 0.205 | -13.864 |
| 63 | | | | | | | | | |
| 64 | 1.653 | 0.030 | 9.185 | 0.120 | 0.672 | 0.004 | 5.557 | 0.125 | |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 65 | 1.536 | 0.016 | 8.713 | 0.081 | 0.642 | 0.022 | 5.672 | 0.078 | 2.676 |
| 66 | 1.475 | 0.012 | 10.022 | 0.092 | 0.720 | 0.037 | 6.797 | 0.084 | 1.216 |
| 67 | 1.599 | 0.016 | 10.691 | 0.316 | 0.804 | 0.063 | 6.688 | 0.209 | 6.872 |
| 68 | 1.528 | 0.092 | 10.508 | 0.616 | 0.710 | 0.010 | 6.878 | 0.578 | 5.595 |
| 69 | 1.534 | 0.022 | 10.811 | 0.088 | 0.729 | 0.028 | 7.047 | 0.117 | 13.378 |
| 70 | 1.577 | 0.025 | 11.230 | 0.210 | 0.729 | 0.019 | 7.122 | 0.175 | 15.932 |
| 71 | | | | | | | | | |
| 72 | 1.629 | 0.027 | 11.045 | 0.169 | 0.718 | 0.026 | 6.778 | 0.154 | 1.338 |
| 73 | 1.683 | 0.024 | 11.397 | 0.302 | 0.737 | 0.035 | 6.772 | 0.204 | 2.311 |
| 74 | 1.557 | 0.025 | 10.059 | 0.284 | 0.755 | 0.026 | 6.462 | 0.209 | 8.331 |
| 75 | 1.523 | 0.018 | 10.473 | 0.226 | 0.725 | 0.034 | 6.876 | 0.169 | 1.764 |
| 76 | 1.657 | 0.024 | 11.586 | 0.364 | 0.807 | 0.026 | 6.990 | 0.241 | -9.669 |
| 77 | 1.648 | 0.018 | 10.544 | 0.089 | 0.716 | 0.046 | 6.397 | 0.087 | -16.480 |
| 78 | 1.631 | 0.022 | 10.679 | 0.098 | 0.696 | 0.060 | 6.547 | 0.107 | |
| 79 | 1.584 | 0.022 | 10.532 | 0.157 | 0.704 | 0.028 | 6.647 | 0.135 | |
| 80 | 1.510 | 0.017 | 9.472 | 0.207 | 0.680 | 0.014 | 6.275 | 0.154 | -16.723 |
| 81 | | | | | | | | | |
| 82 | 1.590 | 0.022 | 10.075 | 0.146 | 0.719 | 0.056 | 6.335 | 0.127 | -16.298 |
| 83 | 1.531 | 0.021 | 9.618 | 0.142 | 0.736 | 0.051 | 6.281 | 0.127 | -11.129 |
| 84 | 1.590 | 0.044 | 11.241 | 0.233 | 0.728 | 0.017 | 7.068 | 0.245 | 13.196 |
| 85 | 1.586 | 0.019 | 10.892 | 0.287 | 0.744 | 0.002 | 6.867 | 0.198 | 4.013 |
| 86 | 1.590 | 0.025 | 11.856 | 0.096 | 0.744 | 0.022 | 7.458 | 0.131 | 15.567 |
| 87 | 1.621 | 0.027 | 11.618 | 0.234 | 0.738 | 0.025 | 7.166 | 0.188 | 4.621 |
| 88 | 1.597 | 0.052 | 10.630 | 0.204 | 0.728 | 0.045 | 6.658 | 0.253 | 5.047 |
| 89 | 1.511 | 0.024 | 10.925 | 0.191 | 0.748 | 0.017 | 7.233 | 0.171 | 9.365 |
| 90 | | | | | | | | | |
| 91 | 1.659 | 0.021 | 11.497 | 0.137 | 0.761 | 0.011 | 6.932 | 0.120 | 4.074 |
| 92 | 1.517 | 0.017 | 9.914 | 0.079 | 0.670 | 0.013 | 6.536 | 0.089 | -11.615 |
| 93 | | | | | | | | | |
| 94 | 1.621 | 0.021 | 10.834 | 0.107 | 0.744 | 0.028 | 6.685 | 0.109 | -4.743 |
| 95 | | | | | | | | | |
| 96 | 1.594 | 0.039 | 11.059 | 0.229 | 0.767 | 0.047 | 6.936 | 0.223 | |
| 97 | 1.675 | 0.041 | 10.698 | 0.279 | 0.700 | 0.021 | 6.387 | 0.229 | -2.797 |
| 98 | 1.495 | 0.019 | 9.949 | 0.143 | 0.700 | 0.015 | 6.654 | 0.128 | 13.865 |
| 99 | 1.489 | 0.019 | 9.801 | 0.087 | 0.637 | 0.009 | 6.582 | 0.101 | 7.358 |
| 100 | 1.425 | 0.018 | 10.686 | 0.638 | 0.631 | 0.016 | 7.500 | 0.457 | 11.067 |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 101 | 1.629 | 0.018 | 10.704 | 0.248 | 0.725 | 0.048 | 6.572 | 0.168 | 9.061 |
| 403 | 1.559 | 0.022 | 10.286 | 0.141 | 0.688 | 0.025 | 6.599 | 0.131 | -7.844 |
| 404 | 1.494 | 0.013 | 9.604 | 0.099 | 0.638 | 0.026 | 6.429 | 0.088 | |
| 405 | 1.579 | 0.016 | 10.335 | 0.134 | 0.708 | 0.021 | 6.544 | 0.107 | |
| 406 | | | | | | | | | |
| 407 | 1.524 | 0.011 | 10.132 | 0.185 | 0.672 | 0.074 | 6.650 | 0.131 | -6.020 |
| 408 | 1.448 | 0.010 | 9.533 | 0.207 | 0.587 | 0.041 | 6.584 | 0.150 | |
| 409 | 1.644 | 0.026 | 10.201 | 0.150 | 0.716 | 0.045 | 6.205 | 0.135 | -10.824 |
| 410 | 1.562 | 0.037 | 11.222 | 0.321 | 0.698 | 0.011 | 7.183 | 0.267 | 7.966 |
| 411 | | | | | | | | | |
| 412 | 1.483 | 0.017 | 10.242 | 0.075 | 0.751 | 0.041 | 6.905 | 0.092 | 13.986 |
| 413 | 1.528 | 0.030 | 10.560 | 0.190 | 0.726 | 0.051 | 6.910 | 0.183 | 15.082 |
| 414 | | | | | | | | | |
| 415 | 1.447 | 0.019 | 9.601 | 0.148 | 0.718 | 0.045 | 6.637 | 0.136 | -0.365 |
| 416 | 1.629 | 0.032 | 11.225 | 0.670 | 0.722 | 0.027 | 6.890 | 0.433 | 8.270 |
| 417 | 1.525 | 0.015 | 10.592 | 0.144 | 0.734 | 0.031 | 6.946 | 0.116 | |
| 418 | 1.596 | 0.026 | 9.763 | 0.086 | 0.734 | 0.052 | 6.117 | 0.111 | -16.723 |
| 419 | 1.540 | 0.019 | 10.243 | 0.102 | 0.716 | 0.012 | 6.650 | 0.104 | |
| 420 | 1.588 | 0.027 | 10.597 | 0.077 | 0.733 | 0.017 | 6.674 | 0.123 | -5.412 |
| 421 | | | | | | | | | |
| 422 | 1.517 | 0.023 | 10.800 | 0.195 | 0.780 | 0.051 | 7.118 | 0.167 | 6.324 |
| 423 | 1.481 | 0.018 | 10.848 | 0.134 | 0.713 | 0.031 | 7.324 | 0.126 | |
| 424 | 1.456 | 0.015 | 10.556 | 0.166 | 0.687 | 0.019 | 7.249 | 0.137 | -2.250 |
| 425 | 1.526 | 0.024 | 10.915 | 0.364 | 0.712 | 0.047 | 7.152 | 0.264 | 1.520 |
| 426 | 1.643 | 0.021 | 12.013 | 0.320 | 0.780 | 0.036 | 7.313 | 0.216 | -12.831 |
| 427 | 1.513 | 0.023 | 10.805 | 0.070 | 0.724 | 0.044 | 7.143 | 0.119 | -4.987 |
| 428 | 1.452 | 0.040 | 20.016 | 0.543 | 0.725 | 0.022 | 13.785 | 0.535 | 0.30 |
| 429 | | | | | | | | | |
| 430 | 1.832 | 0.243 | 26.335 | 1.930 | 0.763 | 0.039 | 14.375 | 2.178 | 11.128 |
| 431 | 1.597 | 0.028 | 11.077 | 0.101 | 0.787 | 0.044 | 6.938 | 0.135 | 11.858 |
| 432 | 1.495 | 0.016 | 10.873 | 0.163 | 0.782 | 0.025 | 7.273 | 0.134 | 13.318 |
| 433 | 1.558 | 0.017 | 11.920 | 0.519 | 0.731 | 0.043 | 7.650 | 0.344 | 3.041 |
| 434 | 1.611 | 0.024 | 11.538 | 0.153 | 0.763 | 0.039 | 7.162 | 0.142 | 5.169 |
| 435 | 1.519 | 0.027 | 11.596 | 0.710 | 0.706 | 0.028 | 7.636 | 0.487 | 11.493 |
| 436 | 1.610 | 0.016 | 11.042 | 0.149 | 0.758 | 0.025 | 6.859 | 0.115 | 2.371 |
| 437 | 1.581 | 0.011 | 10.624 | 0.217 | 0.767 | 0.023 | 6.721 | 0.146 | 6.933 |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 438 | 1.591 | 0.014 | 12.010 | 0.180 | 0.748 | 0.034 | 7.551 | 0.130 | 5.777 |
| 439 | 1.672 | 0.038 | 11.586 | 0.128 | 0.790 | 0.079 | 6.928 | 0.173 | 15.811 |
| 440 | 1.591 | 0.029 | 11.704 | 0.225 | 0.718 | 0.022 | 7.358 | 0.195 | -0.121 |

