

NMR INVESTIGATIONS OF THE ROLE OF INTRINSIC FLEXIBILITY OF THE
TRYPTOPHAN REPRESSOR

by

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of the requirements for the degree

of

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in

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DEDICATION

*To my parents, who encouraged me and put up with me.
Without them, I would never have reached this stage of my life.*

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ABSTRACT

The tryptophan repressor protein regulates intracellular concentration of Tryptophan in *Escherichia coli* by binding to DNA operators and is activated in the presence of high L-Trp concentration by formation of an L-Trp-bound holo-repressor. A Leu to Phe mutation at position 75 generates a temperature-sensitive mutant of TrpR, L75F-TrpR, whereas an Ala to Val mutation only two residue positions further on the protein sequence, at residue position 77, generates a super-repressor mutant of TrpR. Backbone amide dynamics studies on TrpR and the two variants using ^{15}N -NMR relaxation techniques at a magnetic field strength of 600 MHz (^1H Larmor frequency) indicate that all three repressors exhibit comparable diffusion properties, implying that they exhibit very similar global shape, structure, and rotational diffusion properties in both apo- and holo- states, in solution. However, internal backbone amide dynamics of the three apo-repressors reveal small but significant differences in flexibility, which are found primarily for residues spanning the Helix-Turn-Helix DNA-binding domain. These results indicate that the fine-tuning of L-Trp binding interaction is modulated in different ways via small but significant changes in protein flexibility in the two TrpR variants in apo and L-Trp bound forms.

Sulfolobus solfataricus, a model organism for Archaea, lives in extreme thermal and acidic environments such as the hot springs of Yellowstone National Park, and is host to diverse archaeal viruses including *Sulfolobus* spindle shaped virus-1 (SSV1) and *Sulfolobus* spindle shaped virus-Ragged Hills (SSV-RH). SSV viruses exhibit remarkable morphology and genetic diversity, but are poorly understood as many proteins encoded by their genomes have very little sequence homology to proteins of known functions. We have performed detailed backbone dynamics studies to better understand the mode of ligand recognition by E73, a 73-residue, homodimeric protein encoded within SSV-RH genome. Analysis of backbone dynamics measurements obtained for E73 provides evidence for fast time scale dynamics in the proposed nucleic-acid binding site and motion on the microsecond to millisecond time scale in the loop connecting helices α_A and α_B .

INTRODUCTION

Protein-nucleic acid interactions are responsible for the regulation of key biological functions such as transcription, translation, replication, and recombination. Transcription factors (TFs) are the proteins involved in regulation of gene expression. They function through activation by coactivator ligand molecule followed by recognition of specific DNA sequences. In this sense, protein-ligand recognition is a central process in TF function. The mechanism of ligand-recognition by TFs has been a focus of research for several decades primarily due to the strict maintenance of regulation profiles of gene expression these proteins can achieve (1-4). Particularly interesting is that these proteins are not isolated entities *in vivo* and rather interact in a spatially and temporally controlled manner with their respective binding partners. Over the past few decades experimental and computational techniques have been developed that shed light on the mechanisms of these interactions. Our understanding of molecular recognition is still far from perfect. Moreover, an understanding of how TFs perform ligand-recognition in terms of both structure and dynamics is incredibly challenging to obtain because, one, these interactions are controlled by a complex array of intermolecular interactions and two, the experimental observables are an ensemble average of many rapidly exchanging states as observed from the methods typically used to study these systems.

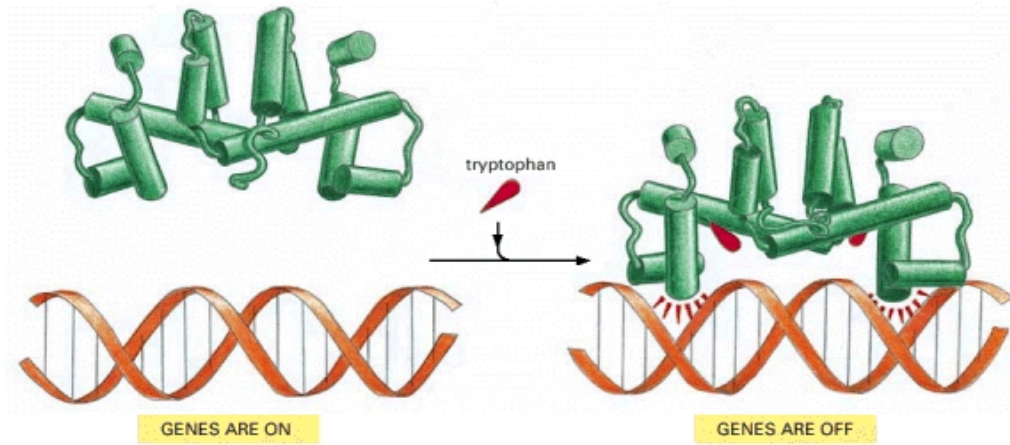
The complexity of interactions between proteins and target molecules is often determined by the considerable flexibility of the protein binding sites and by the structural rearrangements that occur upon binding of the associated molecule. A goal of many biophysical studies is to determine the molecular forces that control biological

interactions and to use this information to rationally manipulate protein function by modifying the protein, the interacting ligand, or both. One of the biggest determinant forces that control protein behavior is its flexibility and controls the dynamics of intermolecular interfaces which can further regulate binding affinity and specificity in molecular recognition.

The Tryptophan Repressor

The *E. coli* Tryptophan repressor (TrpR) is one of the smallest (25 kDa), but the most studied regulatory proteins known to us. It exists as a symmetrical dimer that binds to operator DNA in the presence of L-Tryptophan (L-Trp). Its function is to regulate transcription of genes that control L-Tryptophan (L-Trp) biosynthesis in the cell. The activity of TrpR is modulated by intracellular concentration of its cofactor L-Trp in such a way that when the cell achieves high levels of L-Trp, the inactive, unliganded form of the protein (apo-TrpR) binds to two molecules of L-Trp, which results in the active form (holo-TrpR) which can in turn bind to specific operator DNA sequences pertinent to the biosynthesis of L-Trp, thereby preventing transcription (Figure 1a). Numerous studies have indicated that the intrinsic flexibility of this protein plays an essential role in its L-Trp and DNA recognition properties. Yet, the mechanisms by which motional dynamics mediate its binding properties have remained to be incompletely characterized.

a)



b)

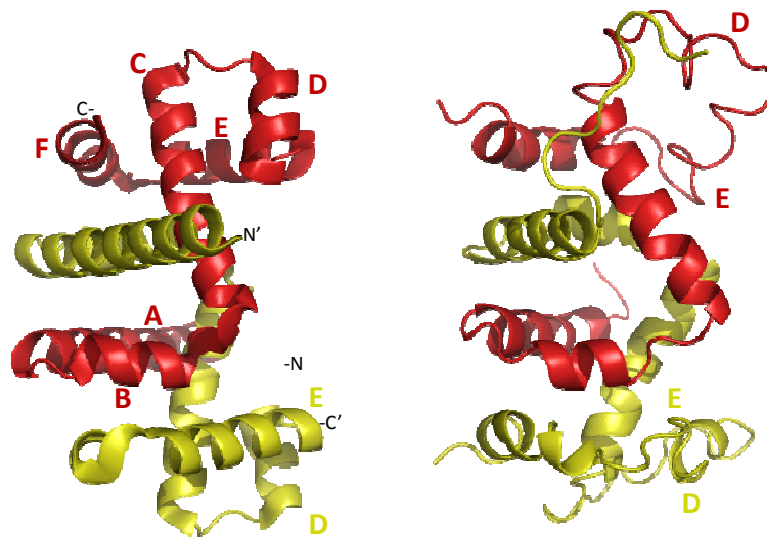


Figure 1. (A) TrpR Functions As A Transcription Factor (Figure Adapted From *Molecular Biology Of The Cell, 4th Edition*) (B) Three-Dimensional Structures Of TrpR Solved By X-Ray Crystallography (PDB ID:1P6Z) (Left) And Nuclear Magnetic Resonance Spectroscopy (1WRT- Only One Model Structure Shown) (Right).

TrpR needs L-Trp to function. In the absence of L-Trp, the apo-repressor displays low affinity for DNA. Upon binding of two L-Trp molecules per dimer, the repressor's binding affinity for operator-specific DNA of several operons responsible for the uptake and biosynthesis of L-Tryptophan and other biological molecules is enhanced significantly. The operons regulated by TrpR include *trpEDCBA*, the DNA operon coding for metabolic enzymes necessary for tryptophan biosynthesis; *trpR*, the gene coding for the tryptophan repressor protein; and the *aroH* and *mtr* operons coding for enzymes for aromatic amino acid biosynthesis and methyl tryptophan resistance, respectively (5-8). The nucleotide sequences of these DNA operator regions share similarities but are not identical. One of the requirements for TrpR function is that the repressor be able to bind DNA with high affinity in response to the metabolic needs of the cell, and to interact specifically with DNA operator sequences to ensure correct selection of the DNA operon(s) whose transcription must be repressed. A key to TrpR function thus involves biophysical characteristics that permit the modulation of the repressor's binding affinity for DNA, specificity, and stoichiometry, (i.e. number of repressor dimer molecules bound per DNA equivalents) (9, 10). For TrpR, these properties are modulated by the binding of the L-Trp co-repressor, which acts as an allosteric effector that alters the repressor's affinity for DNA via the protein's L-Trp cofactor binding sites (10, 11, 12, 13).

The magnitude of the effect of L-Trp binding on TrpR is dependent on the identity of the DNA sequence and on whether the DNA is an operator or non-operator sequence (9, 14, 15). Studies have demonstrated that L-Trp modulates repressor

specificity and not solely affinity, and that both the L-Trp co-repressor and the cognate DNA operator function together to achieve repressor activation. TrpR repressor function does not originate solely from an increase in affinity of the holo-repressor for DNA but rather is modulated by an interplay between affinity, specificity, and cooperativity. These unique characteristics arise from both the intertwined structure of the TrpR protomers in the TrpR dimer, and the extensive flexibility of the TrpR protein (15).

TrpR consists of two identical 108-residue polypeptide chains (11) that fold together to form a homodimer. Structural studies (16-20) have shown that each TrpR monomer is comprised of 6 α -helices, helices A through F (Figure 1b). Helices A, B, C, and F of the two protomers come together to form the hydrophobic core of the TrpR dimer, while helices D and E comprise the helix-turn-helix DNA binding domain of TrpR. The solution NMR structures of WT-TrpR in its apo form have revealed that the helix-D-turn-helix-E DNA binding domain of TrpR in solution is more disordered than in the crystalline state (19, 20) (Figure 1b). DNA binding upon activation of TrpR by L-Trp co-repressor binding is thought to take place via a sequential ordering of the protein's helix-D-turn-helix-E DNA binding region, as inferred from the presence of additional intrahelical ^1H - ^1H NMR NOe connectivities within the helix E region upon formation of the holo TrpR repressor, which are not observed in apo-TrpR (20), and by the observations of slower backbone amide proton $^1\text{H}/^2\text{H}$ exchange rates and changes in ^1H NMR relaxation time constants (21-24). Similar effects are observed for helix D when holo-TrpR binds to DNA operator sequences (25). TrpR thus represents an interesting system for structural biology as this protein is extremely thermostable (T_m of $\sim 90^\circ\text{C}$ with

a free energy of folding of 23 kcal/mole per dimer) (26, 27), yet possesses a highly dynamic structure whose flexibility appears essential for function (21-24, 28, 29).

The Temperature-sensitive Variant, TrpR-L75F

Due to the potential of temperature-sensitive (ts) mutants to yield additional insights into the relationship between TrpR structure, stability, and dynamics, a genetic selection for such mutants was conducted and resulted in the isolation of a TrpR mutant, apo-L75F-TrpR where leucine 75 was replaced by phenylalanine (30). This ts mutant of TrpR was identified and selected using a *trpR*⁻ *E. coli* strain transfected with hydroxylamine-treated plasmid pBKH13 DNA bearing the *trpR* gene (31). The resulting transformants were selected for growth in the presence of 5-methyltryptophan (5-MT) at 42 °C and altered growth at 37 °C (30). 5-MT is an analog of L-Trp that binds to apo-TrpR ~twice more tightly (12), and results in a 5-MT/TrpR pseudorepressor that binds to operator DNA ~10x more tightly than holo-TrpR (32, 33). 5-MT cannot substitute for the amino acid L-tryptophan during protein synthesis, and as a result *E. coli* cells transfected with a functional TrpR starve for L-Trp when grown on minimal media containing 5-MT instead of L-Trp. In contrast, *E. coli* cells containing TrpR mutants which contain amino acid substitution(s) that alter or interfere with TrpR's repressor function survive when grown on minimal media containing 5-MT because the *trpR* operon that controls L-Trp biosynthesis is derepressed and provides L-Trp necessary for cell growth (30, 34). DNA sequencing of the TrpR mutant displaying the ts phenotype described above resulted in the identification of a ts mutant of TrpR containing a single point mutation at residue position 75 at the C-terminus of the first helix of the helix-turn-helix motif, with leucine

75 replaced by phenylalanine, and referred to as L75F-TrpR (30). To verify that the ts phenotype was due only to the mutation at position 75, the coding sequence for L75F was subcloned into pJPR2 vectors (35) that could produce high non-regulated levels of the L75F-TrpR variant. The phenotype of *trpR*⁻ *E. coli* cells transfected with the resulting plasmids (pJPR2.L75F) using the 5-MT temperature screen was identical to that of the original isolate, i.e. growth at 42 °C (which we refer to as the permissive temperature) with altered and weak growth at 37 °C (which we refer to as the non-permissive temperature) as the original *E. coli* cell screen (30). These results indicated that the repressor function of L75F-TrpR is temperature-sensitive, and that this variant is a more effective Trp repressor at 37 °C than at 42 °C (30).

Extensive biophysical and biochemical characterizations of L75F-TrpR revealed that the apo form of L75F-TrpR indicated that this function is not due to a poorly structured protein. In fact L75F-rpR exhibits an increase in apparent α -helicity of ~12 %, a slightly higher urea denaturation mid-point, and a thermal stability identical to that of wild-type TrpR (30) (Figure 2). Fluorescence data indicated that the environment of one or both tryptophan residues of TrpR (Trp19 and Trp99) is more buried in L75F-TrpR compared to WT-TrpR (30) (Figure 2). These data were confirmed by ¹H-NMR and by detection of slower ¹H/²H exchange rates for the spectrally resolved indole ring protons of the two tryptophan side chains in L75F-TrpR compared to those of apo-WT-TrpR. Interestingly, it was shown that L75F-TrpR binds L-Trp with an approximately ten fold lower affinity compared to WT-TrpR (Table 1), and in excess of L-Trp in vitro, the holorepressor's affinity for operator DNA is two to five times weaker (30). It was also

shown that the specificity of L75F-TrpR for operator versus non-operator DNA is ~ 2 fold smaller, although within experimental error of what was measured for WT-TrpR (30).

The Super-repressor Variant TrpR A77V

Mutational studies on TrpR have revealed that the flexibility and structural ordering of the DNA-binding domain is affected by slight changes in amino acid composition, and lead to significant alterations in L-Trp and DNA binding functions of the repressor. Early mutagenesis experiments confirmed the helix-turn-helix model of repressor/DNA recognition for TrpR (31). In these studies, several protein variants were isolated, characterized, and later on classified as “super-repressors” due to their ability to repress gene transcription at limiting L-Trp concentrations where the wild-type repressor is not functional (31). For example, the super-repressor TrpR variant, A77V-TrpR, in which alanine 77 is replaced by valine, exhibited a 10% increased in apparent α -helicity as measured by CD, was slightly more stable to urea denaturation than wild-type repressor, and appeared to be less flexible than apo-WT-TrpR (28, 37). DNA binding studies also showed that the A77V-TrpR variant cannot recognize the full complement of operator sequences normally accessible to WT-TrpR (29). It was thus postulated that the effect of the A to V mutation at residue position 77 is to restrict the internal flexibility of A77V-TrpR, and that decrease in flexibility leads to a restricted specificity of the repressor to a subset of DNA sequences (28).

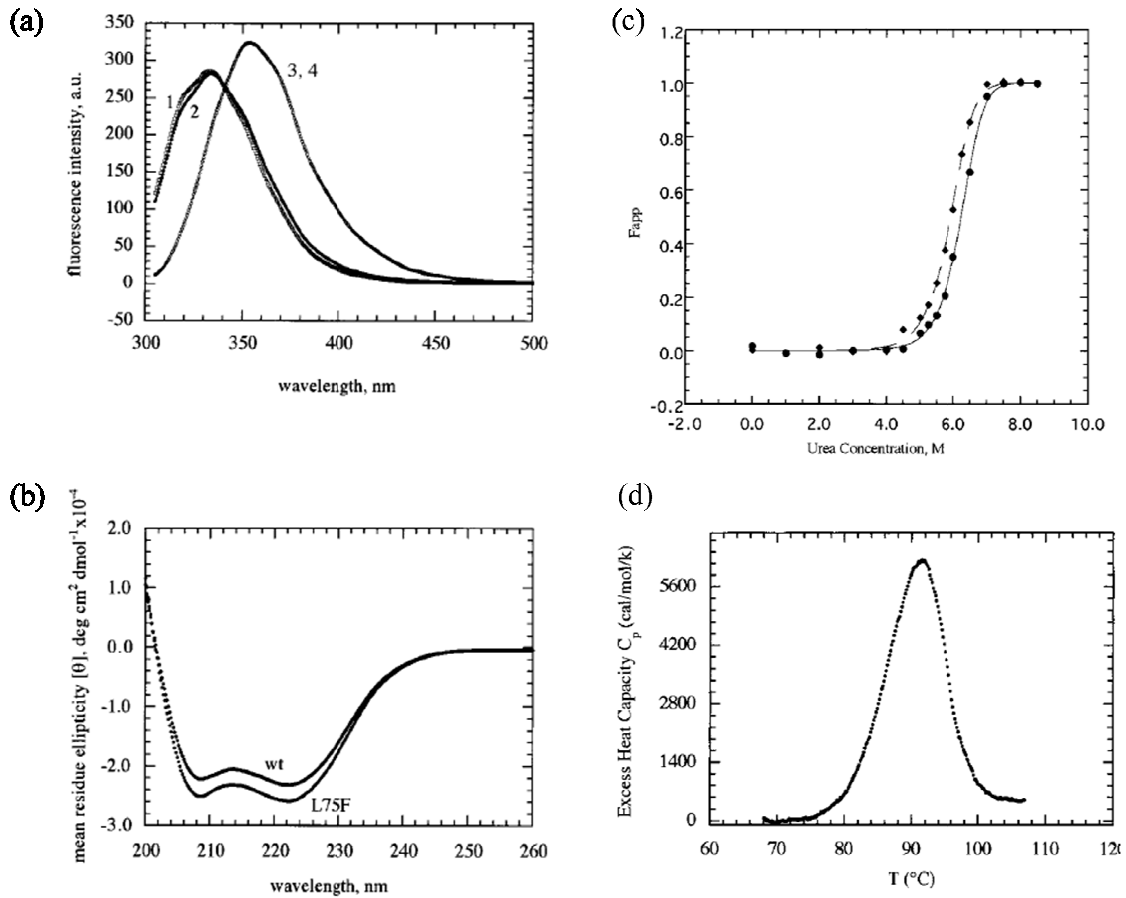


Figure 2. Biophysical Characterization Of Apo-L75F-TrpR And Comparison With Apo-WT-TrpR: (a) Fluorescence emission spectra. Protein concentration 13.7 mM (dimer); 25°C. Each spectrum is an average of five scans with excitation wavelength 295 nm; a.u., arbitrary units. Spectra 1 and 2 are L75F and wild-type proteins, respectively, in P11 buffer; spectra 3 and 4 are L75F and wild-type proteins in P11 buffer/7 M GdnHCl. (b) Far-UV CD spectra. Protein concentration 5.3 mM (dimer) in P11 buffer at 25°C. (c) Urea denaturation. Each point is an average obtained from duplicate experiments in P11 buffer containing 5.3 mM protein (dimer) and the indicated final concentration of urea at 25°C. F_{app} , apparent fraction of unfolded molecules; filled diamonds, wild-type protein; filled circles, L75F protein. (d) Differential scanning calorimetry. Samples of 73 mM protein (dimer) in 10 mM sodium phosphate buffer (pH 7.5), 0.1 M NaCl were scanned from 20 to 118°C under 6 kg/cm² pressure at a rate of 60°C/hour. (Figures and figure legends reproduced from previously published results (36))

Table 1. Thermodynamic Parameters For Binding To L-Trp.

TrpR			Wild-type				L75F	
Temp.	K_d	ΔH	ΔG	$T\Delta S$	K_d	ΔH	ΔG	$T\Delta S$
(°C)	(mM)	(kcal/mol)	(kcal/mol)	(kcal/mol)	(mM)	(kcal/mol)	(kcal/mol)	(kcal/mol)
12	0.014	-11.2	-6.3	-4.9	0.10	-10.0	-5.2	-4.8
18	0.021	-12.9	-6.2	-6.7	0.11	-11.4	-5.3	-6.1
25	0.056	-14.7	-5.8	-8.9	0.21	-12.8	-5.0	-7.8
37	0.11	-16.9	-5.6	-11.3	0.48	-16.3	-4.7	-11.6
44	0.31	-18.7	-5.1	-13.6	0.71	-16.9	-4.6	-12.3
ΔC_p (kcal/mol K)			-0.23				-0.23	

Isothermal titration calorimetry experiments were performed in P11 buffer. K_d is the equilibrium dissociation constant, and ΔG , ΔH , ΔS , and ΔC_p are the free energy-, enthalpy-, entropy- and heat capacity-changes, respectively, for one mole of L-Trp binding per mole of protein dimers. (Data reproduced from previously published report (36))

Surprisingly it was shown that in vitro, apo-A77V-TrpR and holo (L-Trp bound) A77V-TrpR bind L-Trp and operator DNA with the same affinity as apo-WT-TrpR and holo-WT-TrpR, respectively (38). This apparent paradox was resolved by Finucane & Jardetzky who showed, using surface plasmon resonance (SPR) that apo-A77V-TrpR binds to 20 mer consensus DNA at lower protein concentration than does apo-WT-TrpR, and that at equivalent protein concentration, ~ 2x the amount of apo-A77V-TrpR is bound to consensus operator DNA than apo-WT-TrpR (39). These investigators also showed that the holo-WT-TrpR binds at a lower concentration to consensus operator DNA than holo-A77V-TrpR, although the lifetime of the holo-A77V-TrpR:DNA complex is much longer than that of holo-WT-TrpR:DNA (39).