## Table S4b : ΔΔIhh-L67CM:

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | |
| 2 | 1.527 | 0.019 | 11.126 | 0.304 | 0.711 | 0.024 | 7.287 | 0.220 | 11.069 |
| 3 | 1.636 | 0.047 | 11.445 | 0.149 | 0.763 | 0.022 | 6.995 | 0.220 | |
| 4 | 1.599 | 0.036 | 10.776 | 0.044 | 0.769 | 0.017 | 6.741 | 0.152 | -12.833 |
| 5 | 1.515 | 0.028 | 11.628 | 0.129 | 0.780 | 0.060 | 7.673 | 0.164 | 10.826 |
| 6 | 1.663 | 0.031 | 11.028 | 0.107 | 0.774 | 0.031 | 6.630 | 0.139 | -5.899 |
| 7 | 1.576 | 0.036 | 10.766 | 0.156 | 0.810 | 0.061 | 6.832 | 0.183 | 8.393 |
| 8 | 1.532 | 0.026 | 10.785 | 0.453 | 0.739 | 0.018 | 7.042 | 0.319 | -0.365 |
| 9 | 1.585 | 0.031 | 10.049 | 0.263 | 0.726 | 0.054 | 6.340 | 0.207 | 0.851 |
| 10 | 1.672 | 0.035 | 10.475 | 0.252 | 0.754 | 0.034 | 6.266 | 0.200 | -11.738 |
| 11 | | | | | | | | | |
| 12 | 1.621 | 0.029 | 11.005 | 0.229 | 0.751 | 0.018 | 6.790 | 0.187 | 0.426 |
| 13 | 1.573 | 0.045 | 11.054 | 1.540 | 0.808 | 0.041 | 7.028 | 0.999 | -2.919 |
| 14 | 1.688 | 0.054 | 10.681 | 0.460 | 0.736 | 0.010 | 6.329 | 0.339 | -13.319 |
| 15 | 1.460 | 0.021 | 9.922 | 0.093 | 0.770 | 0.035 | 6.796 | 0.116 | -2.494 |
| 16 | 1.563 | 0.022 | 10.415 | 0.267 | 0.741 | 0.034 | 6.664 | 0.196 | -12.954 |
| 17 | 1.569 | 0.029 | 10.339 | 0.264 | 0.763 | 0.029 | 6.588 | 0.207 | -7.298 |
| 18 | 1.493 | 0.028 | 10.588 | 0.142 | 0.729 | 0.036 | 7.092 | 0.161 | |
| 19 | 1.582 | 0.043 | 11.494 | 0.398 | 0.770 | 0.030 | 7.263 | 0.321 | 5.839 |
| 20 | 1.630 | 0.049 | 11.497 | 0.408 | 0.781 | 0.032 | 7.055 | 0.328 | 5.230 |
| 21 | 1.645 | 0.025 | 11.731 | 0.127 | 0.755 | 0.024 | 7.130 | 0.135 | 2.189 |
| 22 | 1.532 | 0.040 | 11.432 | 0.029 | 0.779 | 0.010 | 7.465 | 0.198 | 10.643 |
| 23 | 1.562 | 0.040 | 11.158 | 0.042 | 0.725 | 0.005 | 7.144 | 0.184 | 8.150 |
| 24 | 1.611 | 0.036 | 11.396 | 0.636 | 0.740 | 0.022 | 7.073 | 0.425 | 3.953 |
| 25 | 1.553 | 0.027 | 11.154 | 1.139 | 0.744 | 0.023 | 7.073 | 0.425 | 3.284 |
| 26 | 1.649 | 0.018 | 11.839 | 0.107 | 0.756 | 0.009 | 7.181 | 0.103 | 12.407 |
| 27 | 1.584 | 0.038 | 11.559 | 0.229 | 0.761 | 0.038 | 7.299 | 0.226 | 12.772 |
| | | | | | | | | | |
| 29 | 1.280 | 0.021 | 9.253 | 0.123 | 0.656 | 0.022 | 7.230 | 0.155 | 2.433 |
| 30 | 1.639 | 0.019 | 11.731 | 0.177 | 0.787 | 0.029 | 7.156 | 0.137 | -4.014 |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 31 | 1.601 | 0.017 | 10.403 | 0.186 | 0.772 | 0.004 | 6.497 | 0.135 | 9.548 |
| 32 | 1.584 | 0.040 | 11.216 | 0.139 | 0.707 | 0.067 | 7.083 | 0.199 | 9.366 |
| 33 | 1.580 | 0.031 | 11.125 | 0.940 | 0.739 | 0.041 | 7.042 | 0.610 | |
| 34 | 1.501 | 0.022 | 10.738 | 0.115 | 0.772 | 0.014 | 7.152 | 0.128 | |
| 35 | | | | | | | | | |
| 36 | 1.492 | 0.101 | 11.143 | 0.238 | 0.690 | 0.035 | 7.469 | 0.528 | |
| 37 | 1.514 | 0.046 | 10.511 | 0.129 | 0.752 | 0.033 | 6.944 | 0.226 | 6.264 |
| 38 | 1.565 | 0.043 | 10.525 | 0.755 | 0.728 | 0.005 | 6.726 | 0.516 | -0.547 |
| 39 | 1.565 | 0.026 | 9.846 | 0.379 | 0.747 | 0.026 | 6.293 | 0.264 | -9.305 |
| 40 | 1.536 | 0.026 | 10.443 | 0.180 | 0.733 | 0.020 | 6.800 | 0.165 | -3.710 |
| 41 | 1.471 | 0.024 | 10.470 | 0.135 | 0.748 | 0.034 | 7.116 | 0.147 | 4.136 |
| 42 | 1.450 | 0.066 | 9.283 | 0.235 | 0.778 | 0.017 | 6.401 | 0.334 | 6.447 |
| 43 | 1.556 | 0.018 | 11.305 | 0.494 | 0.749 | 0.026 | 7.264 | 0.328 | 6.386 |
| 44 | | | | | | | | | |
| 45 | 1.606 | 0.022 | 10.749 | 0.139 | 0.820 | 0.010 | 6.692 | 0.125 | -12.224 |
| 46 | 1.609 | 0.020 | 12.593 | 0.325 | 0.761 | 0.036 | 7.827 | 0.223 | 2.372 |
| 47 | 1.574 | 0.030 | 10.785 | 0.036 | 0.765 | 0.012 | 6.854 | 0.132 | 0.669 |
| 48 | 1.489 | 0.026 | 10.631 | 0.119 | 0.743 | 0.041 | 7.141 | 0.148 | 0.851 |
| 49 | 1.525 | 0.012 | 11.009 | 0.172 | 0.761 | 0.043 | 7.222 | 0.126 | |
| 50 | | | | | | | | | |
| 51 | 1.532 | 0.034 | 9.933 | 0.326 | 0.747 | 0.063 | 6.483 | 0.257 | -6.690 |
| 52 | 1.583 | 0.043 | 9.991 | 0.126 | 0.716 | 0.039 | 6.311 | 0.187 | 0.061 |
| 53 | 1.547 | 0.073 | 10.536 | 0.218 | 0.744 | 0.036 | 6.810 | 0.350 | |
| 54 | 1.631 | 0.040 | 9.321 | 0.376 | 0.730 | 0.014 | 5.714 | 0.269 | -12.164 |
| 55 | 1.532 | 0.030 | 9.885 | 0.080 | 0.725 | 0.003 | 6.451 | 0.137 | -12.893 |
| 56 | 1.621 | 0.030 | 10.563 | 0.203 | 0.763 | 0.035 | 6.515 | 0.174 | -0.547 |
| 57 | 1.575 | 0.032 | 10.680 | 0.173 | 0.714 | 0.060 | 6.780 | 0.176 | |
| 58 | 1.561 | 0.015 | 10.292 | 0.517 | 0.790 | 0.015 | 6.594 | 0.337 | 12.589 |
| 59 | | | | | | | | | 10.157 |
| 60 | 1.545 | 0.020 | 10.698 | 0.097 | 0.752 | 0.007 | 6.923 | 0.109 | 6.325 |
| 61 | 1.605 | 0.032 | 10.889 | 0.473 | 0.759 | 0.018 | 6.786 | 0.324 | -0.426 |
| 62 | 1.724 | 0.038 | 10.685 | 1.284 | 0.717 | 0.026 | 6.199 | 0.757 | -12.042 |
| 63 | | | | | | | | | |
| 64 | 1.667 | 0.048 | 9.759 | 1.174 | 0.668 | 0.026 | 5.855 | 0.724 | -5.595 |
| 65 | 1.560 | 0.032 | 8.369 | 0.167 | 0.659 | 0.008 | 5.365 | 0.153 | 2.189 |
| 66 | 1.479 | 0.018 | 9.906 | 0.067 | 0.693 | 0.019 | 6.697 | 0.091 | -1.034 |
| 67 | 1.563 | 0.033 | 10.291 | 0.632 | 0.743 | 0.032 | 6.583 | 0.428 | 4.744 |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 68 | 1.540 | 0.033 | 10.955 | 1.727 | 0.770 | 0.059 | 7.116 | 1.131 | 4.014 |
| 69 | | | | | | | | | -4.865 |
| 70 | 1.602 | 0.024 | 10.807 | 0.467 | 0.759 | 0.003 | 6.748 | 0.308 | 12.407 |
| 71 | | | | | | | | | |
| 72 | 1.638 | 0.034 | 10.995 | 0.557 | 0.771 | 0.034 | 6.713 | 0.368 | -0.669 |
| 73 | 1.637 | 0.042 | 11.235 | 0.174 | 0.767 | 0.029 | 6.865 | 0.204 | 2.737 |
| 74 | 1.541 | 0.037 | 11.237 | 0.950 | 0.770 | 0.045 | 7.294 | 0.641 | 5.717 |
| 75 | 1.580 | 0.022 | 10.443 | 0.145 | 0.749 | 0.024 | 6.611 | 0.130 | 1.703 |
| 76 | 1.682 | 0.019 | 10.928 | 0.394 | 0.762 | 0.024 | 6.496 | 0.245 | -7.785 |
| 77 | 1.641 | 0.015 | 10.441 | 0.086 | 0.769 | 0.014 | 6.363 | 0.079 | -12.529 |
| 78 | 1.672 | 0.033 | 10.692 | 0.274 | 0.715 | 0.021 | 6.395 | 0.207 | 2.250 |
| 79 | 1.622 | 0.028 | 10.287 | 0.077 | 0.699 | 0.036 | 6.341 | 0.120 | 1.338 |
| 80 | 1.511 | 0.026 | 9.427 | 0.733 | 0.722 | 0.025 | 6.238 | 0.497 | -12.893 |
| 81 | | | | | | | | | |
| 82 | 1.624 | 0.025 | 9.911 | 0.242 | 0.739 | 0.030 | 6.102 | 0.176 | -12.529 |
| 83 | 1.554 | 0.028 | 10.033 | 0.403 | 0.771 | 0.018 | 6.455 | 0.284 | -7.724 |
| 84 | 1.621 | 0.045 | 10.692 | 0.188 | 0.739 | 0.018 | 6.597 | 0.216 | 9.792 |
| 85 | 1.615 | 0.023 | 11.396 | 0.072 | 0.783 | 0.047 | 7.055 | 0.109 | 3.467 |
| 86 | 1.595 | 0.020 | 11.446 | 0.033 | 0.799 | 0.023 | 7.176 | 0.092 | 11.616 |
| 87 | 1.563 | 0.030 | 11.295 | 0.261 | 0.779 | 0.014 | 7.227 | 0.217 | 3.223 |
| 88 | 1.532 | 0.031 | 10.403 | 0.110 | 0.751 | 0.020 | 6.791 | 0.154 | 4.014 |
| 89 | 1.565 | 0.047 | 10.615 | 0.096 | 0.757 | 0.007 | 6.783 | 0.214 | 7.541 |
| 90 | | | | | | | | | |
| 91 | 1.654 | 0.037 | 11.297 | 0.069 | 0.771 | 0.011 | 6.832 | 0.160 | 2.433 |
| 92 | 1.526 | 0.020 | 9.920 | 0.087 | 0.715 | 0.022 | 6.502 | 0.104 | -9.792 |
| 93 | | | | | | | | | |
| 94 | 1.656 | 0.027 | 11.237 | 0.422 | 0.762 | 0.029 | 6.787 | 0.278 | -8.393 |
| 95 | | | | | | | | | |
| 96 | 1.553 | 0.038 | 10.934 | 0.290 | 0.796 | 0.044 | 7.040 | 0.254 | 3.102 |
| 97 | 1.649 | 0.036 | 9.711 | 0.440 | 0.738 | 0.043 | 5.888 | 0.296 | -2.068 |
| 98 | 1.462 | 0.021 | 9.669 | 0.163 | 0.690 | 0.013 | 6.615 | 0.146 | 11.555 |
| 99 | 1.489 | 0.041 | 9.678 | 0.097 | 0.631 | 0.006 | 6.498 | 0.189 | |
| 100 | 1.427 | 0.035 | 9.126 | 0.535 | 0.645 | 0.005 | 6.395 | 0.406 | 8.879 |
| 101 | 1.620 | 0.027 | 10.706 | 0.647 | 0.744 | 0.012 | 6.609 | 0.414 | 8.210 |
| 403 | 1.586 | 0.023 | 10.317 | 0.106 | 0.701 | 0.006 | 6.504 | 0.117 | -5.595 |
| 404 | 1.494 | 0.030 | 9.805 | 0.234 | 0.636 | 0.017 | 6.565 | 0.205 | 3.710 |
| 405 | | | | | | | | | |