Taken together, these data suggested that although the substitution of a leucine for a phenylalanine at position 75 is a conservative substitution in a solvent accessible area of the protein, the mutation does not solely result in a minor change in the vicinity of the site of mutation but rather generates non-local perturbations that were postulated to be dynamic in origin with subtle but important consequences on TrpR repressor function (30). However, the detailed mechanisms by which a single point amino acid replacement of a residue located on solvent accessible surface loop leads to global changes in L75F-TrpR have been difficult to establish. The data already available for these two mutants supports the notion that the dynamics features of the helix D-turn-helix E DNA binding domain of TrpR are a critical source of adaptability that allow the protein to recognize a range of operator sequences, while maintaining the ability to reject closely related DNA targets. This is in contrast to the initial view derived from X-ray structures of TrpR that suggested only the orientations of helices D and E were important for operator binding (17, 18). However, in spite of extensive biochemical and biophysical studies of TrpR and TrpR variants, the precise mechanisms by which altered flexibility leads to altered TrpR function are poorly understood.

Research Goals

In particular, the study reported in this dissertation aims to better understand how changes in backbone flexibility affect the L-Trp binding properties of the three different apo-repressors. The two TrpR variants (apo-L75F-TrpR and apo-A77V-TrpR) were chosen in part because they are both results of a single conservative amino acid

substitution in a solvent accessible loop (two residues apart in the protein sequence), yet display opposite phenotypes in terms of L-Trp binding properties and differ considerably from apo-WT-TrpR. Apo-L75F-TrpR has been characterized as a ts mutant which at the permissive temperature of 42°C allows cell growth on minimal media containing 5-MT, while cells producing apo-WT-TrpR starve for L-Trp (30). In contrast to the reduced TrpR function of apo-L75F-TrpR, apo-A77V-TrpR displays enhanced TrpR function and increased repressor activity and regulation of the *trp* operator in vivo (29). For this reason, apo-A77V-TrpR has been designated a super-repressor (39-41).

In addition, the sites at which a conservative amino acid substitution has occurred in the two TrpR mutants (residue position 75 and residue position 77, respectively), and which manifest in very distinct L-Trp binding properties are separated in the TrpR sequence by only one residue, and occur on a solvent accessible surface loop of the HTH domain. Despite their distinct phenotypes, both apo-L75F-TrpR and apo-A77V-TrpR possess very similar biophysical characteristics including a ~10% apparent increase in α -helical content compared to apo-WT-TrpR, and a small increase in chemical stability as implicated from CD and urea denaturation experiments (28, 30, 37), while all three proteins remain highly thermostable with almost identical T_m of ~ 90 °C (30).

NMR AND PROTEIN DYNAMICS

The internal environment of a living cell is an ever changing variable and as re-emphasized by recent structure reports, proteins prefer to stay in motion to be functional and sample several conformations over time (42-45). This flexibility imparts them with an extra ability to interact with other interaction partners. The beginning and end states of such interactions, which are often unbound (apo) and bound (holo and active), are easy to observe by structural analysis but the transient states are short-lived and are hard to capture for a detailed analysis. This makes it difficult to perform thorough analysis of binding kinetics of ligands on the surface of proteins through structural techniques and requires the use of dynamics-based analyses to be performed to explain true determinants of the dynamic process under investigation. Moreover, since biochemical function in biomolecules invariantly depends upon the transduction of information by conformational changes. These changes can be very subtle and hard to monitor but are required by protein in order to fold, bind ligands and perform molecular recognition to carry out its function. Therefore, it is imperative to study dynamics of the proteins and relate them to structure to get a complete picture of the function.

Developments in biochemical research and state-of-the-art technology have allowed time-based measurement of kinetic parameters of protein interactions. However, studies involving them have mostly focused on enzyme kinetics followed by measurement of turnover rates. Although direct measurement of binding kinetics has been made possible by more recent advancements such as biochemical assays, isotope labeling, Q-sense and surface plasmon resonance, only a few studies have been able to

dissect the pathway of protein–ligand interactions and explore the intermediate steps of mechanism. This is because the transition states are always lowly populated and the current techniques are still limited to extract motions in high resolution. This restriction has, on one side, led to accumulation of a wealth of knowledge about slow timescale (μs to ms) motions and their mechanisms leading to protein functions, but on the other side, resulted in a scarcity of understanding of how fast timescale motions (ps - ns) which encompass rapid loop reorientation, libration, vibration and side chain rotations affect protein function. Typically, these motions on fast timescales (ps - ns) contribute to the entropy of the system where as motions on the slow timescale (μs - ms) include concerted motions and larger scale conformational changes of the protein molecule.

It is well known that the protein active sites are vulnerable and minute perturbation in the active site could result in drastic changes in the energetics. NMR is well suited to study such sensitive protein systems and is known to be a powerful tool that aids study of site-specific dynamics of proteins on a wide range of timescales. Commonly used experiments and the timescales they are sensitive to are summarized in Figure 3. Dynamics by NMR is a large field by itself and provides a link between structure, function, and thermodynamics. The traditional approach of using NMR to study dynamic properties of a protein system is through relaxation experiments, which are discussed in the following section.

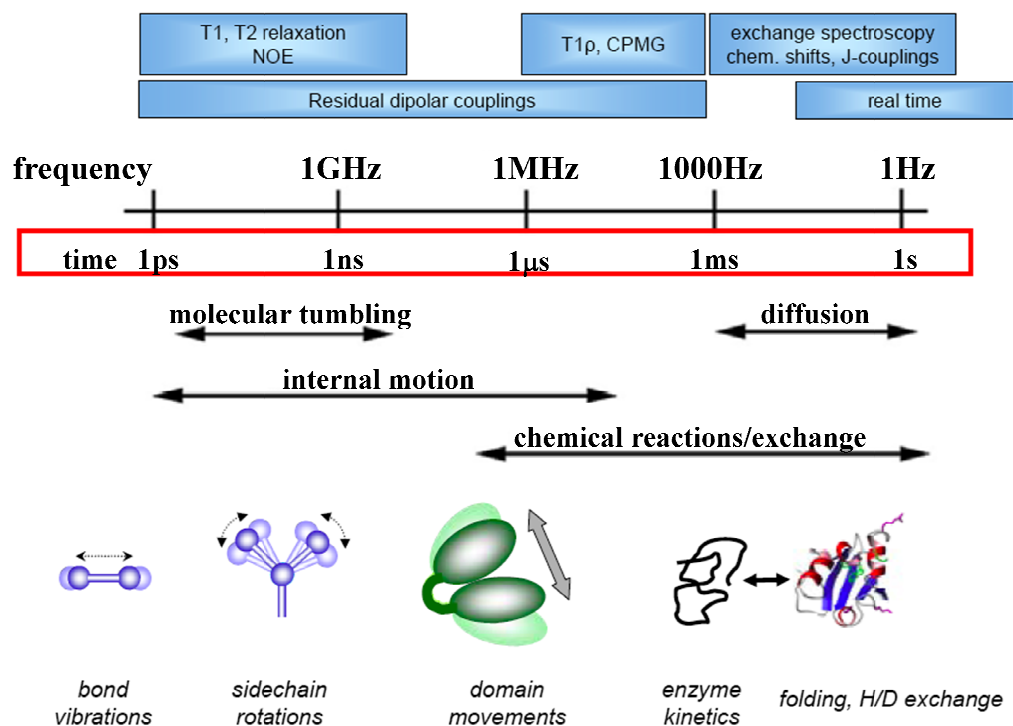


Figure 3. NMR Methods For Protein Dynamics: The above figure shows the range of motions measured by Nuclear Magnetic Resonance. Different time-scale motion is sensitive to specific Nuclear Spin Relaxation Measurements.

Relaxation

Relaxation is the property through which spins return to equilibrium. The stimulation for relaxation comes from fluctuating field surrounding the nucleus. Since each spin has a distinct chemical environment around it, the relaxation parameters are affected differently for each one of them. Typically, T_1 , T_2 and ρ constants are measured to derive relaxation kinetics of spins from NMR.

Relaxation Parameters

Longitudinal Spin Relaxation Time Constant, T_1

The longitudinal relaxation reflects a thermodynamic process in which the net energy of the nuclei becomes lost due to a transfer with the surroundings. Therefore, the time constant, T_1 , is the time nuclear spins take to lose their ‘total’ magnetization to regain their equilibrium magnetization (defined as M_z^0). It is traditionally called as the spin-lattice relaxation as was first described in solid state NMR. The rate of return to thermal equilibrium can be measured using a 180° -time delay- 90° pulse sequence. A 180° RF inversion pulse inverts the net magnetization to maximum magnitude along the negative z axis. A variable time t exists before the subsequent pulse, in which the sample begins to return to its equilibrium state. The 90° read pulse is then used to re-establish coherence and read the resultant net magnetization. The build-up of equilibrium magnetization, I_0 can be represented as shown in Figure 4. Following a 90° pulse, the recovery of bulk magnetization in the equilibrium +z axis can be given by:

$$I(t) = I_0 \{1 - \exp(-t/T_1)\} \quad (1)$$

where $I(t)$ and I_0 are the intensities of a given peak at a relaxation time delay t and at $t = 0$ msec, respectively; and T_1 is the Longitudinal relaxation time constant. The pulse schemes used for heteronuclear $^1\text{H}/^{15}\text{N}$ systems are illustrated in Figure 5.

Transverse Spin Relaxation Time Constant, T_2

Transverse relaxation refers to the loss of coherence of magnetization in the xy plane. This loss of coherence occurs as the result of the transfer of spin energy between

the two nuclei under investigation. For this reason the process is also called spin-spin relaxation. Similar to spin-lattice relaxation, loss of signal over times is an exponential decay process.

For longitudinal and transverse relaxation, the decay of signal intensities is fitted to an exponential decay:

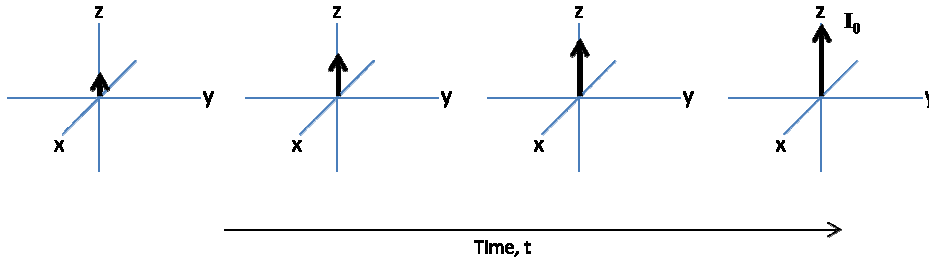
$$I(t) = I_0 \exp (-t/T_2) \quad (2)$$

where T_2 is the relaxation time constant measured in xy plane. Usually, several (6–10) time points per relaxation curve are used to determine the relaxation time constants. In addition to these points, multiple experiments are recorded for 2–3 relaxation delays.

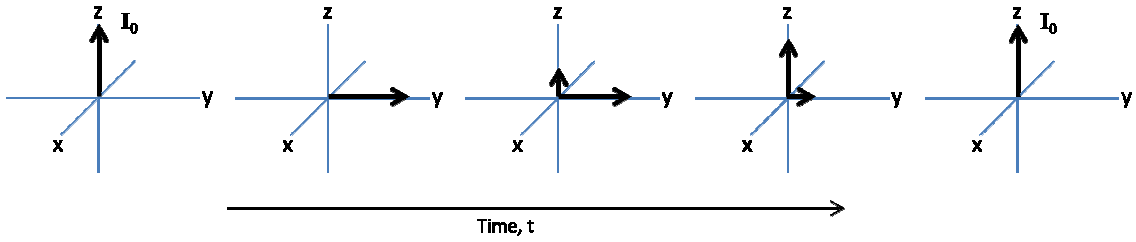
The T_2 relaxation time constants are very important to precisely monitor changes experienced by the spins since they can directly measure inhomogeneity in the field and can also be related to the linewidths of the nuclear resonance lines, which in turn are representative of the molecular weight of the molecule in solution. The linewidth at half-height can be expressed in terms of T_2 as:

$$\Delta\nu = \Delta\omega/2\pi = 1/(\pi T_2) \quad (3)$$

(a)



(b)



(c)

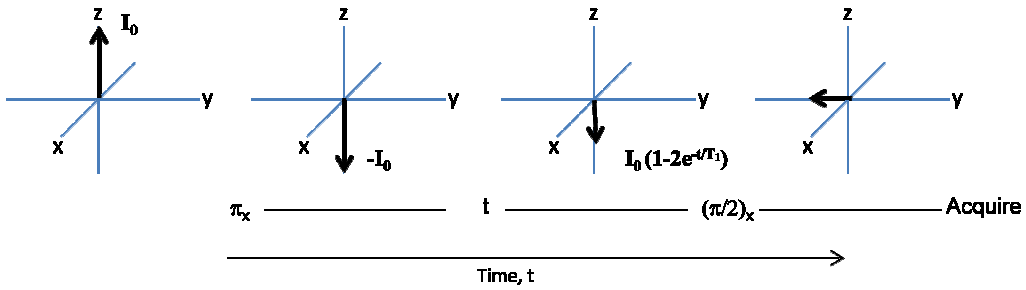


Figure 4. Schematic Representation Of Bulk Magnetization Observed Over Time: (a) Build-up of magnetization I_0 (classically referred to as M_0). (b) Reappearance of the z-magnetization. (c) The inversion recovery method (used in the studies reported in this dissertation). (Redrawn from (46))



Figure 5. Pulse Sequences Used For The Measurement Of ^{15}N (a) T_1 and (b) T_2 Relaxation Times, And (c) For Measurement Of The $^{15}\text{N}\{^1\text{H}\}$ nOe In Our Studies. (47) These pulse sequences can be thought of (and read) as a schematic plot in time (increasing horizontally, rightwards) to perform each run of the relaxation experiments. The narrow and wide bars indicate pulses applied at 90° and 180° respectively. Phase cycling was used to suppress spectral artifacts in the data. This was accomplished by acquiring data with different pulse and receiver phases. ϕ represents the phase of applied excitation pulse and x or y indicates the position of the receiver. ϕ undergoes variations (cycling) throughout the pulse sequence. The order of variation is set to result in the minimization of artifacts after summation of the data from all of the scans in the cycle. Gradient pulses were also included in the pulse schemes to obtain coherence transfer selection but are not visible in the plots shown here. Pulses shown in striped boxes and labeled as SL and SCR represent high power pulses (short, ~ 2 ms and long, ~ 12 ms, respectively) refer to spin-lock and scrambling pulses respectively and are included to obtain higher coherence selection by dephasing of H_2O magnetization. To increment the relaxation delay, T , the loop counters N (for scheme a) and K (for scheme b) are incremented. In sequences a) and b), the T period is where the variable delay is implemented (inversion recovery). The composite pulse in duration T gets rid of the cross-correlation between dipolar coupling and chemical shift anisotropy by periodic application of 180° ^1H pulses during the T_1 or T_2 relaxation period of duration T . In sequence c), the 120° high-power pulses are applied during the entire delay between scans (3 s) at 20-ms intervals, to maintain a saturated state of the ^1H reservoir. For obtaining the reference spectrum without nOe, the 120° pulses were omitted and, instead, two scrambling pulses of ~ 10 ms each, spaced by 1 ms, are introduced immediately prior to the 90° ^{15}N pulse in order to eliminate the H_2O signal.

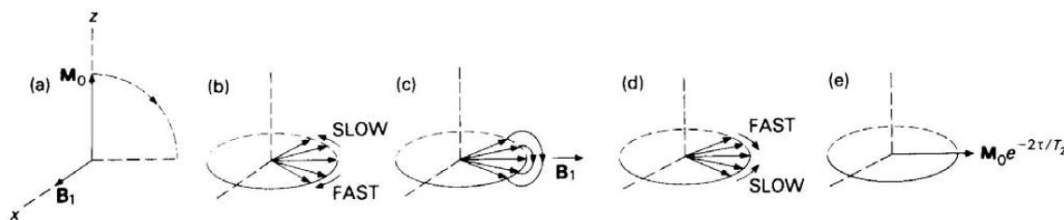


Figure 6. The Spin-echo Effect, Which Forms The Basis Of T_2 Relaxation Measurement Experiments: (a) a 90° pulse puts M_0 into the y direction, (b) The spins fan out followed by a loss of coherence, (c) a 180° pulse interchanges slow and fast spins at time τ , (d) refocusing occurs, (e) the echo at time 2τ . (Figure adopted from (48))

Nuclear Overhauser Effect (nOe)

nOe is one of the most important phenomena involved in nuclear spin relaxation, and is used in protein dynamics studies to gain information about the highest energy spin transitions that result from extremely rapid motions of the protein molecule. nOe is directly proportional to cross-relaxation, σ which is a consequence of the dipolar interaction between the ^{15}N nucleus and its attached proton (discussed in detail in the next section) and is given by:

$$\sigma_{\text{NH}} = 1/T_1 (\text{nOe} - 1) \gamma_{\text{N}}/\gamma_{\text{H}} \quad (4)$$

Rearrangement of the above equation suggests that the measured nOe is equal to $(1 + \sigma \cdot T_1)$, in which T_1 refers to the longitudinal relaxation time of the ^{15}N nucleus. Most NH bond vectors in the protein undergo slow molecular reorientation with respect to the static magnetic field, which results in a high value of σ . However when regions of the protein are flexible these NH bond vectors experience rapid reorientation leading to a low σ value. When measuring the nOe of a ^{15}N labeled protein, one sees both positive and negative values which convey local flexibility of NH bond vectors within the structure. The lower the nOe value, the more flexible the NH bond vector is. nOe enhancements

were determined as the ratio of signal intensities in the proton saturated (Figure 7b) and no saturation experiments.

$$\text{NOE} = (I_{\text{sat}}) / (I_{\text{eq}}) \quad (5)$$

where I_{sat} is the intensity of a peak with proton saturation and I_{eq} is the intensity without proton saturation.

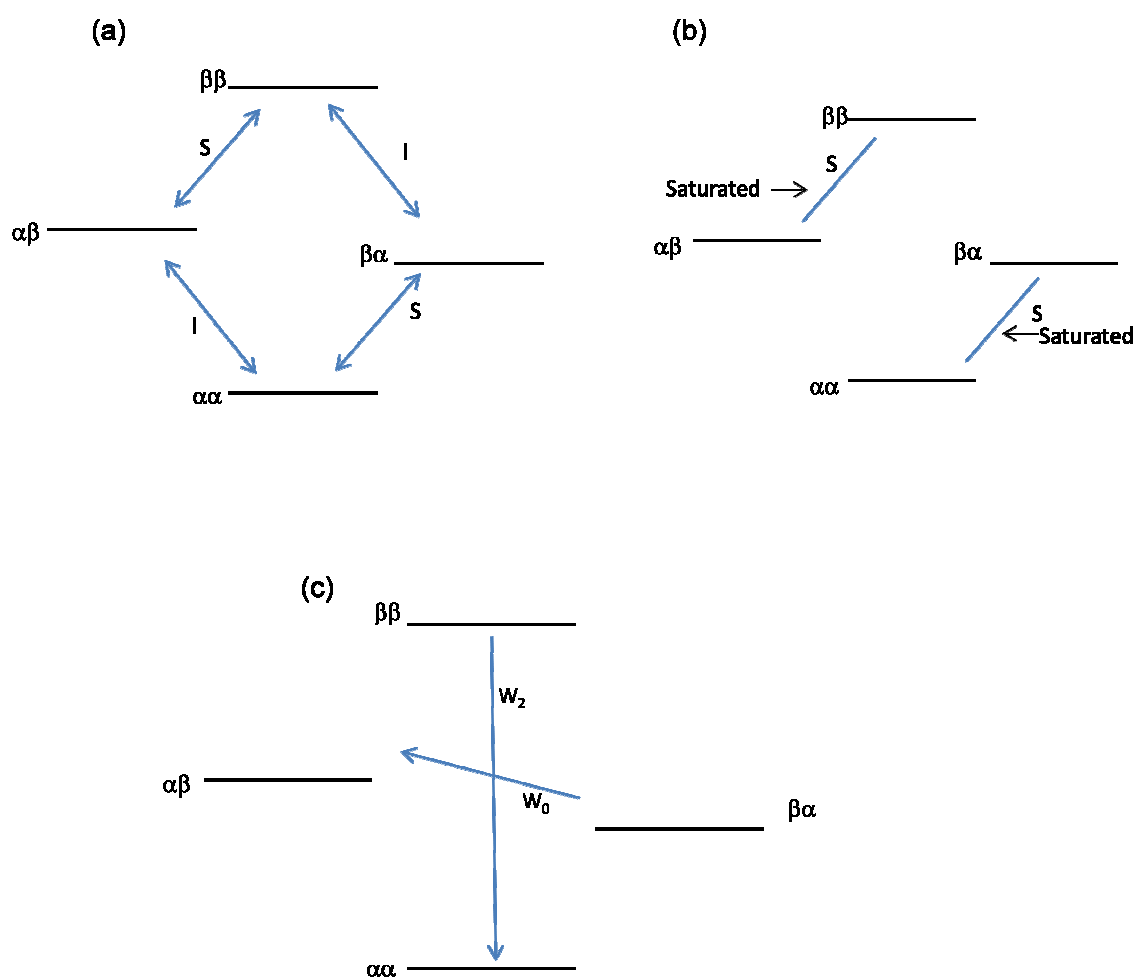


Figure 7. Restoration Mechanisms In Relaxation nOe Experiments: (a) Population and energy levels of a two-spin system. (b) Populations of the levels immediately following saturation of the S transitions. (c) Relaxation pathways immediately following saturation of the S transitions. W_2 and W_0 represent probabilities of double and zero quantum spin transitions respectively. (Redrawn from (46))

Mechanisms Of Relaxation

The two mechanisms that give rise to relaxation are Chemical shift anisotropy and Dipole-dipole interaction as described below. Besides these, there are also other factors contributing to relaxation such as scalar interaction, cross correlations, paramagnetic relaxation and chemical exchange.