| Res. | $R_1(s^{-1})$ | $\Delta R_1(S^{-1})$ | $R_2(S^{-1})$ | $\Delta R_2(S^{-1})$ | NOE | ΔNOE | $R_2/R_1$ | $\Delta R_2/R_1$ | RDC (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 406 | 1.459 | 0.032 | 9.912 | 0.157 | 0.673 | 0.032 | 6.793 | 0.182 | -0.365 |
| 407 | 1.499 | 0.016 | 10.192 | 0.089 | 0.696 | 0.046 | 6.800 | 0.093 | -5.109 |
| 408 | 1.471 | 0.024 | 9.249 | 0.142 | 0.584 | 0.032 | 6.288 | 0.142 | -10.339 |
| 409 | 1.636 | 0.036 | 10.499 | 0.162 | 0.716 | 0.030 | 6.419 | 0.171 | -12.833 |
| 410 | 1.581 | 0.015 | 11.261 | 0.764 | 0.755 | 0.015 | 7.124 | 0.488 | 5.717 |
| 411 | 1.530 | 0.032 | 10.757 | 0.081 | 0.711 | 0.002 | 7.029 | 0.157 | 12.042 |
| 412 | 1.473 | 0.019 | 9.497 | 0.453 | 0.749 | 0.017 | 6.449 | 0.318 | 10.765 |
| 413 | 1.557 | 0.043 | 11.208 | 1.296 | 0.740 | 0.024 | 7.197 | 0.855 | 11.130 |
| 414 | | | | | | | | | |
| 415 | 1.461 | 0.032 | 9.420 | 0.100 | 0.725 | 0.014 | 6.446 | 0.158 | -1.885 |
| 416 | 1.622 | 0.036 | 10.974 | 0.094 | 0.781 | 0.008 | 6.766 | 0.159 | 8.150 |
| 417 | 1.516 | 0.024 | 10.058 | 0.155 | 0.715 | 0.002 | 6.636 | 0.147 | 1.034 |
| 418 | 1.597 | 0.037 | 9.765 | 0.087 | 0.746 | 0.005 | 6.114 | 0.150 | -14.292 |
| 419 | 1.566 | 0.028 | 10.327 | 0.261 | 0.696 | 0.023 | 6.596 | 0.205 | -2.554 |
| 420 | 1.599 | 0.018 | 10.420 | 0.148 | 0.755 | 0.025 | 6.518 | 0.119 | -3.163 |
| 421 | 1.526 | 0.026 | 10.682 | 0.142 | 0.715 | 0.006 | 7.000 | 0.150 | |
| 422 | 1.557 | 0.019 | 10.551 | 0.112 | 0.772 | 0.010 | 6.775 | 0.109 | 3.406 |
| 423 | 1.499 | 0.019 | 10.735 | 0.189 | 0.732 | 0.035 | 7.161 | 0.155 | 0.304 |
| 424 | 1.468 | 0.020 | 10.738 | 0.088 | 0.704 | 0.054 | 7.316 | 0.115 | -1.825 |
| 425 | 1.572 | 0.048 | 11.698 | 0.455 | 0.697 | 0.053 | 7.439 | 0.367 | -0.061 |
| 426 | 1.668 | 0.035 | 13.922 | 1.701 | 0.799 | 0.032 | 8.349 | 1.035 | -10.52 |
| 427 | | | | | | | | | |
| 428 | 1.585 | 0.044 | 21.728 | 0.669 | 0.693 | 0.067 | 13.707 | 0.567 | -4.44 |
| 429 | | | | | | | | | |
| 430 | 1.583 | 0.209 | 27.638 | 2.646 | 0.820 | 0.015 | 17.457 | 2.846 | 10.157 |
| 431 | 1.602 | 0.034 | 10.856 | 0.152 | 0.771 | 0.019 | 6.776 | 0.171 | 11.069 |
| 432 | 1.484 | 0.036 | 10.789 | 0.055 | 0.755 | 0.028 | 7.273 | 0.178 | 8.940 |
| 433 | 1.592 | 0.028 | 11.275 | 1.794 | 0.798 | 0.050 | 7.080 | 1.133 | -1.277 |
| 434 | 1.585 | 0.027 | 11.498 | 0.349 | 0.839 | 0.015 | 7.254 | 0.252 | 2.007 |
| 435 | 1.524 | 0.051 | 11.375 | 1.689 | 0.788 | 0.026 | 7.465 | 1.136 | 8.819 |
| 436 | 1.624 | 0.019 | 11.257 | 0.314 | 0.759 | 0.024 | 6.932 | 0.209 | 0.547 |
| 437 | 1.589 | 0.026 | 10.719 | 0.134 | 0.825 | 0.020 | 6.745 | 0.139 | 5.352 |
| 438 | 1.666 | 0.024 | 11.818 | 0.310 | 0.790 | 0.022 | 7.094 | 0.212 | 4.561 |
| 439 | | | | | | | | | |
| 440 | 1.630 | 0.022 | 12.895 | 0.450 | 0.783 | 0.020 | 7.910 | 0.296 | -1.216 |