Dipole-Dipole Interactions

There is a magnetic dipole associated with each spin $\frac{1}{2}$ nuclear spin which varies with the orientation of the bond vector connecting a spin pair. Each spin member induces a field on the other which fluctuates with the molecular motion and internal structural rearrangements. This gives rise to fluctuations in magnetic field which can induce relaxation of that spin. In our studies, relaxation of ^1H and ^{15}N spin $\frac{1}{2}$ nuclei has been used to derive motions experienced by the bond vector relating them. Hence, the contribution to relaxation in our measurements comes from a dipole-dipole interaction between ^1H and ^{15}N nuclear spins. In addition, the ^{15}N nucleus induces a field over ^1H which fluctuates over time and this fluctuation in field can induce relaxation of ^{15}N spin if frequency of fluctuating field matches it (Figure 8). The field induced by one spin over the other is given by

$$B_{\text{dipole}}(t) = \frac{\gamma h}{2\pi r(t)^3} (3\cos^2\theta(t) - 1) \quad (6)$$

When $\theta = 0$, B_{dipole} is at maximum and the dipole field is zero when $\theta \approx 54^\circ$. Molecular tumbling changes the relative orientation of the two spins, resulting in a change of B_{dipole} . This in turn changes the energy associated with this additional field which is obtained from

$$E = h\omega/2\pi = (h/2\pi)\gamma_s B_{\text{dipole}} \quad (7)$$

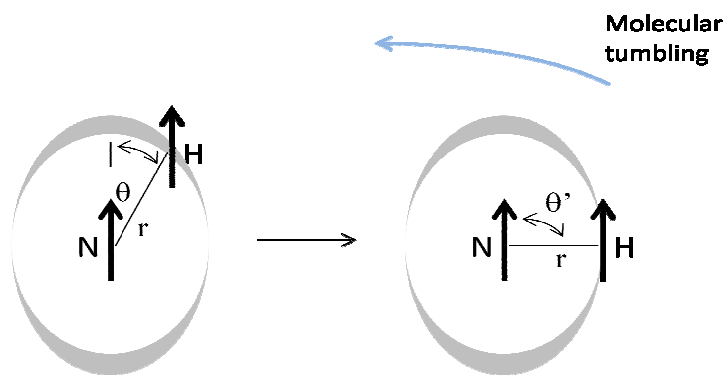


Figure 8. Dipole-Dipole Coupling. The magnetic field generated by the amide nitrogen generates an additional field, B_{dipole} at the amide proton. The strength of this field depends on the relative orientation of the two spins, as illustrated by the gray crescent shapes. (Figure redrawn from(48))

Chemical Shift Anisotropy

Every spin on the protein is present in a distinct chemical environment. This could be due to heterogeneity in the electron density distribution, variation in shielding due to movements of electrons and the orientation of the spin with respect to the molecule. As the molecule tumbles, variations in the chemical environment give rise to fluctuations in the field surrounding it that can induce relaxation of the spin states too. The chemical shift anisotropy is illustrated in Figure 9, where the different local magnetic fields of the nuclei in an anisotropic N-H bond is shown as a function of orientation with respect to the applied field, B_0 .

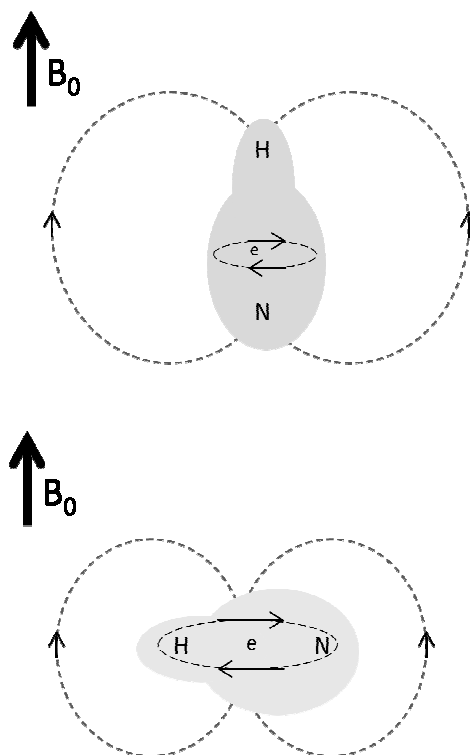


Figure 9. Origins Of Chemical Shift Anisotropy In An N-H Bond: The circulation of electrons in a plane perpendicular to the bond describes a smaller area than circulation in a plane containing the bond. (Redrawn from (46))

As depicted in the figure, the origin of the chemical shift is that the moving electric charges of the electron cloud around a nucleus induce a local magnetic field which opposes the applied field. Thus, the effective field at the nucleus is:

$$B_{\text{eff}} = B_0 (1 - \sigma)$$

The nucleus is said to be shielded, and the extent of shielding is given by the shielding constant, better known as the chemical shift tensor, σ . There are three principal components of the shift tensor, σ_{11} , σ_{22} and σ_{33} , and the isotropic shift tensor σ_{iso} is given by:

$$\sigma_{\text{iso}} = (1/3) (\sigma_{11} + \sigma_{22} + \sigma_{33})$$

The shift tensor, σ is related to Larmor frequency, ω_0 , as follows:

$$\omega_0 = (\gamma/2\pi) B_0 (1 - \sigma)$$

and to chemical shift:

$$\delta = 10^6 (\sigma_{\text{ref}} - \sigma_{\text{sample}})$$

Spectral Density

Molecular motions can be exceedingly complex. Hence, the relaxation data obtained can be encoding information from several timescales of motion. Interpretation of relaxation data in physical terms thus involves derivation of spectral density functions as the primary step to obtain accurate information about the prevalence of motions at each frequency. In simplified terms, spectral density can be defined as a measure of power at a particular frequency that is available from the surrounding of each nuclear spin to induce relaxation in a spin system and there for the spectral density plots against frequencies can identify strong frequency variations from weak frequency variations.

Spectral Density And Autocorrelation Function

Molecular tumbling can be thought to affect the memory the spin has of the environment it was in. This memory can be accessed through the correlation function. The correlation function tells us how similar a parameter of our system is at time t to the same parameter at another time $(t + \tau)$.

$$C(\tau) = \overline{f(t) f(t + \tau)} \quad (8)$$

where the terms under bar represent the average field experienced by all the spins in the sample as a function of time. The orientation of the ^{15}N – ^1H bond vector changes as the molecules tumbles in solution due to Brownian motion. The magnitude of the change depends on how fast the molecule tumbles. The simplest form of correlation function for a molecule undergoing tumbling, $C(\tau)$ is given by

$$C(\tau) = C(0) \exp(-|\tau|/\tau_c) \quad (9)$$

Where τ_c represents the correlation time (or the ‘overall’ tumbling time) i.e. the time the diffusing molecule takes to reorient itself in the same position in the three dimensional space. In other words, the correlation function describes the rate ($1/\tau_c$) by which an induced dipole moves in solution and τ_c , the correlation time, essentially measures the time for a molecule to tumble 1 radian in any direction. A large correlation time suggests slow tumbling of a molecule in solution. The correlation time is often described for diffusion on a solvent by the Stoke-Einstein equation as:

$$\tau_c = \frac{4\pi\eta r^3}{3k_B T} \quad (10)$$

where η represents viscosity of solvent, r represents the effective hydrodynamic radius of the solute, k_B represents Boltzmann constant and T represents temperature. The correlation function is a function of time. So, it can be Fourier transformed to give a function of frequency or the spectral density function. Spectral density gives an approximation of the extent of motion present at each frequency and is denoted as $J(\omega)$

$$C(\tau) \rightarrow \text{Fourier Transform} \rightarrow J(\omega) \quad (11)$$

Therefore, in terms of τ_c ,

$$J(\omega) = 2 [\tau_c C(0)/(1 + \omega^2 \tau_c^2)] \quad (12)$$

where ω denotes the Larmor frequency of the nuclear species (in rad/s); the spectral density function $J(\omega)$ has significant intensity over the frequency range $0 \leq \omega \leq \tau_c^{-1}$; and $C(0)$ is a normalization constant whose value can be calculated from equation (9).

Rapidly fluctuating field has a short correlation time, and the spectral density map is broad (Figure 10). Similarly if the field fluctuates slowly, the correlation time is long,

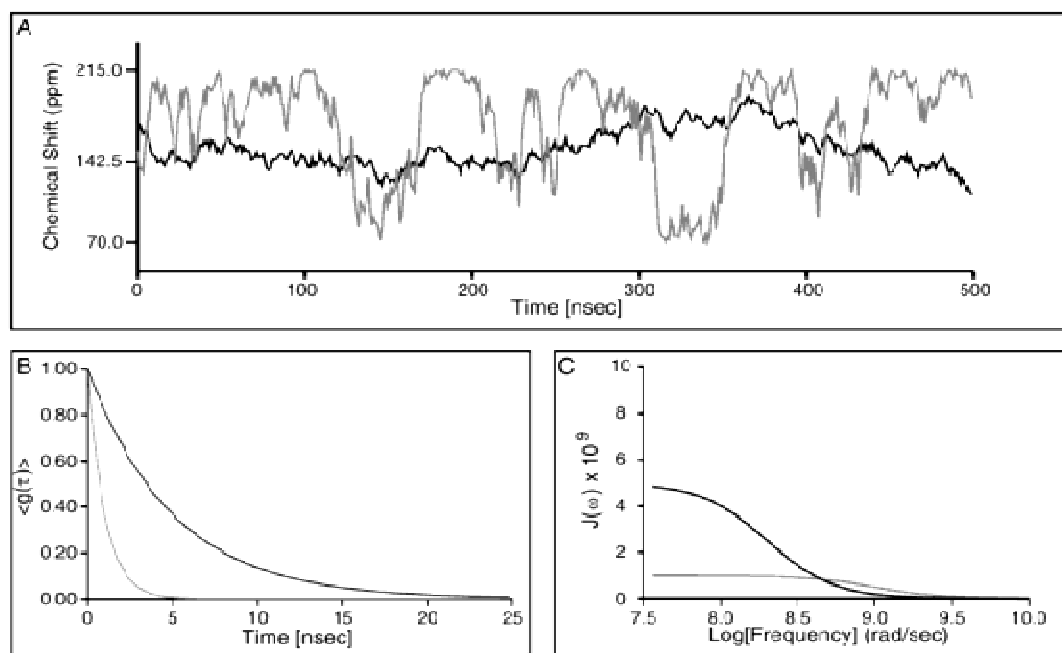


Figure 10. Magnetic Field Fluctuations And The Spectral Density Function: The relationship between random magnetic field fluctuations, the auto-correlation function and the spectral density function is shown. A) The fluctuation in magnetic field for a small protein (grey line, $\tau_c = 1$ nsec) and a large protein (black line, $\tau_c = 5$ nsec) are shown. B) Auto-correlation function depends on molecular weight. Decay is faster for smaller proteins. C) Spectral density functions reflect the change in molecular weight. Note that the larger protein has a greater intensity of spectral density at lower frequencies. (Figure taken from *Fundamentals of protein NMR spectroscopy* by Gordon S. Rule, T. Kevin Hitchens).

and the spectral density function is narrow. Therefore as the correlation time decreases the spectral density is redistributed to higher frequencies.

The spectral density function is evaluated by the intensity of the magnetic field fluctuations at five different frequencies, i.e., ω_H , $\omega_H+\omega_X$, $\omega_H-\omega_X$, ω_X and 0. All of these angular frequencies represent specific time-scale motions. The $\omega_H+\omega_N$, ω_H and $\omega_H-\omega_N$ frequencies measure motions on the picosecond time-scale, ω_N measures motions on the nanosecond timescale and 0 measures slower (milliseconds) time-scale motions. The frequency of the fluctuating field can affect any of these angular frequencies and cause relaxation in the spin system.

The frequency of fluctuating fields is measured in terms of relaxation rate by the following expressions (Abragam, 1961) which express relaxation parameters in terms of frequencies of motion and dipolar coupling and chemical shift anisotropy constants using spectral density:

$$1/T_1 = (d^2/4) \{J(\omega_H-\omega_N) + 3J(\omega_N) + 6J(\omega_H+\omega_N)\} + c^2 J(\omega_N) \quad (13)$$

$$1/T_2 = (d^2/8) \{4J(0) + J(\omega_H+\omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H+\omega_N)\} + (c^2/6) \{3J(\omega_N) + 4J(0)\} + R_{ex} \quad (14)$$

$$nOe = 1 + (d^2)(\gamma_H/\gamma_N) \{6J(\omega_H+\omega_N) - J(\omega_H-\omega_N) - J(\omega_H-\omega_N)\} T_1 \quad (15)$$

where γ_H and γ_N represent gyromagnetic ratios, ω_H and ω_N represent Larmor frequencies, d^2 represents dipolar coupling between the nuclei and follows the expression $d^2 = 0.1 \gamma_H^2 \gamma_N^2 h^2 / (4\pi^2) [1/r_{NH}^6]$, γ_H and γ_N are the gyromagnetic ratio of the two spins and r_{NH} is the internuclear distance of an amide bond (1.02 Å), c^2 represents chemical shift anisotropy and is defined as $c^2 = (2/15) \omega_N^2 (\sigma_{\parallel} - \sigma_{\perp})^2$ where $\sigma_{\parallel}$ and $\sigma_{\perp}$ are the parallel and

perpendicular components of ^{15}N chemical shift tensor respectively and $(\sigma_{\parallel} - \sigma_{\perp}) = -160$ ppm, and R_{ex} represents the conformational exchange to R_2 (or $1/T_2$).

Interpretation Of NMR Relaxation Data

The spectral density functions $J(\omega)$ are modulated by the overall global reorientation of the protein molecule, as well as by the internal motions of individual NH bond vectors. Direct measurements of the three relaxation parameters (^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOes) do not provide sufficient information to uniquely determine the spectral density functions at the five frequencies depicted above. In order to simplify the problem and to gain insights into the molecular motions that may be contributing to relaxation of backbone amides, two different approaches were followed: 1) Reduced Spectral Density Mapping (49, 50) and 2) Model-free Analysis (51) using FastModelFree which is an interface to ModelFree program developed by the Palmer group.

Reduced Spectral Density Mapping

Reduced spectral density mapping is the most direct method of analyzing relaxation parameters. It was first described by Peng and Wagner (50, 52) and then modified by Farrow et al (49) for application to data collected at a single magnetic field strength by exploiting the assumption that the high frequency spectral density terms that contribute to the relaxation processes are of approximately equal magnitude, i.e. $J(\omega_{\text{H}} \pm \omega_{\text{N}}) \approx J(\omega_{\text{H}})$, and therefore may be replaced by a single equivalent term. This method uses measured ^{15}N T_1 , ^{15}N T_2 , and ^{15}N - $\{^1\text{H}\}$ nOe to estimate the magnitude of the

spectral density function at 0, ^1H , and ^{15}N angular frequencies. In turn, $J(0)$, $J(\omega_{\text{H}})$, and $J(\omega_{\text{N}})$ are directly related to molecular motion through equation 12. For instance, according to equation 12, $J(\omega)$ at 0 frequency is equal to $2/5 \tau_c$. This relationship represents an upper limit on $J(0)$, which is usually reduced by fast internal motions that may result from anisotropic rotational motions of the N-H bond vector. It is also important to note that chemical exchange that is in the microsecond to millisecond range contributes positively to $J(0)$ but this effect can be attenuated by measuring T_2 under spin lock conditions ($T_{1\rho}$). Altogether, reduced spectral density mapping is a robust approach for analyzing flexible sections of proteins because it does not depend on having a model of molecular motions under investigation and hence involves the least amount of assumption.

Model-free Analysis

Another approach that can be applied to perform analysis of NMR relaxation data is called the Model-free formalism developed by Lipari and Szabo (53, 54). This approach translates NMR relaxation data into several models of motion and assumes that the correlation function at any time, $C_I(t)$, for internal motions can be expressed as follows:

$$C_I(t) = S^2 + (1-S^2) \exp(-t/\tau_e) \quad (16)$$

where S^2 represents the generalized order parameter whose values range from 0 to 1, and which indicates the degree of spatial restriction for rapid motional reorientation of the N-H bond vector, ($S^2=1$ means fully restricted, $S^2 = 0$ means unrestricted isotropic internal motions), τ_e is the effective correlation time of these rapid local internal fluctuations.

For a spherical protein with overall rotational correlation time τ_m , Fourier transformation of $C_l(t)$ gives rise to a spectral density function of the form:

$$J(\omega) = (S^2 \tau_m) / (1 + \omega^2 \tau_m^2) + [(1 - S^2) \tau] / (1 + \omega^2 \tau^2) \quad (17)$$

where $1/\tau = 1/\tau_m + 1/\tau_e$ (18)

An important assumption of the Lipari-Szabo formalism is that global reorientation of the molecule in solution and internal motions in the proteins are independent of each other and can be separated into two characteristic time constants τ_m and τ_e . This is generally valid for amide NH bond vectors located in well-ordered core regions of a protein but may be less applicable to NH bond vector motions in loop or largely disordered protein segments.

In the case of TrpR, the protein displays a substantial deviation from spherical shape, and this anisotropy must be taken into account when considering the effects of molecular tumbling on the ^{15}N NMR relaxation. In this case, the spectral density can be expressed as a sum of Lorentzian functions that are related to the rotational diffusion coefficients D_{xx} , D_{yy} , and D_{zz} . The overall reorientation of TrpR is best described by rotational reorientation of a prolate ellipsoid, with a characteristic axially symmetric diffusion tensor with two unique rotational diffusion coefficients parallel ($D_{\parallel}$) and perpendicular ($D_{\perp}$) to the unique axis of the molecule (23). For axially symmetric anisotropic diffusion, the functional form for the spectral density function is described as (55, 56) :

$$J(\omega) = S^2 \{A_1 \tau_1 / (1 + \omega^2 \tau_1^2) + A_2 \tau_2 / (1 + \omega^2 \tau_2^2) + A_3 \tau_3 / (1 + \omega^2 \tau_3^2)\} + (1 - S^2) \tau / (1 + \omega^2 \tau^2) \quad (19)$$

with the coefficients

$$A_1 = 0.75 \sin^4 \alpha, A_2 = 3 \sin^2 \alpha \cos^2 \alpha, \text{ and } A_3 = (1.5 \cos^2 \alpha - 1)^2 \quad (20)$$

and α is the angle between the N-H bond vector and the unique axis ($D_{\parallel}$) of the diffusion tensor, and where the correlation times τ_1 , τ_2 , and τ_3 depend on the rotational diffusion rates as follows:

$$\tau_1 = 1/(4D_{\parallel} + 2D_{\perp}), \tau_2 = 1/(D_{\parallel} + 5D_{\perp}), \text{ and } \tau_3 = 1/6D_{\perp} \quad (21)$$

The parameter τ in equation 19 is dominated by the time constant describing the fast internal motions, τ_e , but also depends on the time constant describing the overall anisotropic motion, τ_m .

$$1/\tau = 1/\tau_e + 1/\tau_m \quad (22)$$

$$\text{and} \quad \tau_m = 1/(6 D_{\text{eff}}) = 1/(2D_{\parallel} + 4D_{\perp}) \quad (23)$$

where D_{eff} is approximately one-third of the trace of the diffusion tensor (47, 56).

Equation (23) reveals that the spectral density function governing the relaxation properties of backbone amides will depend on the principal values of the diffusion tensor, $D_{\parallel}$ and $D_{\perp}$, and on the N-H bond vector orientation with respect to $D_{\parallel}$. The effects of rotational diffusion anisotropy ($D_{\parallel}/D_{\perp}$), and N-H bond vector orientation with respect to $D_{\parallel}$, on ^{15}N - T_1 and ^{15}N - T_2 relaxation time constants for a prolate ellipsoid have been well described (57). When the N-H bond vector angle (α) with respect to $D_{\parallel}$ is less than the magic angle 54.7° , the effect of rotational anisotropic diffusion for a prolate ellipsoid manifests itself in larger ^{15}N - T_1 and smaller ^{15}N - T_2 values than what is seen for ^{15}N - ^1H amides in a molecule undergoing isotropic rotational diffusion (57). Thus as anisotropy increases, ^{15}N - $T_1/^{15}\text{N}$ - T_2 ratios increase for ^{15}N - ^1H amides oriented at $\alpha < 54.7^\circ$ and

decrease for ^{15}N - ^1H bond vectors oriented at $\alpha > 54.7^\circ$ with respect to $D_{||}$. Using these known trends, FastModelFree calculates in an iterative fashion the principal components of the rotational diffusion tensors for the apo-L75F-TrpR, apo-WT-TrpR, and apo-A77V-TrpR. Initial estimates of the diffusion tensors are determined from PDB structural coordinate files and further refined using measured ^{15}N - T_1 and ^{15}N - T_2 values, excluding those of backbone amides with anomalous values (see selection criteria below) and minimizing the following χ^2 function:

$$\chi^2 = \sum_i [(T_{1i}/T_{2i})_{\text{exp}} - (T_{1i}/T_{2i})_{\text{calc}}]^2 / \sigma_i^2 \quad (24)$$

where the summation, $\sum_i$, extends over all ^{15}N - ^1H backbone amides whose relaxation parameters fit the selection criteria outlined below; $(T_{1i}/T_{2i})_{\text{exp}}$ and $(T_{1i}/T_{2i})_{\text{calc}}$ correspond to experimentally measured and theoretical ^{15}N - T_1 / ^{15}N - T_2 ratios, respectively, for the i^{th} ^{15}N - ^1H bond vector; and σ_i^2 is the square of the error in experimentally measured ^{15}N - T_1 / ^{15}N - T_2 ratios calculated according to the procedure described in Nicholson et al (58).

Approximation Of Diffusion Parameters:

The overall rotational correlation time τ_m for global reorientation of apo-L75F-TrpR, apo-WT-TrpR, and apo-A77V-TrpR were obtained from ^{15}N - T_1 / ^{15}N - T_2 ratios by minimizing equation 24 and excluding residues with the following ^{15}N NMR relaxation characteristics (57): Residues with a significantly lower ^{15}N - $\{^1\text{H}\}$ -nOe (i.e. $I/I_0 < 0.65$) were excluded because for those residues, the assumption that motions on the τ_c timescale do not contribute to ^{15}N - T_1 relaxation is invalid (51). Residues undergoing conformational exchange that shortens ^{15}N - T_2 significantly were also excluded. Those were recognized as having shorter ^{15}N - T_2 values without a concomitant increase in ^{15}N -

T_1 (47) and confirmed using $T_1\rho$ experiments and excluding residues with $^{15}\text{N}-T_1\rho/^{15}\text{N}-T_2$ ratios > 1 . Consequently, a residue n with specific $^{15}\text{N}-T_1$ and $^{15}\text{N}-T_2$, $T_{1,n}$ and $T_{2,n}$ respectively, is excluded from τ_m calculations if its $T_1\rho/T_2$ ratio is greater than one and/or if both of the following conditions are satisfied (47):

$$\langle T_2 \rangle - T_{2,n} > \text{SD} \quad (25)$$

(SD= 1 standard deviation from the mean)

and
$$(T_{2,n} - \langle T_2 \rangle) / T_{2,n} > [3 (\langle T_1 \rangle - T_{1,n})] / T_{1,n} \quad (26)$$

where $\langle T_1 \rangle$ and $\langle T_2 \rangle$ are the average T_1 and T_2 values, averaged over all backbone amides that have an $^{15}\text{N}-\{^1\text{H}\}-\text{nOe} > 0.65$.

From the mean of 10% trimmed $^{15}\text{N}-T_1/^{15}\text{N}-T_2$ ratios and the criteria described above, initial estimates of τ_m (~ 10 ns for TrpR samples) and the anisotropic diffusion ratios, $D_{\parallel}/D_{\perp}$, (~ 1.12 for TrpR samples) are typically calculated and used as initial input to FastModelFree. Following determination of the global diffusion parameters, FastModelFree calculations, performed according to the protocol described in Mandel et al. (59), internal motional parameters can be obtained in the form of S^2 generalized order parameters, internal correlation time for N-H bond vector motion, τ_e , and chemical exchange contribution to the relaxation, R_{ex} . The program uses minimization of the following χ^2 equation in order to extract dynamics parameters:

$$\chi^2 = [(T_1^{\text{exp}} - T_1^{\text{calc}})^2 / \sigma_{T_1}^2] + [(T_2^{\text{exp}} - T_2^{\text{calc}}) / \sigma_{T_2}^2] + [(n\text{Oe}^{\text{exp}} - n\text{Oe}^{\text{calc}}) / \sigma_{n\text{Oe}}^2] \quad (27)$$

where “exp” and “calc” denote the experimental and calculated values of $^{15}\text{N}-T_1$, $^{15}\text{N}-T_2$ and $^{15}\text{N}-\{^1\text{H}\}-\text{nOe}$, respectively. The term σ^2 denotes the square of the experimental uncertainty associated with the corresponding relaxation parameter, and is based on

Monte Carlo analysis of independently acquired data sets (58). The calculated values of $^{15}\text{N-T}_1$, $^{15}\text{N-T}_2$, and $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ are determined by holding τ_m constant and varying S^2 and τ_e until a minimum is reached in the χ^2 calculations.

Derivation Of Order Parameter And Fitting Of Motional Models

Generalized order parameters (S^2) report on the amplitude of fast (ps-ns) internal motions experienced by the N-H bond vectors of backbone amides. Low S^2 values indicate high flexibility and large amplitude motions. ($D_{\parallel}/D_{\perp}$) ratios can then be used to calculate any anisotropy in the global overall reorientation of the protein molecule in solution (all TrpR samples were best modeled as an axially symmetric prolate ellipsoid as shown in Figure 11). Following calculation of the anisotropy in diffusion properties, FastModelFree fitting of the relaxation data is performed to extract internal dynamics parameters and to establish which models describing the internal motions best fitted the data.

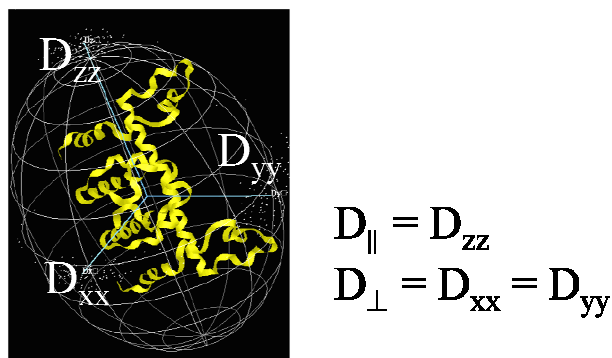


Figure 11. TrpR Molecule Fit Into An Axially Symmetric, Prolate Ellipsoid Model Of Diffusion. The two distinct tensors of diffusion, $D_{\parallel}$ and $D_{\perp}$ that govern rotation of the TrpR molecule in solution.

This approach is analogous to the ModelFree analysis of NMR relaxation parameters and the model selection approach of Mandel and co-workers (59).

As a next step, ^{15}N NMR relaxation data are fitted to one of five possible models invoking various combinations of ModelFree parameters (59). During this analysis, complex forms (models 3-5) of the ModelFree Lipari-Szabo formalism which invoke combinations of τ_e , R_{ex} , and motions on fast and slow time scales (S_f^2 , S_s^2 , τ_s) are only included if the simplest models (models 1-2) fail to fit the experimental data as determined by F-test statistics (59).

BACKBONE DYNAMICS OF THE APO-TRPR FORMS

^{15}N NMR Relaxation Results

^{15}N Relaxation Measurements Of Apo-WT-TrpR

A complete list of all the ^{15}N NMR relaxation parameters measured for backbone amides of apo-WT-TrpR is included in Table 8. Figure 12 depicts the average ^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOe relaxation values for all measurable amide residues of apo-WT-TrpR with error bars representing $\pm$ one standard deviation between triplicate sets of measurements. As is the case for apo-L75F-TrpR, backbone amides located in the hydrophobic core of apo-WT-TrpR (i.e. core helices A, B, C, and F) exhibited uniform ^{15}N - T_1 and ^{15}N - T_2 trends averaging to values of $835.5 \text{ ms} \pm 42.0 \text{ ms}$ and $75.0 \text{ ms} \pm 5.9 \text{ ms}$, respectively (for 43 amides included in the calculations). Backbone amides located in helix D of apo-WT-TrpR yielded slightly lower average ^{15}N - T_1 of $739.1 \pm 34.5 \text{ ms}$ (with 3 amides used in the calculation) and elevated ^{15}N - T_2 of $87.2 \pm 12.3 \text{ ms}$ (for 2 amides) when compared to the average ^{15}N - T_1 and ^{15}N - T_2 values observed backbone amides located in the core helices of apo-WT-TrpR. Backbone amides residing within helix E yielded average ^{15}N - T_1 and ^{15}N - T_2 values of $755.1 \pm 41.6 \text{ ms}$ (number of 6 amides) and $78.2 \pm 5.0 \text{ ms}$, (number of 6 amides), which are not significantly different from average ^{15}N - T_1 and ^{15}N - T_2 values observed for backbone amides in core helices. Overall, the pattern of ^{15}N - T_1 and ^{15}N - T_2 values measured over the polypeptide sequence of apo-WT-TrpR was similar to that obtained for apo-L75F-TrpR with a few slight differences observed for amides within residue stretch 60-80. The average ^{15}N - $\{^1\text{H}\}$ -nOe value for

amide residues within the hydrophobic core (helices A, B, C, and F) of apo-WT-TrpR was found to be 0.79 ± 0.04 (for 45 amides). Backbone amides located within helix D-turn-helix E region display lower $^{15}\text{N}\{-^1\text{H}\}$ -nOe values, than those measured for backbone amides of core helices but differences were also observed between the $^{15}\text{N}\{-^1\text{H}\}$ -nOe trends of helix D amides and those of helix E.

Backbone amides located in helix D exhibit $^{15}\text{N}\{-^1\text{H}\}$ -nOe values lowest of all helices averaging to 0.58 ± 0.06 (for 3 amides), progressively decreasing for amides located at the N-terminal end of helix D to those located at the C-terminal end of helix D. Backbone amides located in helix E exhibited a slightly higher average $^{15}\text{N}\{-^1\text{H}\}$ -nOe value of 0.68 ± 0.04 (for 6 amides), with $^{15}\text{N}\{-^1\text{H}\}$ -nOe values progressively increasing from the N- to C-terminal end of helix E. While $^{15}\text{N}\{-^1\text{H}\}$ -nOe values were on average higher for helix E amides than those of helix D amides, they remain considerably lower than corresponding $^{15}\text{N}\{-^1\text{H}\}$ -nOe values (≥ 0.8) of amides residing in core helices A, B, C and F.

^{15}N Relaxation Measurements Of Apo-L75F-TrpR

^{15}N -T₁, ^{15}N -T₂, and $^{15}\text{N}\{-^1\text{H}\}$ -nOe profiles for apo-L75F-TrpR are shown in Figure 13. A complete listing of all relaxation parameters measured for apo-L75F-TrpR is included in Table 9. Figure 13 depicts the average ^{15}N -T₁, ^{15}N -T₂, and $^{15}\text{N}\{-^1\text{H}\}$ -nOe relaxation values for all measurable amide residue of apo-L75F-TrpR with error bars representing $\pm$ one standard deviation between triplicate sets of measurements. The ^{15}N -T₁ and ^{15}N -T₂ NMR relaxation time constants of NH residues located in the core helices

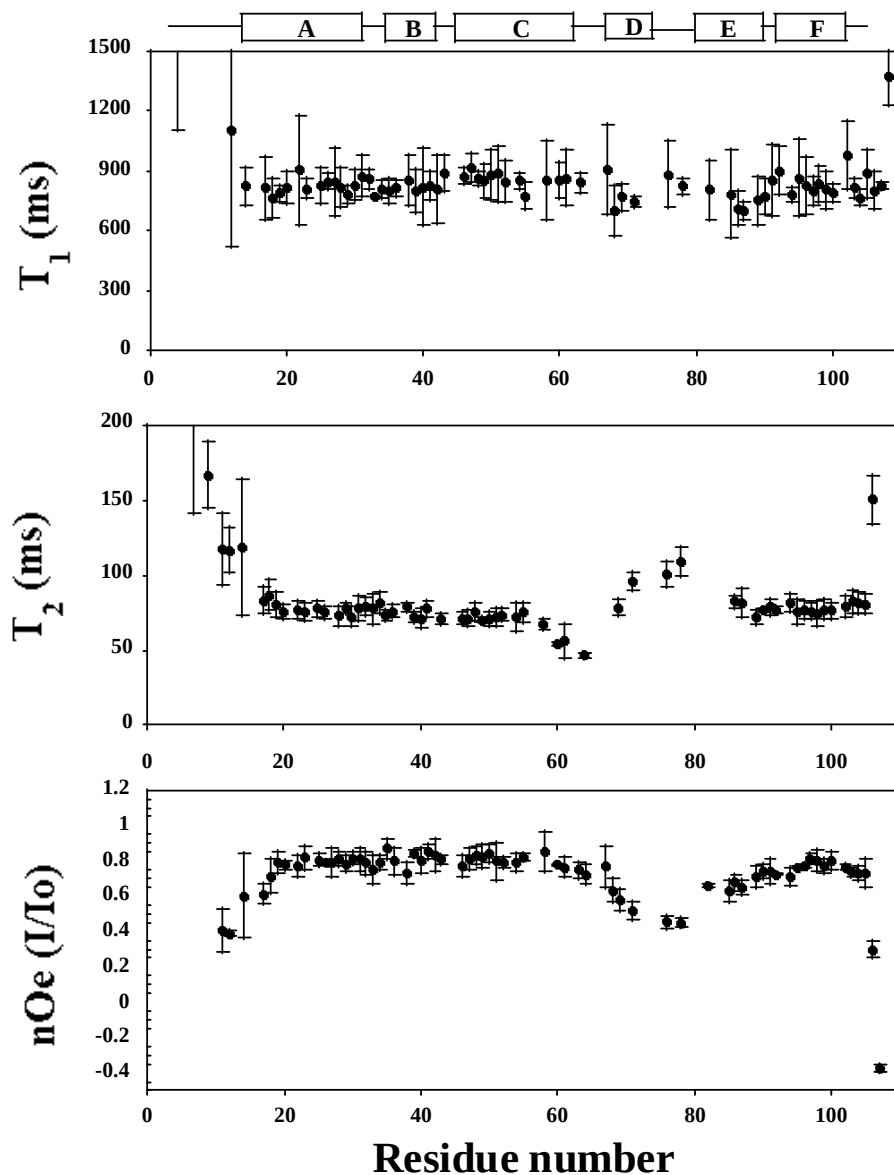


Figure 12. ^{15}N NMR Relaxation Parameters (a) T_1 ; (b) T_2 ; and (c) $n\text{Oe}$ Measured For Apo-L75F-TrpR At 45°C And pH 5.7 and $\sim 1\text{mM}$ (dimer) protein concentration. Note that the T_1 , T_2 , and NOE values are quite uniform for backbone amides located in the core A, B, C, and F helices of the protein, with T_1 and T_2 values of ~ 850 msec, and ~ 75 msec. $n\text{Oe}$ values are depressed (< 0.8) for backbone amides in the helix D-turn-helix E region as well as for residues in the flexible N- and C-termini. Error bars correspond to spread in the data in the triplicate measurements acquired for each relaxation parameter.

residues 16-32; helix B: residues 35-42; helix C: residues 45-63 and helix F: residues 93-103) of the protein were found to be quite uniform, yielding an average $^{15}\text{N-T}_1$ value of $836.7 \text{ ms} \pm 36.9 \text{ ms}$ and an average $^{15}\text{N-T}_2$ value of $73.3 \text{ ms} \pm 3.8 \text{ ms}$ (with 52 NH bond vectors used in the calculations). $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ NMR relaxation time constants of NH residues located in helix D (residues 68-74) exhibited slightly depressed $^{15}\text{N-T}_1$ and slightly elevated $^{15}\text{N-T}_2$ values, with average $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ relaxation time constants of $781.6 \text{ ms} \pm 54.1 \text{ ms}$ and $83.0 \text{ ms} \pm 3.1 \text{ ms}$ (with 6 NH bond vectors included in the calculations), respectively. Similar measurements for NH residues located in helix E (residues 81-90) yielded average $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ values of $798.4 \text{ ms} \pm 47.7 \text{ ms}$ and $74.3 \text{ ms} \pm 14.7 \text{ ms}$ (for 8 NH bond vectors), respectively, which were quite comparable to average $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ values calculated for core residues. Backbone amides located at the N- and C-termini of apo-L75F-TrpR exhibited large $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ values, as expected for very flexible ends of a protein.

Similarly, heteronuclear $^{15}\text{N}\{-^1\text{H}\}$ -nOe values were quite uniform for backbone amides located in the core helices of apo-L75F-TrpR, averaging to 0.79 ± 0.05 (with 52 NH bond vectors included in the calculation), while $^{15}\text{N}\{-^1\text{H}\}$ -nOe values of $^{15}\text{N}/^1\text{H}$ amides located in the helix D-turn-helix E domain of the protein were shown to be significantly lower (by ~ 0.2 nOe units), although the lower $^{15}\text{N}\{-^1\text{H}\}$ -nOe values were not uniform across helices D and E, averaging to 0.64 ± 0.02 (with 6 NH bond vectors included in the calculations) for helix D amides, and 0.56 ± 0.12 (with 8 NH bond vectors included in the calculations) for helix E amides. While $^{15}\text{N}\{-^1\text{H}\}$ -nOes for amides in the D/E region were lower than $^{15}\text{N}\{-^1\text{H}\}$ -nOes found for amides located in the core

helices, a higher variation and progressive downward trends in $^{15}\text{N}\{-^1\text{H}\}$ -nOe values for helix E amides were observed, with amides at the C-terminal end of helix E displaying higher $^{15}\text{N}\{-^1\text{H}\}$ -nOe than that of amides closer to the N-terminal end of helix E and adjacent to the turn region of the helix D-turn-helix E domain (Figure 13, bottom panel). Amides located in the N- and C-terminal ends of the protein exhibited large and negative $^{15}\text{N}\{-^1\text{H}\}$ -nOe values in all six apo and holo forms of TrpR, consistent with the observed large and positive ^{15}N - T_1 and ^{15}N - T_2 trends that are characteristic of very flexible N- and C-termini and will not be repeatedly discussed individually for all TrpR forms.

^{15}N Relaxation Measurements Of Apo-A77V-TrpR

^{15}N - T_1 , ^{15}N - T_2 , and heteronuclear $^{15}\text{N}\{-^1\text{H}\}$ -nOe trends observed for apo-A77V-TrpR are shown in Figure 14, and follow similar but not strictly identical patterns as those observed for backbone amides of apo-L75F-TrpR and apo-WT-TrpR. A complete list of all the ^{15}N NMR relaxation parameters measured for apo-A77V-TrpR is included in Table 10. Figure 14 depicts average ^{15}N - T_1 , ^{15}N - T_2 , and $^{15}\text{N}\{-^1\text{H}\}$ -nOe relaxation values for all measurable amide residues of apo-A77V-TrpR with error bars representing $\pm$ one standard deviation between triplicate sets of measurements. Similar to apo-L75F-TrpR and apo-WT-TrpR, the ^{15}N - T_1 and ^{15}N - T_2 NMR relaxation time constants of NH residues located in the core helices (A, B, C, and F) of the protein were found to be uniform, yielding average ^{15}N - T_1 and ^{15}N - T_2 values of $839.7 \text{ ms} \pm 53.8 \text{ ms}$ and of $75.4 \text{ ms} \pm 6.1 \text{ ms}$, (for 47 NH bond vectors included in the calculations), respectively. ^{15}N - T_1 and ^{15}N - T_2 NMR relaxation time constants of NH residues located in the helix D yielded averages

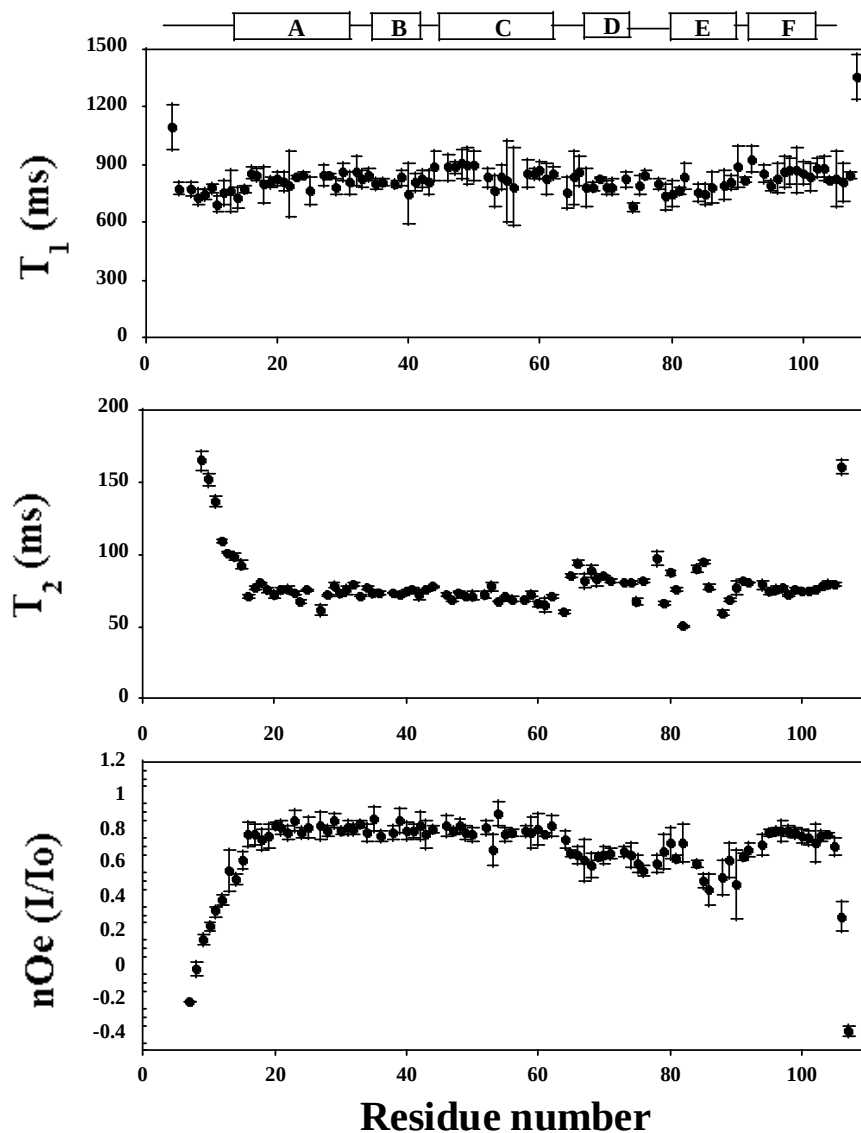


Figure 13. ^{15}N NMR Relaxation Parameters (a) T_1 ; (b) T_2 ; And (c) nOe Measured For Apo-WT-TrpR at 45°C And pH 5.7 And ~1mM (Dimer) Protein Concentration. Note that the T_1 , T_2 , and NOE values are quite uniform for backbone amides located in the core A, B, C, and F helices of the protein, with T_1 and T_2 values of ~ 850 msec, and ~ 75 msec. nOe values are depressed (< 0.8) for backbone amides in the helix D-turn-helix E region as well as for residues in the flexible N- and C-termini. Error bars correspond to spread in the data in the triplicate measurements acquired for each relaxation parameter.

of $812.7 \text{ ms} \pm 32.7 \text{ ms}$ and $82.8 \text{ ms} \pm 8.9 \text{ ms}$ (with 7 helix D NH bond vectors included in the calculations), respectively. Similarly for helix E amides, average $^{15}\text{N-T}_1$

^{15}N Relaxation Measurements Of Apo-A77V-TrpR

$^{15}\text{N-T}_1$, $^{15}\text{N-T}_2$, and heteronuclear $^{15}\text{N-}\{^1\text{H}\}$ -nOe trends observed for apo-A77V-TrpR are shown in Figure 14, and follow similar but not strictly identical patterns as those observed for backbone amides of apo-L75F-TrpR and apo-WT-TrpR. A complete list of all the ^{15}N NMR relaxation parameters measured for apo-A77V-TrpR is included in Table 10. Figure 14 depicts average $^{15}\text{N-T}_1$, $^{15}\text{N-T}_2$, and $^{15}\text{N-}\{^1\text{H}\}$ -nOe relaxation values for all measurable amide residues of apo-A77V-TrpR with error bars representing $\pm$ one standard deviation between triplicate sets of measurements. Similar to apo-L75F-TrpR and apo-WT-TrpR, the $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ NMR relaxation time constants of NH residues located in the core helices (A, B, C, and F) of the protein were found to be uniform, yielding average $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ values of $839.7 \text{ ms} \pm 53.8 \text{ ms}$ and of $75.4 \text{ ms} \pm 6.1 \text{ ms}$, (for 47 NH bond vectors included in the calculations), respectively. $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ NMR relaxation time constants of NH residues located in the helix D yielded averages of $812.7 \text{ ms} \pm 32.7 \text{ ms}$ and $82.8 \text{ ms} \pm 8.9 \text{ ms}$ (with 7 helix D NH bond vectors included in the calculations), respectively. Similarly for helix E amides, average $^{15}\text{N-T}_1$ and $^{15}\text{N-T}_2$ values were calculated to be $800.4 \text{ ms} \pm 16.7 \text{ ms}$ and $78.6 \text{ ms} \pm 3.7 \text{ ms}$ (with 3 helix E NH bond vectors included in the calculations), respectively.