***10. Table S5. Model-free analysis results for ΔΔIhh-V67CM and ΔΔIhh-L67CM using relaxation data acquired at 600 MHz NMR.***

| | ΔΔIhh-V67CM | ΔΔIhh-L67CM |
|---|---|---|
| Total analyzed | 120 residues | 120 residues |
| Model 1: $S^2$ ($\tau_e$=0, $R_{ex}$=0, $S_f^2$=1) | 81 residues | 81 residues |
| Model 2: $S^2$, $\tau_e$ ($R_{ex}$=0, $S_f^2$=1) | 19 residues | 25 residues |
| Model 3: $S^2$, $R_{ex}$($\tau_e$=0, $S_f^2$=1) | 9 residues<br>G5, R18, E20, R26, F49, E426, T430, A433, and V438 | 7 residues<br>G5, H30, V46, E424, L428, T430, and N440 |
| Model 4: $S^2$ ,$\tau_e$, $R_{ex}$ ($S_f^2$=1) | 6 residues<br>K27, I29, R92, R419, E424, and L428 | 0 residues |
| Model 5: $S^2$ ,$\tau_e$, $S_f^2$ ($R_{ex}$=0) | 2 residues<br>G64 and A65 | 4 residues<br>R54, G65, T100, and A418 |
| Unassigned | 3 residues: H30, V98, R405 | 3 residues: V23, T77, Y79 |
| $\tau_m$ | 7.70 ns | 7.77 ns |
| $D_{II}/D_{\perp}$ | 0.86 | 0.96 |
| $S^2$ (Ave) | 0.89±0.01 | 0.89±0.01 |

### *11. Model-free results for ΔΔIhh-V67CM and ΔΔIhh-L67CM. (Table S6);*

Secondary structure elements are given in the column marked "2°". Model "0" indicates that no model was deemed appropriate for the data. Residues that not analyzed include residues that were not assigned due to severe exchange broadening ("X"), praline residues ("P"), and spectral overlap ("OL"). All relaxation data were acquired at 298 K, pH 7.0.

## Table S6a; ΔΔIhh-V67CM

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 1 | β | X | | | | | |
| 2 | β | 1 | 0.91 | 0.02 | | | |
| 3 | β | 1 | 0.93 | 0.01 | | | |
| 4 | β | 1 | 0.93 | 0.01 | | | |
| 5 | | 3 | 0.89 | 0.02 | | | 1.36±0.22 |
| 6 | β | 1 | 0.94 | 0.01 | | | |
| 7 | β | 1 | 0.93 | 0.02 | | | |
| 8 | β | 1 | 0.88 | 0.01 | | | |
| 9 | β | 1 | 0.92 | 0.03 | | | |
| 10 | β | 1 | 0.92 | 0.01 | | | |
| 11 | | P | | | | | |
| 12 | | 2 | 0.93 | 0.01 | | 108±139 | |
| 13 | | 1 | 0.91 | 0.01 | | | |
| 14 | | 1 | 0.93 | 0.01 | | | |
| 15 | β | OL | | | | | |
| 16 | β | 1 | 0.91 | 0.01 | | | |
| 17 | β | 1 | 0.86 | 0.02 | | | |
| 18 | β | 3 | 0.85 | 0.01 | | | 1.05±0.21 |
| 19 | β | 2 | 0.92 | 0.01 | | 65±12 | |
| 20 | α | 3 | 0.91 | 0.01 | | | 1.07±0.25 |
| 21 | α | 1 | 0.96 | 0.01 | | | |
| 22 | α | 1 | 0.94 | 0.01 | | | |
| 23 | α | OL | | | | | |
| 24 | α | 1 | 0.93 | 0.01 | | | |
| 25 | α | 1 | 0.94 | 0.02 | | | |
| 26 | | 3 | 0.94 | 0.01 | | | 0.65±0.24 |
| 27 | | 4 | 0.88 | 0.01 | | 29±5 | 1.12±0.17 |
| 28 | | P | | | | | |
| 29 | β | 4 | 0.74 | 0.01 | | 30±5 | 0.64±0.19 |
| 30 | β | 0 | | | | | |
| 31 | β | 1 | 0.91 | 0.01 | | | |
| 32 | β | 1 | 0.93 | 0.01 | | | |
| 33 | β | 2 | 0.88 | 0.01 | | 103±41 | |
| 34 | β | 1 | 0.91 | 0.02 | | | |
| 35 | β | OL | | | | | |
| 36 | | 1 | 0.88 | 0.02 | | | |
| 37 | | 1 | 0.89 | 0.01 | | | |
| 38 | | 2 | 0.91 | 0.01 | | 45±8 | |
| 39 | β | 1 | 0.88 | 0.01 | | | |
| 40 | β | 1 | 0.90 | 0.01 | | | |
| 41 | β | 1 | 0.87 | 0.01 | | | |
| 42 | β | 2 | 0.83 | 0.02 | | 34±14 | |
| 43 | β | 1 | 0.92 | 0.01 | | | |

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 44 | β | P | | | | | |
| 45 | β | 1 | 0.92 | 0.01 | | | |
| 46 | β | 1 | 1.00 | 0.01 | | | |
| 47 | β | 1 | 0.90 | 0.01 | | | |
| 48 | β | 1 | 0.89 | 0.01 | | | |
| 49 | β | 3 | 0.87 | 0.01 | | | 0.50±0.21 |
| 50 | β | 1 | 0.88 | 0.02 | | | |
| 51 | β | 2 | 0.83 | 0.02 | | 67±29 | |
| 52 | β | 2 | 0.86 | 0.01 | | 56±22 | |
| 53 | β | 2 | 0.89 | 0.01 | | 65±10 | |
| 54 | β | 1 | 0.89 | 0.01 | | | |
| 55 | β | 1 | 0.88 | 0.01 | | | |
| 56 | β | 1 | 0.92 | 0.01 | | | |
| 57 | β | OL | | | | | |
| 58 | β | 1 | 0.90 | 0.00 | | | |
| 59 | β | 1 | 0.88 | 0.01 | | | |
| 60 | β | 1 | 0.91 | 0.01 | | | |
| 61 | β | 1 | 0.90 | 0.01 | | | |
| 62 | β | 1 | 0.95 | 0.01 | | | |
| 63 | | X | | | | | |
| 64 | | 5 | 0.76 | 0.01 | 0.90±0.01 | 1165±114 | |
| 65 | β | 5 | 0.68 | 0.01 | 0.85±0.01 | 1249±265 | |
| 66 | β | 1 | 0.85 | 0.01 | | | |
| 67 | β | 1 | 0.93 | 0.01 | | | |
| 68 | β | 2 | 0.87 | 0.03 | | 56±357 | |
| 69 | β | 1 | 0.89 | 0.01 | | | |
| 70 | β | 2 | 0.91 | 0.01 | | 66±33 | |
| 71 | | P | | | | | |
| 72 | | 1 | 0.93 | 0.01 | | | |
| 73 | β | 1 | 0.96 | 0.01 | | | |
| 74 | β | 1 | 0.89 | 0.01 | | | |
| 75 | β | 1 | 0.88 | 0.01 | | | |
| 76 | β | 1 | 0.95 | 0.01 | | | |
| 77 | β | 1 | 0.92 | 0.01 | | | |
| 78 | β | 1 | 0.92 | 0.01 | | | |
| 79 | | 1 | 0.90 | 0.01 | | | |
| 80 | β | 2 | 0.82 | 0.01 | | 51±10 | |
| 81 | β | OL | | | | | |
| 82 | β | 1 | 0.88 | 0.01 | | | |
| 83 | β | 1 | 0.85 | 0.01 | | | |
| 84 | β | 2 | 0.92 | 0.02 | | 82±129 | |
| 85 | | 2 | 0.90 | 0.01 | | 52±9 | |
| 86 | | 1 | 0.97 | 0.01 | | | |
| 87 | | 1 | 0.95 | 0.01 | | | |
| 88 | | 1 | 0.90 | 0.02 | | | |
| 89 | | 1 | 0.89 | 0.01 | | | |
| 90 | | OL | | | | | |
| 91 | β | 1 | 0.96 | 0.01 | | | |
| 92 | β | 4 | 0.81 | 0.01 | | 52±9 | 0.57±0.14 |
| 93 | β | OL | | | | | |
| 94 | β | 1 | 0.92 | 0.01 | | | |
| 95 | β | OL | | | | | |

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 96 | β | 1 | 0.92 | 0.01 | | | |
| 97 | β | 2 | 0.92 | 0.02 | | 117±157 | |
| 98 | | 0 | | | | | |
| 99 | | 2 | 0.82 | 0.01 | | 66±7 | |
| 100 | | 2 | 0.80 | 0.01 | | 57±10 | |
| 101 | | 1 | 0.93 | 0.01 | | | |
| 403 | β | 1 | 0.88 | 0.01 | | | |
| 404 | β | 2 | 0.81 | 0.01 | | 74±13 | |
| 405 | β | 0 | | | | | |
| 406 | β | OL | | | | | |
| 407 | β | 1 | 0.88 | 0.01 | | | |
| 408 | β | 1 | 0.82 | 0.01 | | | |
| 409 | β | 1 | 0.90 | 0.01 | | | |
| 410 | β | 2 | 0.90 | 0.02 | | 86±54 | |
| 411 | β | OL | | | | | |
| 412 | β | 1 | 0.85 | 0.01 | | | |
| 413 | β | 1 | 0.88 | 0.01 | | | |
| 414 | | P | | | | | |
| 415 | | 1 | 0.82 | 0.01 | | | |
| 416 | β | 1 | 0.94 | 0.02 | | | |
| 417 | β | 1 | 0.88 | 0.01 | | | |
| 418 | β | 1 | 0.86 | 0.01 | | | |
| 419 | β | 4 | 0.83 | 0.01 | | 40±9 | 0.78±0.16 |
| 420 | β | 2 | 0.89 | 0.01 | | 58±20 | |
| 421 | β | OL | | | | | |
| 422 | β | 1 | 0.89 | 0.01 | | | |
| 423 | β | 1 | 0.88 | 0.01 | | | |
| 424 | β | 4 | 0.81 | 0.01 | | 43±11 | 1.00±0.21 |
| 425 | β | 1 | 0.89 | 0.01 | | | |
| 426 | β | 3 | 0.92 | 0.01 | | | 1.45±0.33 |
| 427 | | 1 | 0.90 | 0.01 | | | |
| 428 | | 4 | 0.80 | 0.03 | | 29±12 | 10.72±0.62 |
| 429 | β | X | | | | | |
| 430 | β | 3 | 1.00 | 0.08 | | | 14.25±2.20 |
| 431 | β | 1 | 0.92 | 0.01 | | | |
| 432 | β | 1 | 0.88 | 0.01 | | | |
| 433 | β | 3 | 0.89 | 0.01 | | | 1.42±0.51 |
| 434 | | 1 | 0.95 | 0.01 | | | |
| 435 | β | 1 | 0.89 | 0.02 | | | |
| 436 | β | 1 | 0.93 | 0.01 | | | |
| 437 | β | 1 | 0.92 | 0.01 | | | |
| 438 | β | 3 | 0.92 | 0.01 | | | 0.94±0.19 |
| 439 | β | 1 | 0.96 | 0.01 | | | |
| 440 | β | 1 | 0.95 | 0.01 | | | |