Heteronuclear $^{15}\text{N-}\{^1\text{H}\}$ -nOe values were quite uniform for backbone amides located in the core helices of apo-A77V-TrpR, averaging to 0.79 ± 0.04 (47 NH bond vectors included in the calculation), while $^{15}\text{N-}\{^1\text{H}\}$ -nOe values of $^{15}\text{N}/^1\text{H}$ amides located

in helices D and E regions yielded average $^{15}\text{N}\{-^1\text{H}\}$ -nOe values of 0.70 ± 0.02 (for 7 helix D NH bond vectors included in the calculation) and 0.77 ± 0.02 (for 3 helix E NH bond vectors included in the calculation) respectively. The lower $^{15}\text{N}\{-^1\text{H}\}$ -nOe values measured for backbone amides in the D-E region of apo-A77V-TrpR are not as low as those observed for corresponding backbone amides of apo-WT-TrpR and apo-L75F-TrpR. This difference in $^{15}\text{N}\{-^1\text{H}\}$ -nOe trends supports the notion that substitution of alanine to valine at residue position 77 has altered the flexibility of the A77V-TrpR mutant when compared to apo-WT-TrpR. The $^{15}\text{N}\{-^1\text{H}\}$ -nOe data shown in Figure 14 (bottom panel) indicated that amides located within the helix D-turn-helix E DNA binding domain of apo-A77V-TrpR display more dynamically restrained ps-ns internal motions, than corresponding amides of apo-WT-TrpR and apo-L75F-TrpR.

Comparison of Relaxation Trends Between The Apo-TrpR Forms

The measured ^{15}N - T_1 relaxation values observed for all three apo-repressors are within the theoretically expected range for the 25 kDa size of TrpR. ^{15}N - T_1 trends were uniform across the protein sequence for all three apo-TrpR proteins, indicating that the three proteins have very similar global properties. Backbone amides residing within the N- and C- termini displayed very similar trends among all three apo-repressors, with significantly larger ^{15}N - T_1 values than those measured for amides in core helices, indicating that the N- and C-terminal ends of the three proteins are highly flexible and lack well-defined stable structure.

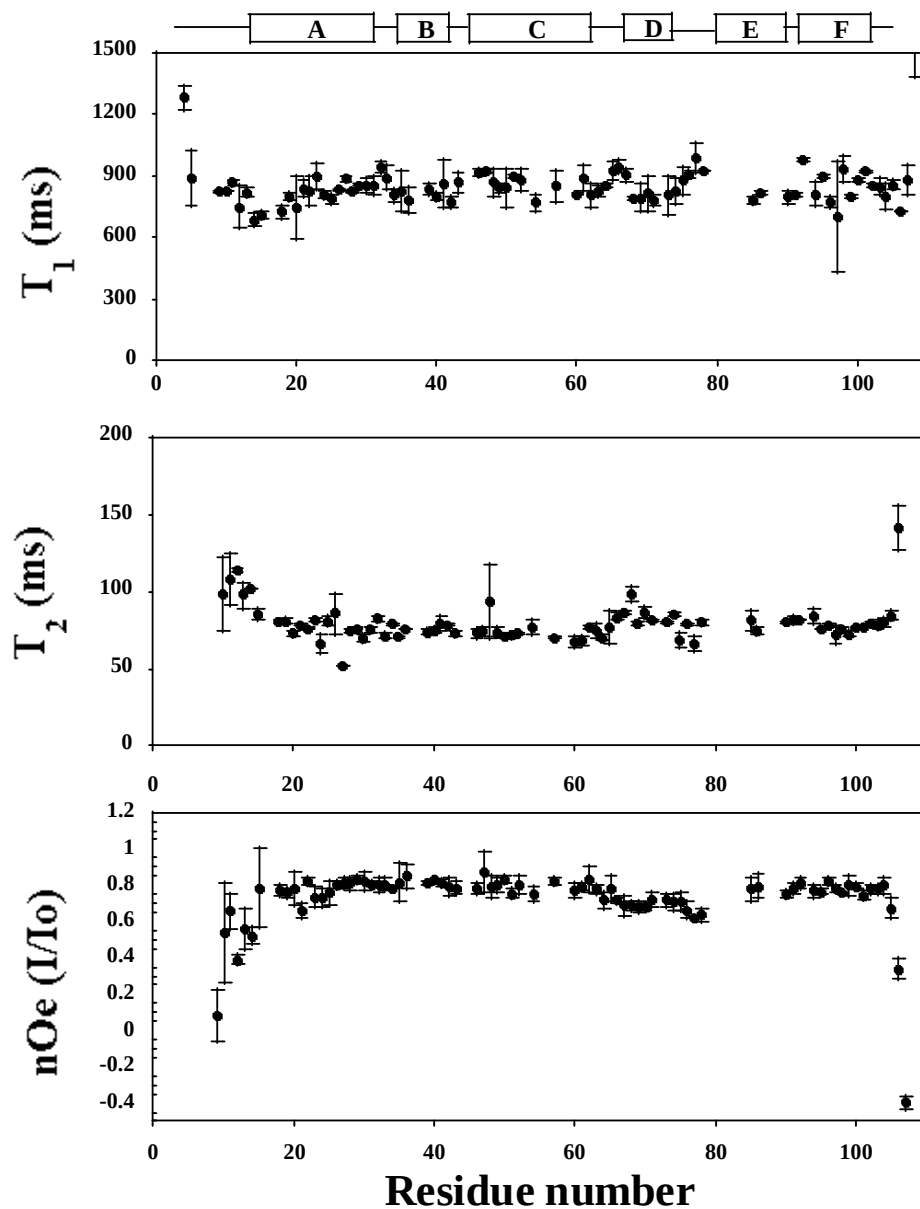


Figure 14. ^{15}N NMR Relaxation Parameters (a) T_1 ; (b) T_2 ; And (c) $n\text{Oe}$ Measured For Apo-A77V-TrpR At 45°C And pH 5.7 And $\sim 1\text{mM}$ (Dimer) Protein Concentration. Note that the T_1 , T_2 , and NOE values are quite uniform for backbone amides located in the core A, B, C, and F helices of the protein, with T_1 and T_2 values of ~ 850 msec, and ~ 75 msec. $n\text{Oe}$ values are depressed (< 0.8) for backbone amides in the helix D-turn-helix E region as well as for residues in the flexible N- and C-termini. Error bars correspond to spread in the data in the triplicate measurements acquired for each relaxation parameter.

Comparison of ^{15}N - T_2 data obtained for apo-TrpR-WT and apo-TrpR-L75F

(Figure 15) revealed that both proteins exhibit similar trends with uniform and comparable ^{15}N - T_2 values for backbone amides located within the core helices (A-C and F) of both apo-repressors. High ^{15}N - T_2 values were observed for amides located at the N- and C- terminal ends of both proteins, as expected for these very flexible sections of the two proteins. ^{15}N - T_2 values for amides located in the helix-D-turn-helix-E region varied for both apo-WT-TrpR and apo-L75F-TrpR. The variation in the ^{15}N - T_2 data could be categorized into two groups based on the residue amide positions. The first group is located at the C-terminal end of helix-C and the turn following helix C for which lower than average ^{15}N - T_2 values were observed when comparing ^{15}N - T_2 trends between apo-WT-TrpR and apo-L75F-TrpR (Figure 15). The depression in ^{15}N - T_2 values obtained for residues E60, L61 and G64 of apo-WT-TrpR indicated possible contributions from chemical exchange that may be occurring for backbone amides located at C-terminal region of helix C and the turn region following helix C. A second group of unusual ^{15}N - T_2 values clustered within the helix D-turn-helix E domain. For these residues a much higher spread in ^{15}N - T_2 values were observed compared to the uniform ^{15}N - T_2 trends seen for amides in the core helices of the two apo-repressors. Interestingly, a low ^{15}N - T_2 value of $50.6 \text{ ms} \pm 0.4 \text{ ms}$ was measured for the amide of I82 of apo-L75F-TrpR indicating the possibility of chemical exchange occurring at the N-terminal end of helix E.

Overall, the ^{15}N - T_2 relaxation patterns observed for backbone amides located within residue stretch 68-90 of apo-WT-TrpR are difficult to analyze on a per residue

basis, due to the paucity of reliable ^{15}N - T_2 data which arises from significant NMR resonance overlaps of amides located within this region. Nevertheless, the high dispersion in ^{15}N - T_2 trends observed for amides within the helix D/E region of both apo-WT-TrpR and apo-L75F-TrpR indicates the presence of inherent mobility on broad timescales for these amides of both apo-repressors. In contrast, comparison of ^{15}N - T_2 trends for amide residues in apo-L75F-TrpR and apo-A77V-TrpR reveals very comparable relaxation profiles (Figure 17), including highly similar ^{15}N - T_2 trends for amides located in core helices A, B, C, and F, and helix D. The comparison of relaxation data between apo-A77V-TrpR and apo-L75F-TrpR for backbone amides of helix E is limited due to many NMR resonance overlaps and the resulting scarcity of data in this region for apo-A77V-TrpR. Of the two data points which can be compared, the ^{15}N - T_2 value was slightly greater for apo-L75F-TrpR at residue G85 (94.7 ± 1.3 ms) and similar for residue S86 (77.4 ± 1.7 ms). Amide residues located at N- and C- terminal ends of both apo TrpR variants exhibit very large ^{15}N - T_2 values, as expected for such flexible regions.

Comparison Of The ^{15}N - $\{^1\text{H}\}$ Heteronuclear nOe Profiles

The ^{15}N - $\{^1\text{H}\}$ -nOe profiles for both apo-L75F-TrpR and apo-WT-TrpR are very similar for amides located within the N- and C- termini and the protein core helices A, B, C, and F, corresponding to residues 7-64 and 96-107, respectively (Figure 15). Minor differences were observed but their significance is questionable. For example, R54 located in helix C of apo-L75F-TrpR exhibited a high ^{15}N - $\{^1\text{H}\}$ -nOe value of 0.94 ± 0.03 . In contrast, a slightly lower ^{15}N - $\{^1\text{H}\}$ -nOe of 0.80 ± 0.05 was observed for R54 NH

of apo-WT-TrpR. Overall, elevated $^{15}\text{N}\{-^1\text{H}\}$ -nOe values of ~ 0.8 observed for amides located within the core helices A-C and F indicate that the N-H bond vectors of such residues experience restricted ps-ns timescale motional fluctuations.

Backbone amides located within the helix D-turn-Helix E domain of apo-WT-TrpR and apo-L75F-TrpR exhibit lower $^{15}\text{N}\{-^1\text{H}\}$ -nOes compared to those found for amides in the core helices (A, B, C, and F) of the two apo-repressors. These lower $^{15}\text{N}\{-^1\text{H}\}$ -nOe trends indicate that backbone amides in helix D-turn-Helix E region of the two proteins are more flexible than amides in the helical core of the two proteins. There are also differences between $^{15}\text{N}\{-^1\text{H}\}$ -nOe patterns found for helix D amides versus those of helix E.

Backbone amides located in helix D of apo-WT-TrpR exhibited lower $^{15}\text{N}\{-^1\text{H}\}$ -nOe values as compared to corresponding amides in apo-L75F-TrpR (Figure 15), suggesting that amides of helix D are more flexible on the ps-ns timescale in apo-WT-TrpR than in apo-L75F-TrpR. Comparison of $^{15}\text{N}\{-^1\text{H}\}$ -nOe profiles for amides located in the helix E region of apo-L75F-TrpR and apo-WT-TrpR reveals interesting results. Helix E amides of apo-L75F-TrpR displayed greater ps-ns flexibility than the corresponding N-H bond vectors in apo-WT-TrpR, as indicated by a sharp lowering of $^{15}\text{N}\{-^1\text{H}\}$ -nOe values for helix E amides of apo-L75F-TrpR, yielding an average $^{15}\text{N}\{-^1\text{H}\}$ nOe of 0.56 ± 0.12 (with 8 NH bond vectors included in the calculation). In comparison, corresponding helix E amides of the apo-WT-TrpR yielded an average $^{15}\text{N}\{-^1\text{H}\}$ -nOe of 0.68 ± 0.04 (6 NH bond vectors included in the calculation), suggesting that although both proteins have a flexible helix E domain ($^{15}\text{N}\{-^1\text{H}\}$ -nOe < 0.8), the helix E

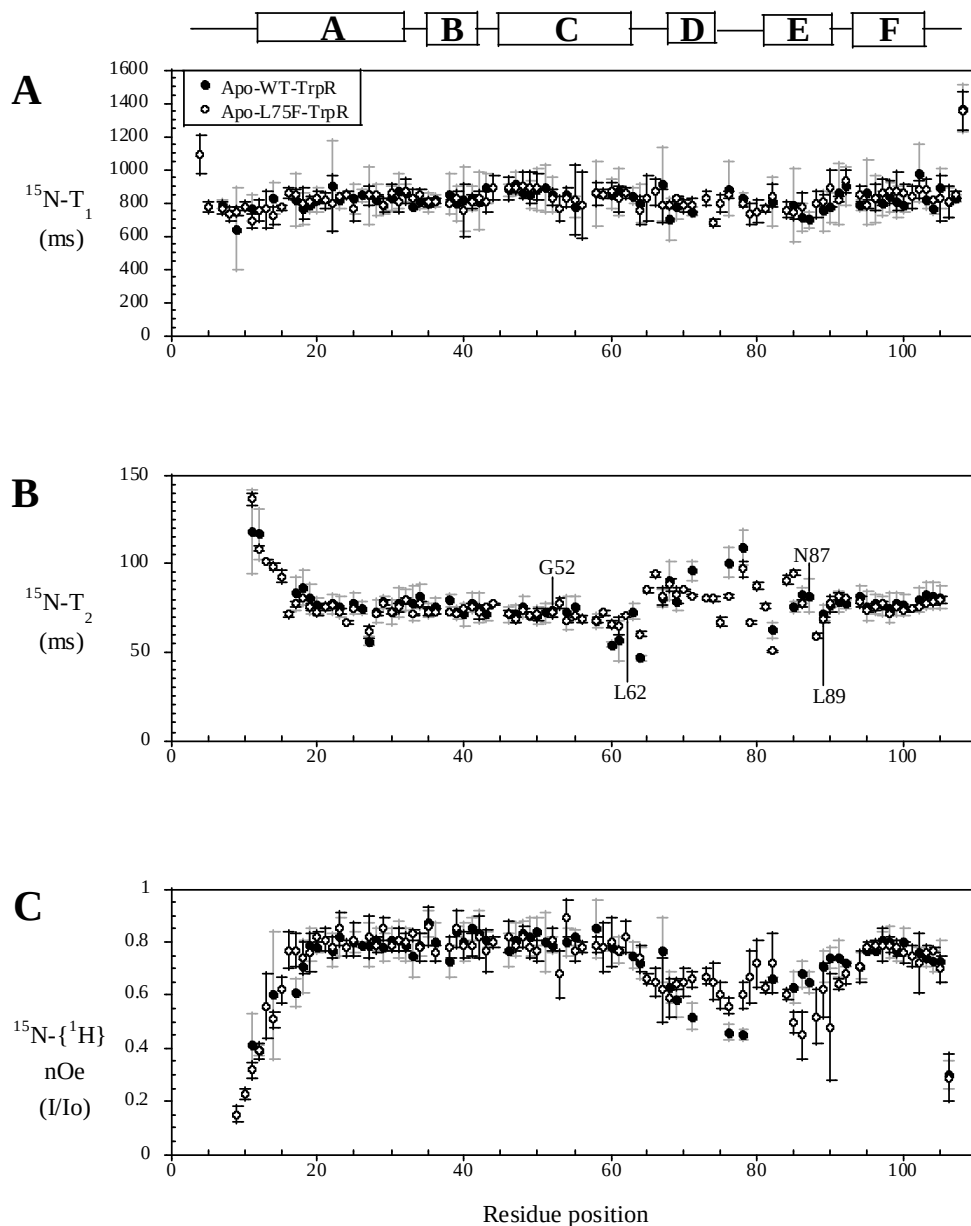


Figure 15. Comparison Of $^{15}\text{N}-T_1$ And $^{15}\text{N}-T_2$ And $^{15}\text{N}-\{^1\text{H}\}$ Heteronuclear nOe_Trends Between Apo-L75F And Apo-WT-TrpR. The scatter in the data for backbone amides in the helix-D-turn-helix-E region (more pronounced for T_2 than T_1 measurements) reflect the anisotropic overall reorientation of the proteins in solution and the possibly complex (ps-ns) motions that this part of the TrpR experiences. This motional complexity also complicates a rigorous interpretation of S^2 generalized order parameter data for backbone atoms in this region of the protein (see text for details). nOes show high ps-ns flexibility in helix E of apo-L75F-TrpR and helix D of apo-WT-TrpR.

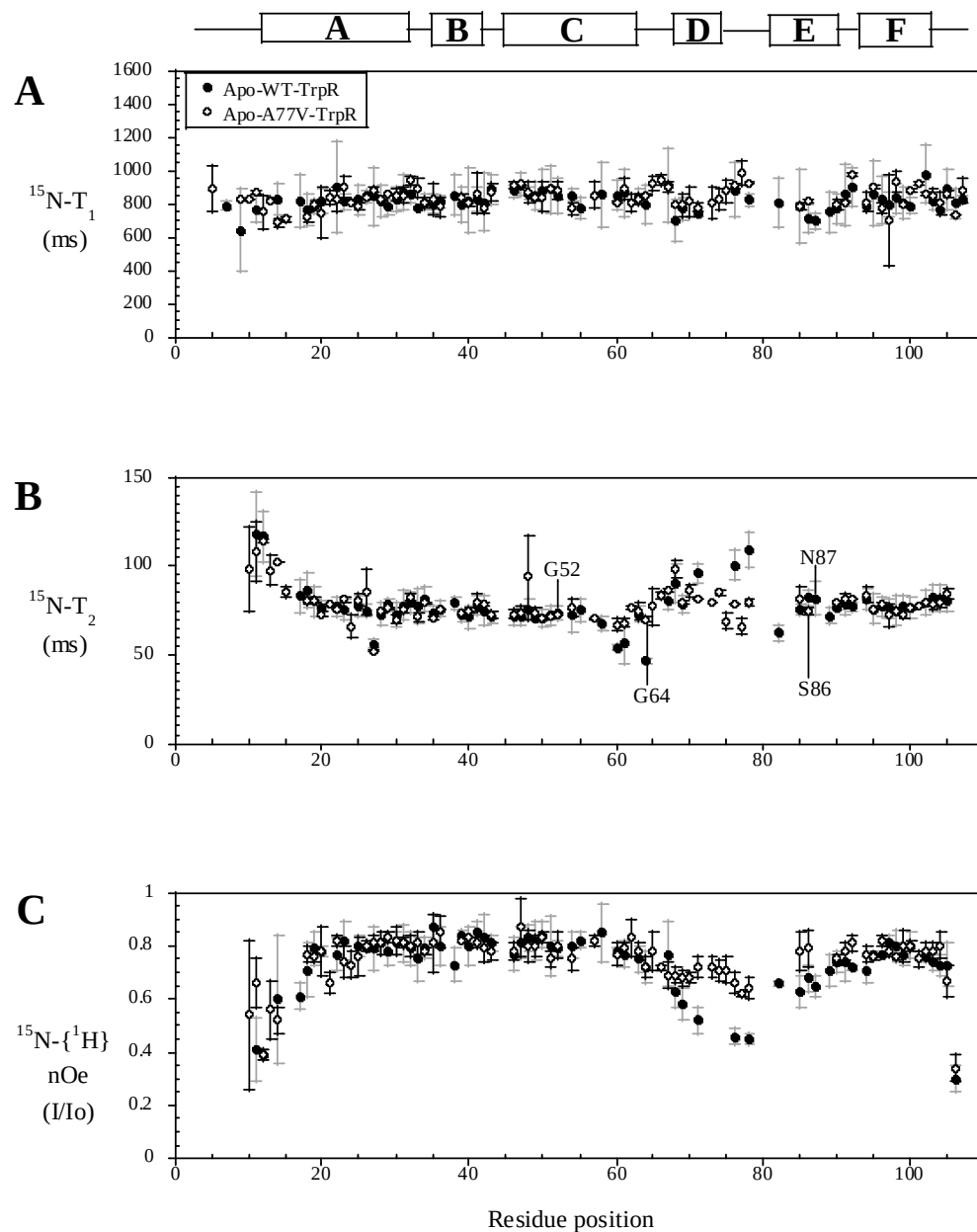


Figure 16. Comparison Of $^{15}\text{N}-T_1$ And $^{15}\text{N}-T_2$ And $^{15}\text{N}-\{^1\text{H}\}$ Heteronuclear nOe_Trends Between Apo-WT And Apo-A77V-TrpR. The nOe values for N-H bond vectors of this region are significantly higher for apo-A77V-TrpR compared to those of apo-WT-TrpR, indicating that the helix D-turn-helix E domain of apo-A77V is significantly less flexible than that of apo-WT-TrpR across both the D and E regions. Interpretation of S^2 generalized order parameter data for backbone atoms in this region of the protein (see text for details).

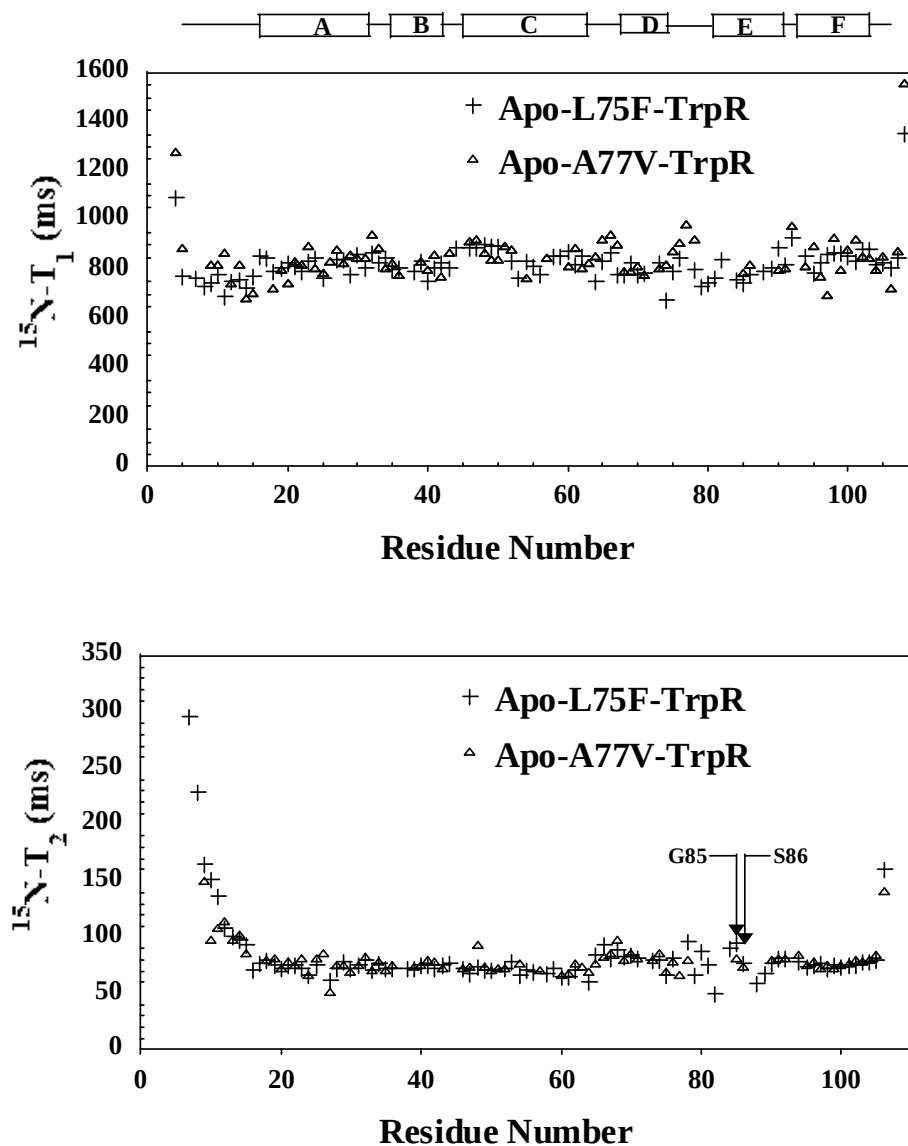


Figure 17. Comparison Of ^{15}N - T_1 Trends Of Apo-L75F-TrpR And Apo-A77V-TrpR. A small scatter in ^{15}N - T_1 profiles is observed for backbone amides located within the helix D-turn-helix E domain of the three proteins. The scatter in the ^{15}N - T_2 data of backbone amides located in the helix-D-turn-helix-E region (more pronounced for ^{15}N - T_2 than ^{15}N - T_1 measurements) reflects the occurrence of more complex backbone amide motions in this region.

of apo-L75F-TrpR may be slightly more flexible (as suggested by a lower average ^{15}N - $\{^1\text{H}\}$ -nOe of 0.56) compared to that of apo-WT-TrpR (average ^{15}N - $\{^1\text{H}\}$ -nOe of 0.68).

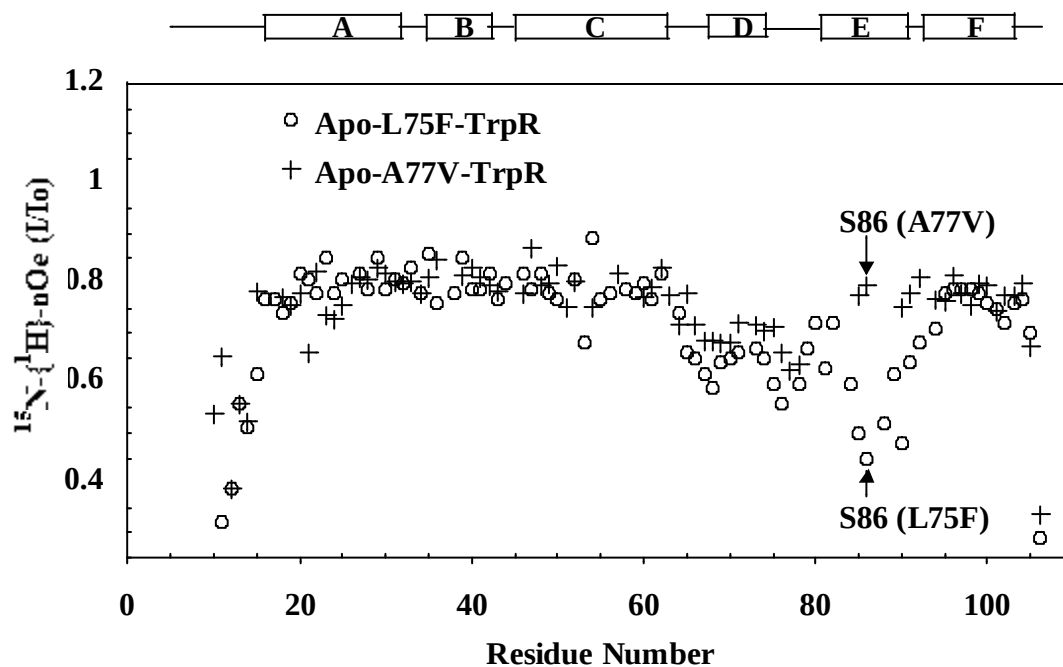


Figure 18. Comparison Of The $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ Profiles Of Apo-L75F-TrpR And Apo-A77V-TrpR. As seen for apo-L75F-TrpR and apo-WT-TrpR, $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ patterns are quite uniform for backbone amides located in the core helices and flexible ends of the proteins. The $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ trends for residues in the helix D region are comparable between the two TrpR variants, while the $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ s measured for backbone amides in the E domain of apo-A77V-TrpR are significantly higher than those of corresponding amides of apo-L75F-TrpR. The $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ values for backbone amides in the E domain of A77V-TrpR are comparable to those found for residues in the core helices, indicating that the helix D-turn-helix E domain of apo-A77V-TrpR is significantly less flexible than its counterpart in apo-L75F-TrpR, and appears to be as motionally restricted (in terms of ps-ns internal motions) as core helices of the super-repressor.

$^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ profiles obtained for amides located in the N- and C-termini and core helices (helices A-C and F) of apo-L75F-TrpR and apo-A77V-TrpR are highly similar between the two apo-repressors (Figure 18). However, as noticed while comparing apo-L75F-TrpR and apo-WT-TrpR above, R54 located in helix C of apo-L75F-TrpR has a higher $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ value than its corresponding counterparts in apo-A77V-TrpR. As stated above, the observed $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ for R54 in apo-L75F-TrpR

corresponds to a value of 0.94 ± 0.03 . In contrast, $^{15}\text{N}\{-^1\text{H}\}$ -nOe data obtained for R54 in apo-A77V-TrpR revealed a lower value of 0.75 ± 0.04 , consistent with $^{15}\text{N}\{-^1\text{H}\}$ -nOe enhancements obtained for the surrounding amides. Comparison of $^{15}\text{N}\{-^1\text{H}\}$ nOe profiles for amides located within the helix D-turn-helix E domain of apo-L75F-TrpR and apo-A77V-TrpR also revealed interesting differences that suggest a possible rationale as to why these two TrpR variants possess such distinct L-trp co-repressor binding affinities.

Although $^{15}\text{N}\{-^1\text{H}\}$ -nOe profiles for helix D amides were very similar between the two apo-repressors, with perhaps slightly higher $^{15}\text{N}\{-^1\text{H}\}$ -nOe values for apo-A77V-TrpR residues, significant differences were observed for amides located within helix E. $^{15}\text{N}\{-^1\text{H}\}$ nOe values were found to be significantly lower for backbone amides located within helix E of apo-L75F-TrpR compared to corresponding amides in apo-A77V-trpR. These data indicate that N-H bond vectors in helix E of apo-L75F-TrpR are more flexible and experience a higher level of ps-ns internal fluctuations compared to corresponding amides in the helix E domain of apo-A77V-TrpR. The differences in $^{15}\text{N}\{-^1\text{H}\}$ -nOe enhancements are most pronounced for helix E residue S86 in apo-L75F-TrpR (with an $^{15}\text{N}\{-^1\text{H}\}$ -nOe of 0.39 ± 0.07) compared to an $^{15}\text{N}\{-^1\text{H}\}$ nOe enhancement of 0.79 ± 0.07 for S86 of apo-A77V-TrpR.

The $^{15}\text{N}\{-^1\text{H}\}$ -nOe profiles of amides located in the N- and C-termini of both apo-A77V-TrpR and apo-WT-TrpR were very comparable, displaying large negative or small positive values ($^{15}\text{N}\{-^1\text{H}\}$ -nOe < 0.4) (Figure 16). Close correlations between $^{15}\text{N}\{-^1\text{H}\}$ nOe trends were also observed for N-H residues located within the core helices (A, B, C, and F) of the two apo-repressors. However, significant differences in $^{15}\text{N}\{-^1\text{H}\}$ nOe

trends were observed for backbone amides located in the helix D-turn-helix E domain of the two proteins. $^{15}\text{N}\{-^1\text{H}\}$ -nOes were found to be substantially lower for amides located within helix D of apo-WT-TrpR (averaging to 0.58 ± 0.06) than those located in helix D of apo-A77V-TrpR (averaging to 0.70 ± 0.02). Helix E amides followed similar trends in $^{15}\text{N}\{-^1\text{H}\}$ -nOes with the enhancement values being lower in apo-WT-TrpR (averaging to 0.68 ± 0.04) than in apo-A77V-TrpR (averaging to 0.77 ± 0.02). $^{15}\text{N}\{-^1\text{H}\}$ -nOe differences were most pronounced for amides of the helix D, indicating that this helix is the most flexible in apo-WT-TrpR on the ps-ns timescale. Two residues located in helix E of apo-A77V-TrpR (G85 and S86) are of special interest since they exhibit high $^{15}\text{N}\{-^1\text{H}\}$ -nOe values (0.78 ± 0.07 and 0.79 ± 0.07 respectively) in the range of those obtained for the core helices A, B, C and F, whereas G85 and S86 of apo-WT-TrpR possess considerably lower $^{15}\text{N}\{-^1\text{H}\}$ -nOe values of 0.63 ± 0.06 and 0.68 ± 0.05 , respectively.

Model-free Analysis

Generalized order parameters (S^2) report on the amplitude of fast (ps-ns) internal motions experienced by N-H bond vectors of the backbone amides. Low order parameter values ($S^2 < 0.4$) indicate high flexibility and have been calculated for amide residues located in N- and C-terminal ends for all three apo-TrpR proteins studied. As indicated by the determination of the principal components of the diffusion tensors ($D_{\parallel}/D_{\perp}$) for apo-TrpR-WT and the two apo-TrpR variants, the global overall reorientation of the TrpR protein molecule(s) in solution was best modeled as that of an axially symmetric prolate ellipsoid. FastModelFree fitting of the relaxation data was performed to extract

internal dynamics parameters which revealed dynamic parameters obtained as a function of residue number as tabulated in Tables 20-22.

The ^{15}N NMR relaxation data obtained for apo-WT-TrpR, apo-L75F-TrpR, and apo-A77V-TrpR were fitted successfully to one of five possible combinations of Model-Free parameters under current fitting criteria (59). During relaxation data analysis, complex forms of the Model-Free Lipari-Szabo formalism that include parameters defining effective correlation time for internal motions (τ_e), conformational exchange parameter (R_{ex}), and motions on the fast and slow time scales (S_f^2 , S_s^2 , τ_s) were only included if the simplest model failed to fit the experimental data as determined by F-test statistics (59). The extended model-free formalism (60) or model 5 was used to fit relaxation data for only 4 amide residues of apo-WT-TrpR (6.8% of all satisfactorily fitted residues) namely L71 in helix D, G78 within the turn region following helix D, and S107 and D108 at the C-terminal end of apo-WT-TrpR. The numbers of apo-WT-TrpR amides that fitted to each combination of model-free parameters (and their percentages out of the total number amides fitted to any model) were 40 (or 68.9%) to model 1 (S^2 only), 13 (or 22.4%) to model 2 (S^2 and τ_e), 0 to model 3 (S^2 and R_{ex}), and 1 (or 1.7 %) to model 4 (S^2 , R_{ex} and τ_e).

The number of ^{15}N - ^1H amides residues fitted to model 1 through model 5 (and percentages out of a total 93 amides with available ^{15}N -T₁, ^{15}N -T₂, and ^{15}N -{ ^1H }-nOe relaxation data) for apo-L75F-TrpR were 44 (47.3 %) to model 1, 14 (15.1 %) to model 2, 5 (5.4 %) to model 3, 4 (4.3 %) to model 4, and 19 (20.4 %) to model 5, as reported in Table 21.

Relaxation data for N- and C- terminal residues of apo-L75F-TrpR (residues 7-15, and 106-108, respectively) were fitted better to an extended model (model 5) indicative of highly complex motions on broad timescales, which is not too surprising considering that these segments are highly flexible in solution. Out of the 17 backbone amides of the helix D-turn-helix E domain (i.e. residues 68-90) of apo-L75F-TrpR that could be analyzed, and for which well-resolved NMR peaks could be obtained, only two amides, namely A80 and L89, displayed ^{15}N NMR relaxation data that could best fit to the simplest model of motion (model 1). The relaxation data for 6 residues were best fit to model 2, which invokes an internal correlation time for NH bond vector libration (τ_e), along with S^2 . 8 amide residues (E70, F75, G78, T81, I82, R84, G85 and S88) of apo-L75F-TrpR displayed ^{15}N -T₁, ^{15}N -T₂, and ^{15}N -{ ^1H }-nOe profiles that required more complicated models to fit the data, and out of these, a relatively high number of 5 (29.4 %) residues (E70, G78, T81, R84 and G85) exhibited ^{15}N NMR relaxation parameters that could only be fitted to the most complicated, extended model (model 5) indicating that complex motions on broad timescales are occurring in this region. A fit to model 5 suggests that the internal motions are too complex to be reliably described using ModelFree analysis, and that some of intrinsic assumptions that this approach invokes about the forms of NMR spectral density functions may not be strictly valid in such cases (53, 54).

Similarly, the number of amide residues of apo-A77V-TrpR fitted to model 1 through model 5 (and their percentages out of a total of 80 amides for which reliable ^{15}N -T₁, ^{15}N -T₂ and ^{15}N -{ ^1H }-nOe data could be obtained) was found to be 45 (56.3 %) to

model 1, 5 (6.3 %) to model 2, 9 (11.3 %) to model 3, 9 (11.3 %) to model 4, and 7 (8.8 %) to model 5. However, it should be noted that in the helix D-turn-helix E region (residues 68-90), 71 % of the residues (i.e. 9 amides out of a total of 13 that could be fitted to any model of motion) had ^{15}N NMR relaxation parameters that could be best fitted to either model 1 or model 2 (i.e. simpler models of motion), whereas only 1 residue (Q68) had relaxation data with best fit to model 5. These results also corresponded to a smaller spread of S^2 values for N-H bond vectors of apo-A77V-TrpR belonging to the D/E region, indicating that internal motions of backbone amides in the D/E region of apo-A77V-TrpR are more restricted than corresponding amides in apo-L75F-TrpR and apo-WT-TrpR. Interestingly, two glycine residues located in the turn region between helix D and helix E (i.e. G76 and G78) and the substituted valine at residue position 77 between those two glycines displayed ^{15}N relaxation data best fitted to model 4 (S^2 , R_{ex} and τ_e), indicating that this region undergoes μs -ms conformational exchange, with a higher than average (18 amides best fitted to either model 3 or 4 which report chemical exchange parameters averaging to $2.5 \pm 0.4 \text{ sec}^{-1}$) exchange rate (R_{ex}) of 6 sec^{-1} observed for Val 77.

Our computation of generalized order parameters revealed results consistent with the ^{15}N - $\{^1\text{H}\}$ -nOe profiles observed for amides of the three apo-repressors. Furthermore, model selection has revealed noteworthy differences and subtleties in S^2 trends between the three apo-TrpR proteins. Specifically, major differences in S^2 and model selection were observed for amide residues located within the helix D-turn-helix E region (i.e. the DNA binding domain) of the apo-repressors. In contrast S^2 trends and model selections

were more uniform for amides residing within the core helices (A, B, C and F) of the proteins. It was found that NH bond vectors in core helices are highly restricted in terms of ps-ns internal motions, as indicated by generalized order parameters $S^2 > 0.88$ for core residues of all three apo-TrpR proteins.

If one is to compute average S^2 values for all amides with reliable ^{15}N NMR relaxation data in the helix D-turn-helix E region, S^2 trends appear to be very comparable for all three apo-repressors. However separating S^2 trends for backbone amides of helix D versus those of helix E reveals small but significant variations among the three apo-repressors. An average order parameter computed for backbone amides of helix D in apo-WT-TrpR yields $\langle S^2 \rangle$ of 0.78 ± 0.04 (only 2 NH used in the calculation). This is to be compared with an average $\langle S^2 \rangle$ value 0.83 ± 0.01 for helix D amides of apo-L75F-TrpR (6 amides included in the calculation) and an average $\langle S^2 \rangle$ value 0.82 ± 0.02 for helix D amides of apo-A77V-TrpR (7 amides used in the calculation). While average $\langle S^2 \rangle$ values do not reveal any significant differences between the three apo-repressors, the distribution of S^2 values for helix D amides is greater in apo-WT-TrpR compared to that of apo-L75F-TrpR. There is a slight difference in S^2 values between the two proteins, supporting the observation from ^{15}N - $\{^1\text{H}\}$ nOe trends that helix D amides are more dynamic (on the ps-ns timescale) in apo-WT-TrpR than corresponding helix D amides of apo-L75F-TrpR. Interpretation of S^2 trends is complicated by the fact that ^{15}N NMR relaxation data for most amides in the helix D-turn-helix E region cannot be fitted to models 1 and 2 where ModelFree analysis is most applicable.

An average order parameter computed for NH bond vectors of helix E of apo-WT-TrpR yielded an average $\langle S^2 \rangle$ of 0.87 ± 0.06 (for 6 amides included in the calculation) which is comparable to the average $\langle S^2 \rangle$ values for corresponding residues in apo-L75F-TrpR ($\langle S^2 \rangle = 0.84 \pm 0.03$; 9 amides included in the calculation) and in apo-A77V-TrpR ($\langle S^2 \rangle = 0.91 \pm 0.01$, 4 amides included in the calculation). Perhaps more noteworthy, helix E amides of apo-A77V-TrpR yielded rather high S^2 value, indicative of highly restricted ps-ns motions and of the same magnitude as helix A, B, C and F. Furthermore NMR relaxation data fitting and model selection (most of the helix D amides of apo-A77V-trpR are best fitted to model 1) result in lower uncertainty values in S^2 , supporting the observation that residues in helix D are less flexible in the super-repressor TrpR mutant.