## Table S6b: ΔΔIhh-L67CM

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 1 | β | X | | | | | |
| 2 | β | 1 | 0.90 | 0.01 | | | |
| 3 | β | 1 | 0.95 | 0.01 | | | |
| 4 | β | 1 | 0.90 | 0.00 | | | |
| 5 | | 3 | 0.88 | 0.02 | | | 1.06±0.25 |

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 6 | β | 1 | 0.93 | 0.01 | | | |
| 7 | β | 1 | 0.90 | 0.01 | | | |
| 8 | β | 1 | 0.89 | 0.01 | | | |
| 9 | β | 1 | 0.89 | 0.01 | | | |
| 10 | β | 1 | 0.93 | 0.02 | | | |
| 11 | | P | | | | | |
| 12 | | 1 | 0.93 | 0.01 | | | |
| 13 | | 1 | 0.91 | 0.03 | | | |
| 14 | | 2 | 0.94 | 0.03 | | 698±364 | |
| 15 | β | 1 | 0.84 | 0.01 | | | |
| 16 | β | 1 | 0.89 | 0.01 | | | |
| 17 | β | 1 | 0.89 | 0.01 | | | |
| 18 | β | 1 | 0.88 | 0.01 | | | |
| 19 | β | 1 | 0.93 | 0.02 | | | |
| 20 | α | 1 | 0.95 | 0.02 | | | |
| 21 | α | 1 | 0.97 | 0.01 | | | |
| 22 | α | 1 | 0.95 | 0.00 | | | |
| 23 | α | 0 | | | | | |
| 24 | α | 1 | 0.94 | 0.02 | | | |
| 25 | α | 1 | 0.91 | 0.02 | | | |
| 26 | | 1 | 0.98 | 0.01 | | | |
| 27 | | 1 | 0.95 | 0.01 | | | |
| 28 | | P | | | | | |
| 29 | β | 1 | 0.76 | 0.01 | | | |
| 30 | β | 3 | 0.94 | 0.01 | | | 0.68±0.23 |
| 31 | β | 2 | 0.90 | 0.01 | | 34±6 | |
| 32 | β | 1 | 0.94 | 0.01 | | | |
| 33 | β | 1 | 0.92 | 0.02 | | | |
| 34 | β | 1 | 0.89 | 0.01 | | | |
| 35 | β | OL | | | | | |
| 36 | | 1 | 0.92 | 0.02 | | | |
| 37 | | 1 | 0.88 | 0.01 | | | |
| 38 | | 2 | 0.89 | 0.02 | | 55±16 | |
| 39 | β | 1 | 0.89 | 0.01 | | | |
| 40 | β | 1 | 0.88 | 0.01 | | | |
| 41 | β | 1 | 0.87 | 0.01 | | | |
| 42 | β | 1 | 0.79 | 0.02 | | | |
| 43 | β | 1 | 0.90 | 0.01 | | | |
| 44 | β | P | | | | | |
| 45 | β | 1 | 0.92 | 0.01 | | | |
| 46 | β | 3 | 0.93 | 0.01 | | | 1.47±0.35 |
| 47 | β | 1 | 0.90 | 0.00 | | | |
| 48 | β | 1 | 0.88 | 0.01 | | | |
| 49 | β | 1 | 0.89 | 0.01 | | | |
| 50 | β | OL | | | | | |
| 51 | β | 1 | 0.87 | 0.02 | | | |
| 52 | β | 1 | 0.85 | 0.01 | | | |
| 53 | β | 1 | 0.88 | 0.02 | | | |
| 54 | β | 5 | 0.75 | 0.05 | 0.88±0.03 | 1697±1550 | |
| 55 | β | 2 | 0.84 | 0.01 | | 38±3 | |
| 56 | β | 1 | 0.91 | 0.01 | | | |
| 57 | β | 1 | 0.90 | 0.01 | | | |

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 58 | β | 1 | 0.91 | 0.01 | | | |
| 59 | β | OL | | | | | |
| 60 | β | 2 | 0.89 | 0.01 | | 40±9 | |
| 61 | β | 1 | 0.93 | 0.02 | | | |
| 62 | β | 1 | 0.98 | 0.02 | | | |
| 63 | | X | | | | | |
| 64 | | 1 | 0.95 | 0.03 | | | |
| 65 | β | 5 | 0.65 | 0.02 | 0.84±0.01 | 1478±137 | |
| 66 | β | 2 | 0.83 | 0.01 | | 49±13 | |
| 67 | β | 1 | 0.90 | 0.02 | | | |
| 68 | β | 1 | 0.89 | 0.02 | | | |
| 69 | β | X | | | | | |
| 70 | β | 2 | 0.92 | 0.01 | | 51±13 | |
| 71 | | P | | | | | |
| 72 | | 1 | 0.95 | 0.02 | | | |
| 73 | β | 1 | 0.95 | 0.01 | | | |
| 74 | β | 1 | 0.90 | 0.02 | | | |
| 75 | β | 2 | 0.88 | 0.01 | | 55±23 | |
| 76 | β | 1 | 0.96 | 0.01 | | | |
| 77 | β | 0 | | | | | |
| 78 | β | 1 | 0.94 | 0.02 | | | |
| 79 | | 0 | | | | | |
| 80 | β | 2 | 0.84 | 0.02 | | 43±19 | |
| 81 | β | OL | | | | | |
| 82 | β | 1 | 0.90 | 0.01 | | | |
| 83 | β | 1 | 0.89 | 0.01 | | | |
| 84 | β | 1 | 0.91 | 0.01 | | | |
| 85 | | 1 | 0.95 | 0.01 | | | |
| 86 | | 1 | 0.95 | 0.00 | | | |
| 87 | | 1 | 0.92 | 0.01 | | | |
| 88 | | 1 | 0.87 | 0.01 | | | |
| 89 | | 2 | 0.88 | 0.01 | | 37±8 | |
| 90 | | OL | | | | | |
| 91 | β | 1 | 0.94 | 0.01 | | | |
| 92 | β | 2 | 0.84 | 0.01 | | 52±16 | |
| 93 | β | OL | | | | | |
| 94 | β | 1 | 0.95 | 0.01 | | | |
| 95 | β | OL | | | | | |
| 96 | β | 1 | 0.91 | 0.02 | | | |
| 97 | β | 1 | 0.92 | 0.02 | | | |
| 98 | | 2 | 0.81 | 0.01 | | 46±8 | |
| 99 | | 2 | 0.81 | 0.01 | | 62±5 | |
| 100 | | 5 | 0.75 | 0.03 | 0.84±0.02 | 640±192 | |
| 101 | | 2 | 0.92 | 0.02 | | 68±29 | |
| 403 | β | 2 | 0.87 | 0.01 | | 65±8 | |
| 404 | β | 2 | 0.82 | 0.01 | | 68±13 | |
| 405 | β | OL | | | | | |
| 406 | β | 2 | 0.82 | 0.01 | | 50±21 | |
| 407 | β | 1 | 0.86 | 0.01 | | | |
| 408 | β | 2 | 0.78 | 0.01 | | 82±17 | |
| 409 | β | 2 | 0.89 | 0.01 | | 89±58 | |
| 410 | β | 1 | 0.92 | 0.01 | | | |