Reduced Spectral Density Mapping Results

The ^{15}N NMR relaxation data obtained for apo-WT-TrpR, apo-L75F-TrpR, and apo-A77V-TrpR were also analyzed using the reduced spectral density mapping method (50, 61) besides ModelFree (51, 59, 62). Both types of analysis were undertaken because the region with most noticeable differences was also the most challenging to probe by NMR due to the extensive overlap of NH NMR signals, which precluded a quantitative analysis of resonance intensities and measurements of ^{15}N NMR relaxation parameters for many amides in this region. In addition, many amides in the HTH domain for which ^{15}N NMR relaxation parameters could be measured could not be fit to simple models of motions (i.e model 1: S^2 only; or model 2: S^2 , τ_e) (59), limiting our interpretation of S^2 parameters obtained from FastModelFree analysis for these residues.

The spectral density functions ($J_{\text{eff}}(0)$, $J(\omega_N)$ and $J(0.87\omega_H)$) were calculated for each residue at a single magnetic field strength of 14.1 T. The results are presented in Figures 19 and 20 and a complete tabulation of reduced spectral density functions is available in Table 14-16. As foreshadowed by the relaxation rates, the reduced spectral density functions $J_{\text{eff}}(0)$, $J(\omega_N)$ and $J(0.87\omega_H)$ for NH vectors of the core helices of all three proteins were fairly uniform. This uniformity indicates that the core NH vectors have very similar motional properties. $J(\omega_N)$ profiles were very similar throughout the sequence of all three proteins. These uniform patterns are very consistent with the uniform trends observed in the ^{15}N - T_1 data, and reflect the same insensitivity to residue position as observed in their ^{15}N - T_1 data. The fact that ^{15}N - T_1 trends are consistent with $J(\omega_N)$ profiles is not too surprising considering that $J(\omega_N)$ is a measure of the spectral power of frequencies that contribute significantly to ^{15}N - T_1 relaxation, thus we expect $J(\omega_N)$ profiles to be very comparable to the observed ^{15}N - T_1 trends (63). Very small $J_{\text{eff}}(0)$ and large $J(0.87\omega_H)$ (the latter characteristics of very rapid ps-ns motions) were observed for amides located in the N- and C-terminal ends of the three proteins. These data are very consistent with what is observed for very flexible disordered termini of proteins (63).

Interesting differences in $J_{\text{eff}}(0)$ and $J(0.87\omega_H)$ were observed for backbone amides residing within the helix-D-turn-helix-E domain of the three apo-repressors. As reported by the ^{15}N - $\{^1\text{H}\}$ -nOe trends, elevated values of $J(0.87\omega_H)$ were observed for these residues compared to backbone amides residing in the 6 core α -helices of the proteins. Residues which were not undergoing chemical exchange tended to possess

slightly lower $J(0)$ values than those measured for core amides. Together these data indicate that in all three apo-repressors the HTH domain is more flexible than core elements.

However the patterns were not uniform across the D and E regions and differential flexibility was again observed between the two regions. For example, elevated $J(0.87\omega_H)$ were measured for helix D amides of apo-WT-TrpR compared to $J(0.87\omega_H)$ values measured for corresponding amides of apo-L75F-trpR (Figure 19 bottom panel) and apo-A77V-TrpR (Figure 20 bottom panel), clearly indicating that the helix D region of apo-WT-TrpR is more flexible than helix D of apo-L75F-TrpR and that of apo-A77V-TrpR. The $J(0.87\omega_H)$ patterns are reverse for helix E amides of apo-L75F-TrpR, whereby $J(0.87\omega_H)$ trends are slightly higher than those calculated for helix E amides of apo-WT-TrpR (Figure 19 bottom panel), indicating this region is more flexible in apo-L75F-TrpR than in apo-WT-TrpR.

These data clearly show that the Leu to Phe mutation at residue position 75 reduces the internal backbone motions of helix D amides and enhances the ps-ns motion of helix E amides. In contrast, the $J(0.87\omega_H)$ trends remain lower for helix E amides of apo-A77V-TrpR compared to apo-WT-TrpR (Figure 20 bottom panel), indicating that the Ala to Val substitution at residue position 77 reduces ps-ns internal motions throughout the entire helix D-turn-helix E domain of the super-repressor.

We observed noticeable scatter in $J_{\text{eff}}(0)$ trends for HTH amides of all three apo-repressors (Figures 19 and 20, top panels). Many residues exhibited slightly smaller $J_{\text{eff}}(0)$ than those calculated for core residues, consistent with increased internal motions

occurring in many regions of the HTH domains of the three apo-repressors. However several other residues exhibited significantly large $J_{\text{eff}}(0)$, and indicated that these residues are not only experiencing ps-ns bond vector fluctuations but also slower μs -ms chemical exchange motions. Elevated $J_{\text{eff}}(0)$ were observed for residues E60 and G64 of apo-WT-TrpR and T81 of apo-L75F-TrpR (Figure 19 top panel) which suggest strong R_{exch} contributions to ^{15}N - T_2 relaxation.

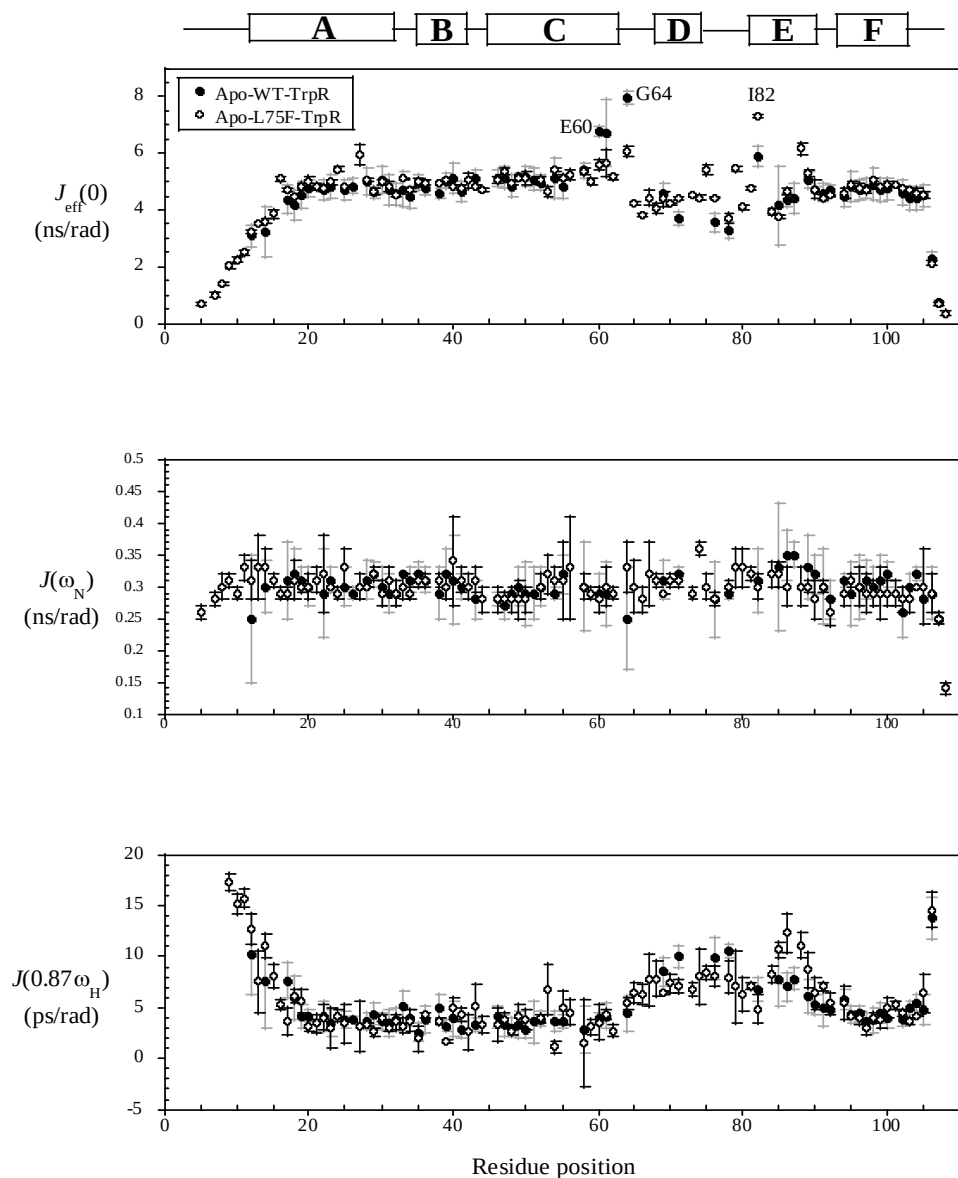


Figure 19. Comparison Plots Of Reduced Spectral Density Functions Calculated For Apo-WT-TrpR (Filled Circles) And Apo-L75F-TrpR (Open Crosses): (top) $J_{\text{eff}}(0)$; (middle) $J(\omega_N)$; and (bottom) $J(0.87\omega_H)$, with secondary structural elements depicted above the plots. $J_{\text{eff}}(0)$ (suspected to include R_{exch}) from both proteins are labeled. Significant differences in $J(0.87\omega_H)$ were observed in the helix-D-turn-helix E motif. Slightly elevated $J(0.87\omega_H)$ for helix D amides of apo-WT-TrpR compared to their counterparts in apo-L75F-TrpR support the notion that helix D of the wild-type apo-repressor is more flexible (in terms of ps-ns internal motions) than helix D of the ts apo-TrpR mutant. The $J(0.87\omega_H)$ pattern is reversed for helix E, indicating increased flexibility of this region in apo-L75F-TrpR compared to apo-WT-TrpR.

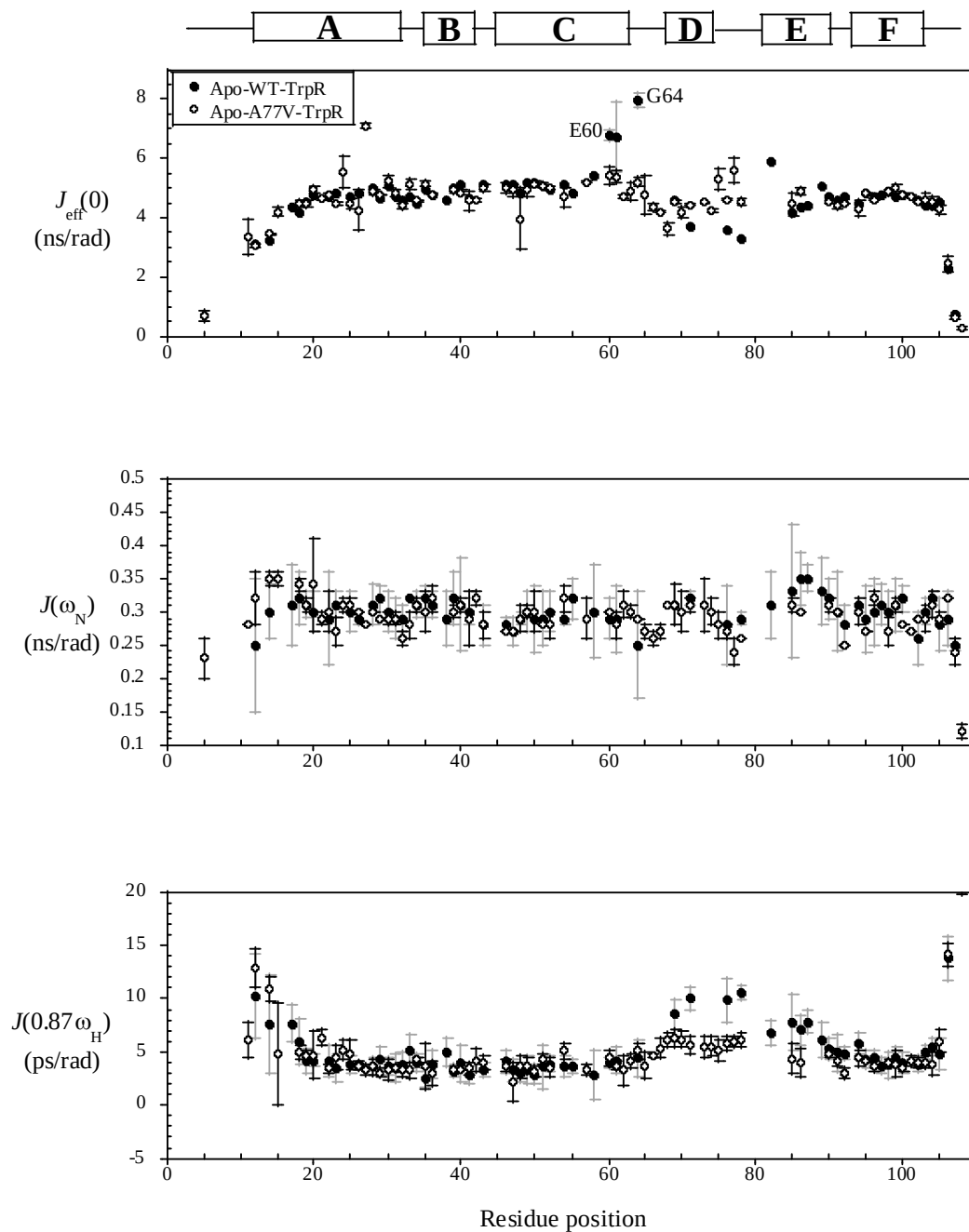


Figure 20. Comparison Plots Of Reduced Spectral Density Functions Calculated For Apo-WT-TrpR (Filled Circles) And Apo-A77V-TrpR (Open Crosses): (top) $J_{\text{eff}}(0)$; (middle) $J(\omega_N)$; and (bottom) $J(0.87\omega_H)$, with secondary structural elements depicted above the plots. Reduced $J(0.87\omega_H)$ trends for backbone amides in the helix-D-turn-helix E region of apo-A77V-TrpR support the notion that the Ala to Val amino acid substitution at residue position 77 decreases the overall ps-ns motional flexibility of the HTH DNA-binding domain of the super-repressor.

PREPARATION OF HOLO-TRPR SAMPLES

To perform comparative studies with holo (L-Trp) forms of the TrpR and its mutants, holo-TrpR samples were made and studied as described below.

Assignment Of Chemical Shifts

Backbone amide resonances of two apo (WT and A77V) were assigned using multidimensional (2D and 3D) heteronuclear (^1H , ^{15}N , ^{13}C) NMR experiments. These include ^{15}N - ^1H HSQC, HNCACB, CBCACONH, and HNCA that upon analysis, permitted the assignment of 85 and 88 backbone amides out of a total of 104 non-proline backbone residues in apo-WT-TrpR and apo-A77V-TrpR, respectively. Resonance overlap obscured the assignment of rest of the resonances. The chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) ^1H signal at 0.0 ppm (64) and analyzed using SPARKY (65). Resonance assignments of backbone atoms (^1H , ^{15}N , $^{13}\text{C}_{\alpha/\beta}$) obtained for apo-WT-TrpR and apo-A77V-TrpR were deposited in the BioMagResBank as entries 17041, 17046, and 17047 and are reported here in Tables 3 and 4. Resonance assignments for apo-L75F-TrpR were obtained from published work (66). In this case 101 out of 104 non-proline residues had been assigned (66). The sample used for these experiments were grown to contain ^{15}N and ^{13}C labels to observe their resonance signals and were concentrated to a final sample concentration of ~1 mM. Similarly, the holo-TrpR chemical shifts were also assigned.

L-Trp Binding Titrations

TrpR binding to L-Trp was measured by monitoring a series of ^{15}N - ^1H HSQC spectra of solutions of TrpR as a function of the concentration of titrating L-Trp. 0.7-1.0 mM ^{15}N labeled TrpR WT, L75F and A77V samples were titrated with increasing amounts of an unlabeled L-Trp stock solution (50 mM L-Trp, 50 mM K_2HPO_4 , pH 5.7). Eight 0.2 molar equivalent aliquots of the L-Trp stock solution were added to an ^{15}N -labeled protein solution (1 mM monomer, buffer same as above) of either WT-TrpR, L75F-TrpR or A77V-TrpR followed by acquisition of 2D ^1H - ^{15}N -HSQC spectrum after each addition to monitor $^1\text{H}/^{15}\text{N}$ chemical shift changes upon L-Trp addition to TrpR samples. The combined amide chemical shift perturbation was calculated for each sample by taking into account the gyromagnetic ratios of the ^{15}N and ^1H nuclei using the equation $\Delta\delta_{\text{av}} = (0.5[\Delta\delta(^1\text{H}^{\text{N}})^2 + (0.2\Delta\delta(^{15}\text{N}))^2])^{1/2}$ where $\Delta\delta(^1\text{H}^{\text{N}})$ and $\Delta\delta(^{15}\text{N})$ are the chemical shift differences for the $^1\text{H}^{\text{N}}$ and ^{15}N atoms between holo- and apo-forms (i.e. $\Delta\delta = (\text{holo-TrpR} - \text{apo-TrpR})$), and $\Delta\delta_{\text{av}}$ is a weighted average of the ^{15}N and $^1\text{H}^{\text{N}}$ chemical shifts as described in Pellechia et al. (67). Formation of holo-TrpR samples was considered complete when further addition of L-Trp resulted in no changes in $^1\text{H}/^{15}\text{N}$ chemical shifts as monitored in the 2D ^1H - ^{15}N correlation HSQC spectra of the three holo-repressors.

Chemical Shift Perturbation Mapping

The change in chemical shifts due to L-Trp binding were monitored and compared for each TrpR sample at the same amounts of L-Trp titrated. In general, chemical shift changes between holo- and apo-TrpR proteins were found to be small for

most amides, indicating that the overall 3D structures of the apo and holo forms of the three TrpR proteins are very similar. However, ~27 residues exhibited significant $^1\text{H}/^{15}\text{N}$ chemical shift changes upon formation of the holorepressors. On the basis of $^1\text{H}/^{15}\text{N}$ chemical shift variations, residues with corresponding $\Delta\delta_{\text{av}}$ values of ≥ 0.2 ppm were identified as those experiencing significant chemical shift variations and selected for further analysis. As can be noticed in the plot, the chemical shifts were affected differently for the three proteins (Figure 21). Deviations were observed in helices B, C, D and E of all three proteins. A broad comparison between the three shows that TrpR-A77V observed the least change in chemical shifts. In Figure 22, residues with significant chemical shift changes are mapped onto the 3D structure of holo-WT-TrpR, highlighting that several residues with perturbed chemical shifts clearly cluster around the protein's L-Trp binding pocket. For example, residues 38–44 (the C-terminal end of helix B) and 47–49 (beginning of helix C) point directly at the amino and carboxyl groups of L-Trp. Residues 51, 52, 55, 56, 58, and 59 of helix C and residues 84–86 of helix E are directly adjacent to the L-Trp binding pocket (6, 7). Interestingly, several amides with perturbed chemical shifts belong to residues that are not part of the protein's L-Trp binding pocket. Such amides include residues 60–64 (which comprise the C-terminal end of helix C), residues 67–69 and 73–78 (which span the turn–helix D–turn region of the HTH DNA-binding domain of TrpR), and residues 89–91 of helix E. These data suggest that for these residues, there is a correlation between the observed chemical shift changes induced by binding of L-Trp to the repressors and the indirect effects such as subtle conformational or slow internal dynamics changes.

Not all three TrpR proteins, however, exhibited similar patterns of $^1\text{H}/^{15}\text{N}$ chemical shift changes (Figure 21). For example, WT-TrpR displayed the greatest number of residues with chemical shift changes above the $\Delta\delta_{\text{av}} \geq 0.2$ ppm threshold, with the largest changes observed for residues located in the turn region spanning helices B and C (i.e., residues 40 and 42–44) and residues in the turn–helix D–turn region (i.e., residues 60, 61, 64, 67, 69, 75, 77, and 78) of TrpR’s HTH DNA-binding domain (Figure 21, top panel). L75F-TrpR displayed the second greatest number of residues with chemical shift changes $\Delta\delta_{\text{av}}$ of ≥ 0.2 ppm. The same residues located in the turn region between helices B and C exhibited significantly perturbed $^1\text{H}/^{15}\text{N}$ chemical shifts in L75F-TrpR as in WT-TrpR (Figure 21, middle panel). In contrast, a second cluster of residues with large chemical shift changes was localized to the turn–helix E region (residues 77, 81, 85, 88, 89, and 91) of L75F-TrpR, suggesting that the binding of L-Trp Lastly, A77V-TrpR displayed the lowest number of amide residues with chemical shift changes ($\Delta\delta_{\text{av}}$) of ≥ 0.2 ppm. Residues with significant $^1\text{H}/^{15}\text{N}$ chemical shift changes were located at the end of helices B, C, and E, similar to what was observed for WT-TrpR, and spanning the L-Trp binding pocket of the repressor. The largest differences between A77V- and WT-TrpR were found for residues in the turn–helix D–turn region. A77V-TrpR had very few residues in this region with perturbed $^1\text{H}/^{15}\text{N}$ chemical shifts compared to wild-type TrpR (Figure 21, bottom panel). Overall, these data indicated that the chemical shift changes observed upon formation of holo-A77V-TrpR are almost exclusively associated with residues lining the L-Trp binding pocket of the repressor. The absence of significant $^1\text{H}/^{15}\text{N}$ chemical shift changes for residues in the helix D–turn–

helix E (HTH) region of A77V-TrpR suggests that the conformation of A77V-TrpR in its apo state closely mimics that of the L-Trp-bound holo form of the protein.