| res | 2° | Model | $S^2$ | $S^2$err | $S_{2f}$ | $\tau_e$(ps) | $R_{ex}(s^{-1})$ |
|---|---|---|---|---|---|---|---|
| 411 | β | 2 | 0.89 | 0.01 | | 69±6 | |
| 412 | β | 1 | 0.85 | 0.01 | | | |
| 413 | β | 1 | 0.91 | 0.02 | | | |
| 414 | | P | | | | | |
| 415 | | 2 | 0.79 | 0.01 | | 31±8 | |
| 416 | β | 1 | 0.92 | 0.01 | | | |
| 417 | β | 2 | 0.85 | 0.01 | | 44±4 | |
| 418 | β | 5 | 0.81 | 0.01 | 0.89±0.01 | 1341±246 | |
| 419 | β | 2 | 0.87 | 0.02 | | 65±22 | |
| 420 | β | 1 | 0.90 | 0.01 | | | |
| 421 | β | 2 | 0.88 | 0.01 | | 58±9 | |
| 422 | β | 2 | 0.89 | 0.01 | | 31±11 | |
| 423 | β | 1 | 0.88 | 0.01 | | | |
| 424 | β | 3 | 0.85 | 0.01 | | | 0.63±0.16 |
| 425 | β | 1 | 0.94 | 0.02 | | | |
| 426 | β | 1 | 0.96 | 0.02 | | | |
| 427 | | OL | | | | | |
| 428 | | 3 | 0.91 | 0.02 | | | 10.93±0.68 |
| 429 | β | X | | | | | |
| 430 | β | 3 | 0.92 | 0.10 | | | 16.61±2.89 |
| 431 | β | 1 | 0.91 | 0.01 | | | |
| 432 | β | 1 | 0.90 | 0.00 | | | |
| 433 | β | 1 | 0.92 | 0.02 | | | |
| 434 | | 1 | 0.93 | 0.01 | | | |
| 435 | β | 1 | 0.89 | 0.03 | | | |
| 436 | β | 1 | 0.94 | 0.01 | | | |
| 437 | β | 1 | 0.90 | 0.01 | | | |
| 438 | β | 1 | 0.97 | 0.01 | | | |
| 439 | β | OL | | | | | |
| 440 | β | 3 | 0.94 | 0.01 | | | 1.75±0.48 |

### *12. References for supporting materials*


1. Makhatadze, G. I., Medvedkin, V. N. & Privalov, P. L. (1990). Partial molar volumes of polypeptides and their constituent groups in aqueous solution over a broad temperature range. *Biopolymers* **30**, 1001-10.
2. Palmer, A. G., III. (2001). NMR probes of molecular dynamics: overview and comparison with other techniques. *Annu. Rev. Bioph. Biom* **30**, 129-155.
3. Tjandra, N., Wingfield, P., Stahl, S. & Bax, A. (1996). Anisotropic rotational diffusion of perdeuterated HIV protease from 15N NMR relaxation measurements at two magnetic fields. *J. Biomol. NMR.* **8**, 273-284.
4. Pawley, N. H., Wang, C., Koide, S. & Nicholson, L. K. (2001). An improved method for distinguishing between anisotropic tumbling and chemical exchange in analysis of 15N relaxation parameters. *J Biomol NMR* **20**, 149-65.
5. Bruschweiler, R., Liao, X. & Wright, P. E. (1995). Long-range motional restrictions in a multidomain zinc-finger protein from anisotropic tumbling. *Science* **268**, 886-9.
6. Lipari, G. & Szabo, A. (1982). Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity. *J. Am. Chem. Soc.* **104**, 4546-4559.
7. Lipari, G. & Szabo, A. (1982). Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 2. Analysis of experimental results. *J. Am. Chem. Soc.* **104**, 4559-4570.
8. Clore, G. M., Szabo, A., Bax, A., Kay, L. E., Driscoll, P. C. & Gronenborn, A. M. (1990). Deviations from the simple two-parameter model-free approach to the interpretation of nitrogen-15 nuclear magnetic relaxation of proteins. *J. Am. Chem. Soc.* **112**, 4989-4991.
9. Cole, R. & Loria, J. P. (2003). FAST-Modelfree: A program for rapid automated analysis of solution NMR spin-relaxation data. *J. Biomol. NMR.* **26**, 203-213.
10. Van Roey, P., Pereira, B., Li, Z., Hiraga, K., Belfort, M. & Derbyshire, V. (2007). Crystallographic and mutational studies of mycobacterium tuberculosis recA mini-inteins suggest a pivotal role for a highly conserved aspartate residue. *J. Mol. Biol.* **367**, 162-173.
11. Barbato, G., Ikura, M., Kay, L. E., Pastor, R. W. & Bax, A. (1992). Backbone dynamics of calmodulin studied by nitrogen-15 relaxation using inverse detected two-dimensional NMR spectroscopy: the central helix is flexible. *Biochemistry* **31**, 5269-78.
12. Woessner, D. E. (1962). Nuclear spin relaxation in ellipsoids undergoing rotational Brownian motion. *J. Chem. Phys.* **37**, 647-54.
13. Mandel, A. M., Akke, M. & Palmer, A. G., III. (1995). Backbone dynamics of Escherichia coli ribonuclease HI: correlations with structure and function in an active enzyme. *J. Mol. Biol.* **246**, 144-63.