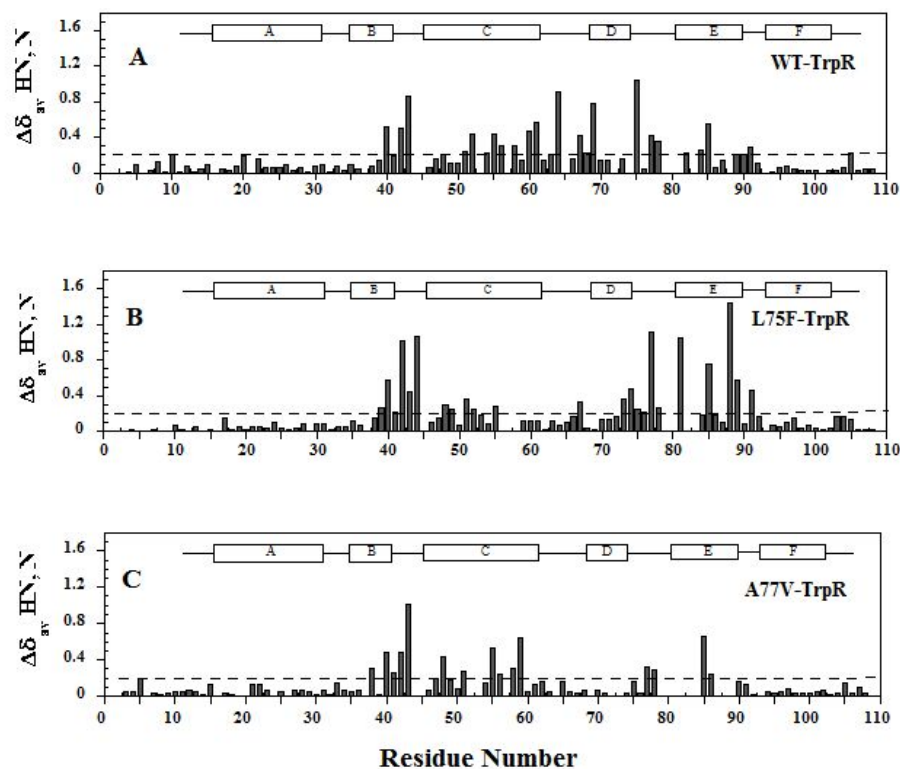


Figure 21: Chemical Shift Changes In TrpR Induced By L-Tryptophan Binding. The chemical shift changes between apo- and holo-TrpR are shown for the TrpR repressors (top) WT, (middle) L75F, and (bottom) A77V. $\Delta\delta_{av}$ is a weighted average of the ^{15}N and $^1\text{H}^{\text{N}}$ chemical shifts calculated as follows: $\Delta\delta_{av} = (0.5[\Delta\delta(^1\text{H}^{\text{N}})]^2 + (0.2\Delta\delta(^{15}\text{N}))^2)^{1/2}$ where $\Delta\delta(^1\text{H}^{\text{N}})$ and $\Delta\delta(^{15}\text{N})$ are the chemical shift differences for the $^1\text{H}^{\text{N}}$ and ^{15}N atoms between apo- and holo-forms.

Further, a comparison of the overlaid 2D $^1\text{H}/^{15}\text{N}$ HSQC spectra of the L-Trp-saturated forms of holo-WT-TrpR and holo-A77V-TrpR revealed an interesting trend and indicated that ~98% of the $^1\text{H}/^{15}\text{N}$ chemical shifts are identical for holo-WT and holo-

A77V TrpR. Any observed chemical shift differences for the two holoproteins corresponded to resonances in holo-A77V-TrpR that are marginally offset in terms of chemical shifts relative to corresponding signals of holo-WT-TrpR. The close resemblance of spectral patterns in the 2D $^1\text{H}/^{15}\text{N}$ HSQC spectra of the two holo-repressors suggests that the conformations of their L-Trp-bound state are almost identical. This is not the case for holo-L75F-TrpR where only ~91% of the observed NH signals overlaid directly with those of holo-WT-TrpR in 2D $^1\text{H}/^{15}\text{N}$ HSQC spectra of these proteins recorded under identical conditions. The remaining ~9% (~9 residues) exhibited small but distinct chemical shift differences from corresponding NH signals of holo-WT-TrpR. These differences were assigned to amides in the HTH domain of TrpR within the stretch of residues 61–79 and amides located in turn regions spanning helix D and next to helix E (i.e., residues 60, 64, 67, 76, 78, 89, and 91). Distinct chemical shifts would be expected for residues close to Phe 75 due to potential ring current effects introduced by the Leu to Phe amino acid substitution in L75F-TrpR. Others are too distant to be experiencing substantial ring current effects, and their distinct chemical shifts most likely originate from small conformational and/or dynamics changes within helices C, D, or E that propagate outward to adjacent turn regions.

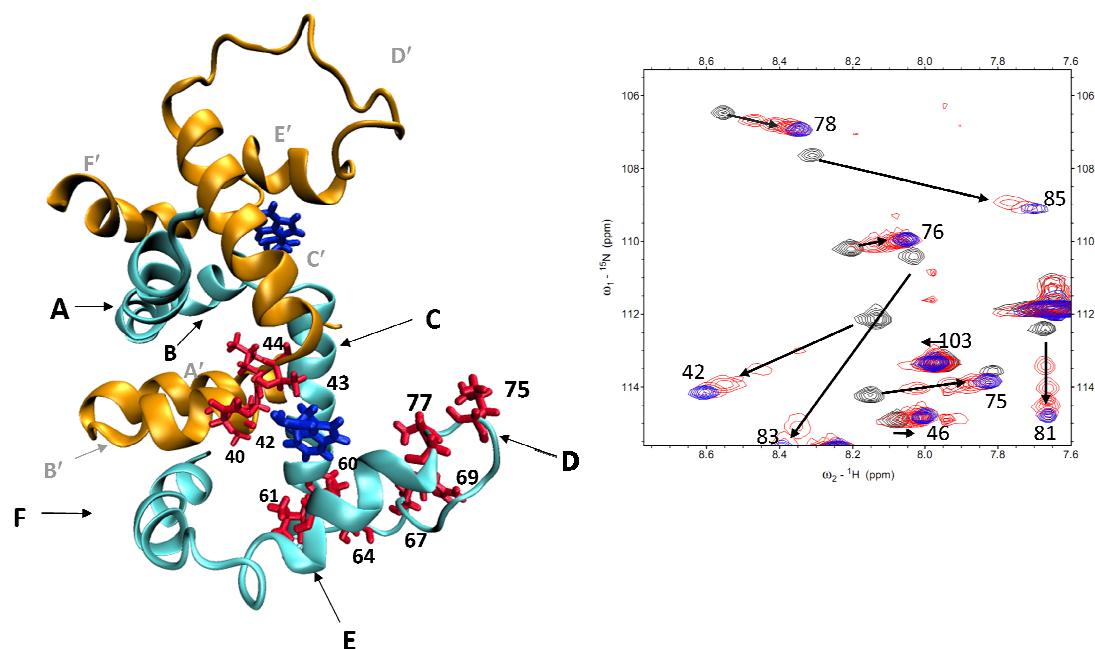


Figure 22: Structural Representation Of Residue Amides Experiencing Chemical Shift Change On Trp-Binding: (Left) Ribbon representation of the structural elements of holo-WT-TrpR. The backbone structures of the two protomers are shown in gold and light blue, with helices A through E labeled. The L-tryptophan co-repressor ligand is shown in blue in stick representation. Side-chains corresponding to amides with most perturbed $^{15}\text{N}/^1\text{H}$ chemical shifts upon L-Trp binding (i.e. residues 40, 42, 43, 60, 61, 64, 67, 68, 69, 75, 77, and 85) are colored red and shown in stick representation. The TrpR protein model was prepared using the molecular graphics program VMD, version 1.8.3 (Humphrey et al., 1996) and the solution solved NMR structure file (PDB ID 1WRS). (Right) Section of an overlay of 2D ^1H - ^{15}N -HSQC spectra displaying the change in chemical shift of several residues upon addition of L-Trp to WT-TrpR. Resonance assignments are shown along with arrows indicating the directions of the chemical shift changes. Contour levels shown in grey correspond to the location of $^1\text{H}/^{15}\text{N}$ resonances of apo-WT-TrpR. Red contour levels indicate the locations of $^1\text{H}/^{15}\text{N}$ resonances which shift as a function of increasing concentration of L-Trp (0.2 to 1.4 mM). The final locations of $^1\text{H}/^{15}\text{N}$ resonances following the addition of 1.6 mM of L-Trp are shown in blue. $^1\text{H}/^{15}\text{N}$ chemical shift changes shown are the result of titration of 0.2mM aliquots of L-Trp into a 1mM (monomer) WT-TrpR protein solution. (68)

Interestingly, small differences in chemical shifts between holo-WT-TrpR and holo-L75F-TrpR were observed for residues located in the C-terminal ends of helices C and E (i.e., residues 56, 60, 89, and 90), consistent with previously observed differences

in $^1\text{H}/^{15}\text{N}$ chemical shifts between the 2D $^1\text{H}/^{15}\text{N}$ spectra of apo-L75F-TrpR and apo-WT-TrpR. On the basis of these data, we thus conclude that the long-range perturbations observed in apo-L75F-TrpR persist in holo-L75F-TrpR.

BACKBONE DYNAMICS OF HOLO TRPR FORMS

^{15}N Relaxation Measurements For Holo-TrpR Samples

^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOe relaxation experiments were performed in triplicate at 318 K (45°C) on the holo forms of TrpR. Standard NMR relaxation pulse sequences were used as described for apo-TrpR samples. To perform relaxation experiments, a sample concentration of ~1mM was used for WT and L75F TrpR forms except for a lower concentration of 0.6–0.7mM used in case of TrpR-A77V to avoid the risk of aggregation. Buffer conditions were same as those used for Chemical Shift Assignment experiments. Out of 104 non-proline residues, relaxation parameters were measured for amides of 80, 76 and 78 residues of holo-L75F, holo-WT and holo-A77V TrpR respectively. A complete listing of all ^{15}N NMR relaxation parameters measured for all three holo-TrpR proteins is included in tables 11-13 and reported in the BMRB (entries 17041, 17046, and 17047).

Relaxation profiles for all holo-TrpR samples are shown in Figures 23-25 and depict the average ^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOe relaxation values for all measurable amides with error bars corresponding to $\pm$ one standard deviation between triplicate sets of measurements. The ^{15}N - T_1 and ^{15}N - T_2 NMR relaxation time constants of NH residues located in the core helices (helix A-C and F) were found to be uniform for all holo-TrpR samples, yielding an average ^{15}N - T_1 value of 802 ms $\pm$ 62 ms and an average ^{15}N - T_2 value of 77 ms $\pm$ 8 ms (with 58 NH bond vectors used in the calculations) for holo-L75F-TrpR, 807 ms $\pm$ 50 ms and an average ^{15}N - T_2 value of 75 ms $\pm$ 5 ms (with 56 NH bond

vectors used in the calculations) for holo-WT-TrpR and $889 \text{ ms} \pm 43 \text{ ms}$ and an average ^{15}N - T_2 value of $73 \text{ ms} \pm 4 \text{ ms}$ (with 55 NH bond vectors used in the calculations) for holo-A77V-TrpR respectively. However, all three holo-forms exhibited a break in uniformity of trend from residue E65 to residue R84. This region corresponds to the turn–helix D–turn–helix E DNA-binding domain of the repressors. The ^{15}N - T_2 profiles of holo-WT-TrpR and holo-A77V-TrpR in this region (Figure 27) were almost identical. ^{15}N - T_2 values for holo-L75F-TrpR amides followed, for the most part, trends similar to those of holo-WT-TrpR and holo-A77V-TrpR, with the exception of two amides (G76 and G78) that displayed significantly higher ^{15}N - T_2 values, suggesting that these two residues are significantly more flexible in L75F-TrpR than in the two other TrpR variants. These two glycines are located in the turn region of the HTH DNA-binding domain next to the amino acid substitution site and may be at the origin of the flexibility differences observed for helix D amides of holo-L75F-TrpR (see below).

The $^{15}\text{N}\{-^1\text{H}\}$ -nOe profiles of backbone amides of holo-WT-TrpR and the two TrpR variants are shown in Figures 23-25, bottom panels. Large decreases in $^{15}\text{N}\{-^1\text{H}\}$ -nOe (< 0.6) were observed for backbone amides in the N- and C-termini of the holo-repressors (residues 4–15 and 105–108, respectively together with the large ^{15}N - T_1 and ^{15}N - T_2 values suggesting large amplitude picosecond to nanosecond motions. $^{15}\text{N}\{-^1\text{H}\}$ -nOes for backbone amides in the core helices were quite uniform, averaging to 0.78 ± 0.06 , for all three holo-repressors, indicating that core amides are motionally restricted in terms of picosecond to nanosecond fluctuations and are part of a rigid structural core for all three TrpR proteins. In contrast, $^{15}\text{N}\{-^1\text{H}\}$ -nOes for residues

located at the end of helix C (starting at residue E65) and progressing toward the beginning of helix E (residue A80) decreased for all three holo-repressors (see Figure 28). Some of the lowest $^{15}\text{N}\{-^1\text{H}\}$ -nOes were measured for backbone amides preceding and directly following helix D (i.e., turn residues) as well as in helix D (e.g., M66, Q68, E70, L71, G76, and G78). This observed decrease in $^{15}\text{N}\{-^1\text{H}\}$ -nOe correlates well with the slight elevation in $^{15}\text{N}\text{-T}_2$ observed for these residues (Figure 27). Altogether, $^{15}\text{N}\text{-T}_1$, $^{15}\text{N}\text{-T}_2$, and $^{15}\text{N}\{-^1\text{H}\}$ -nOes measured for holo-WT-TrpR, holo-L75F-TrpR, and holo-A77V-TrpR are largely within experimental error of each other, indicating that all three holo-repressors have overall very similar ^{15}N NMR relaxation behaviors, except for a few important residues within or in the vicinity of the repressors' HTH DNA-binding domain.

Model-free Analysis

The ^{15}N NMR relaxation parameters of holo-WT-TrpR, holo-L75F-TrpR, and holo-A77V-TrpR were further analyzed using ModelFree and the model selection approach of Mandel and co-workers (59) to further characterize the internal dynamics of the holo repressors occurring on ps-ns and μs -ms timescales. As indicated by comparable principal components of the diffusion tensors ($D_{\parallel}/D_{\perp}$) of holo-WT-TrpR and the two holo-TrpR mutants, the global overall reorientation of the three TrpR proteins in solution was best modeled as an axially symmetric prolate ellipsoid, as was done in case of the

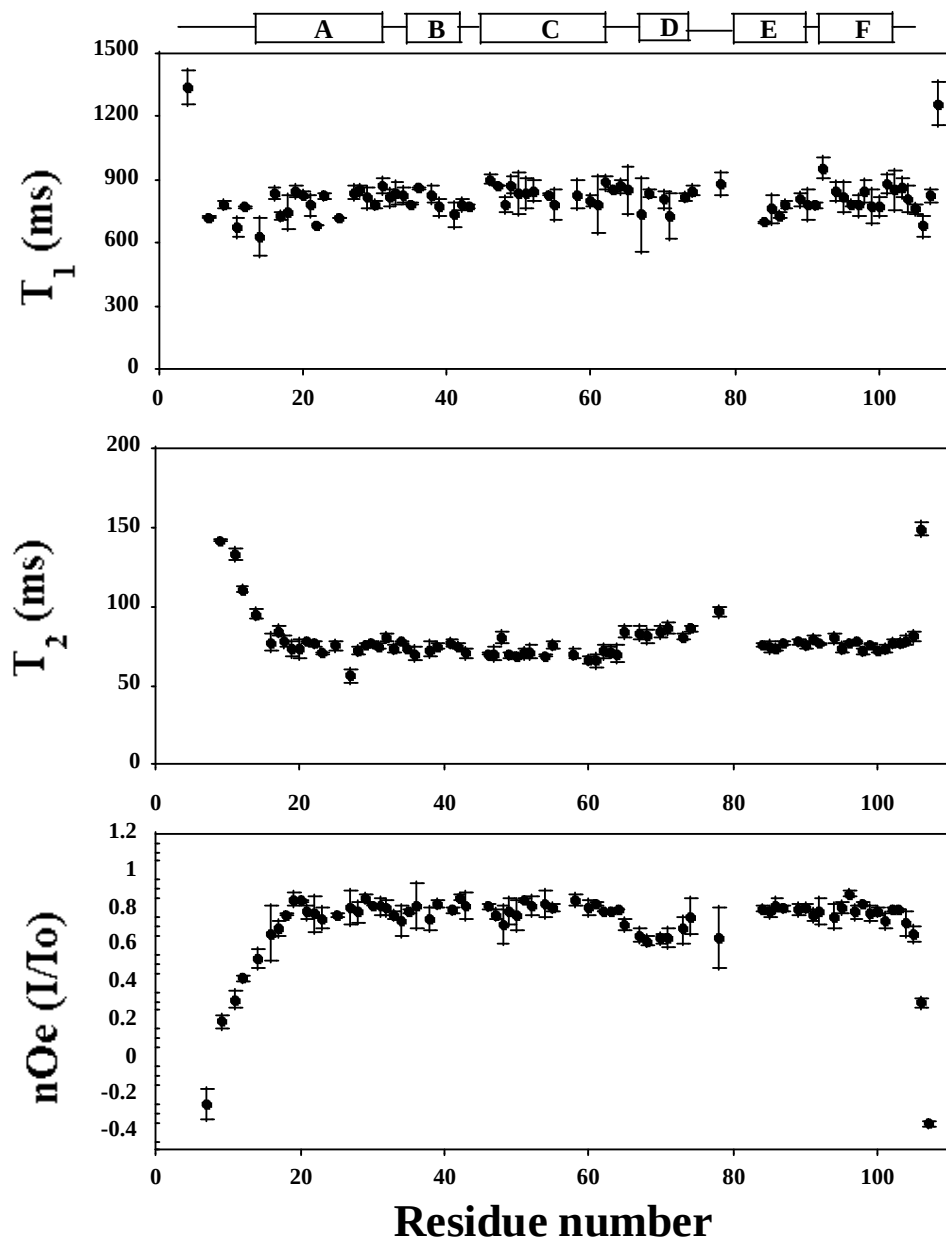


Figure 23. ^{15}N - T_1 , ^{15}N - T_2 And ^{15}N - $\{^1\text{H}\}$ Heteronuclear nOe Profiles Measured For Holo-WT-TrpR Measured At 14.1 T.

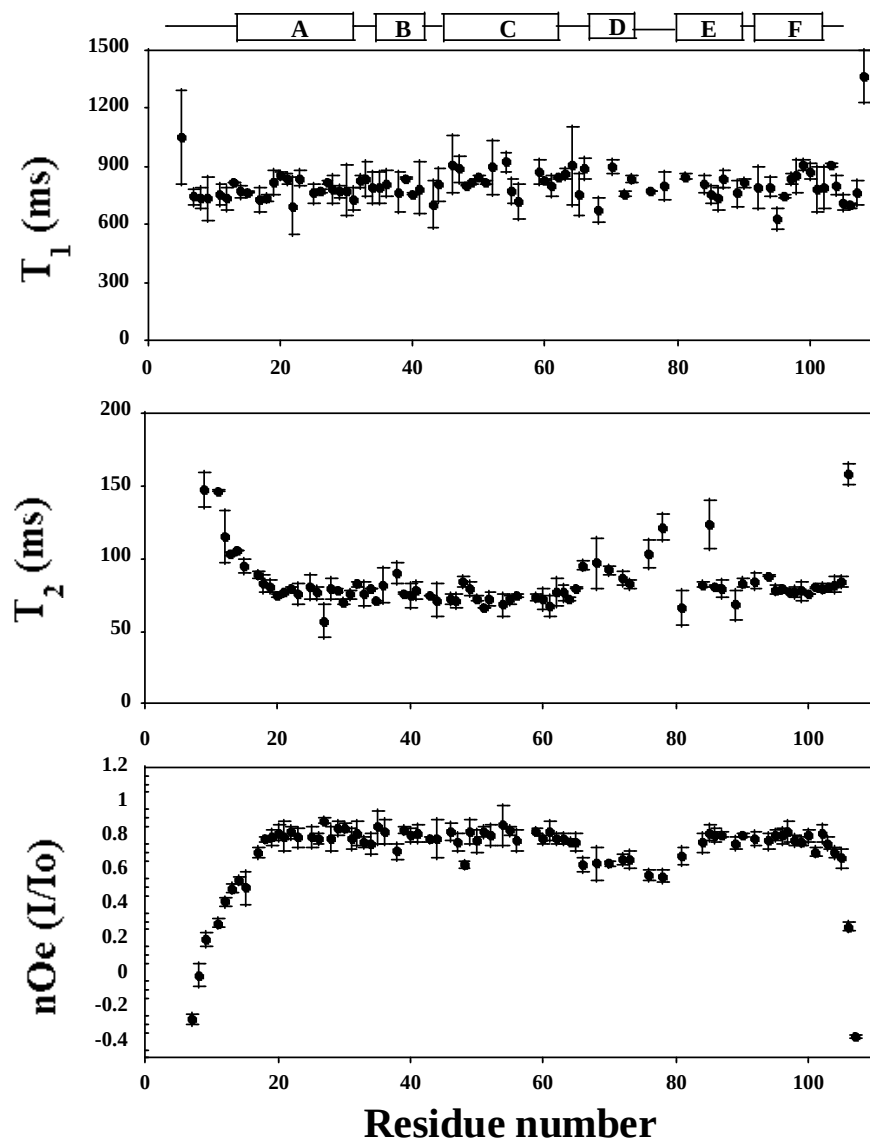


Figure 24. ^{15}N - T_1 , ^{15}N - T_2 And ^{15}N - $\{^1\text{H}\}$ Heteronuclear nOe Profiles Measured For Holo-L75F-TrpR Measured At 14.1 T.

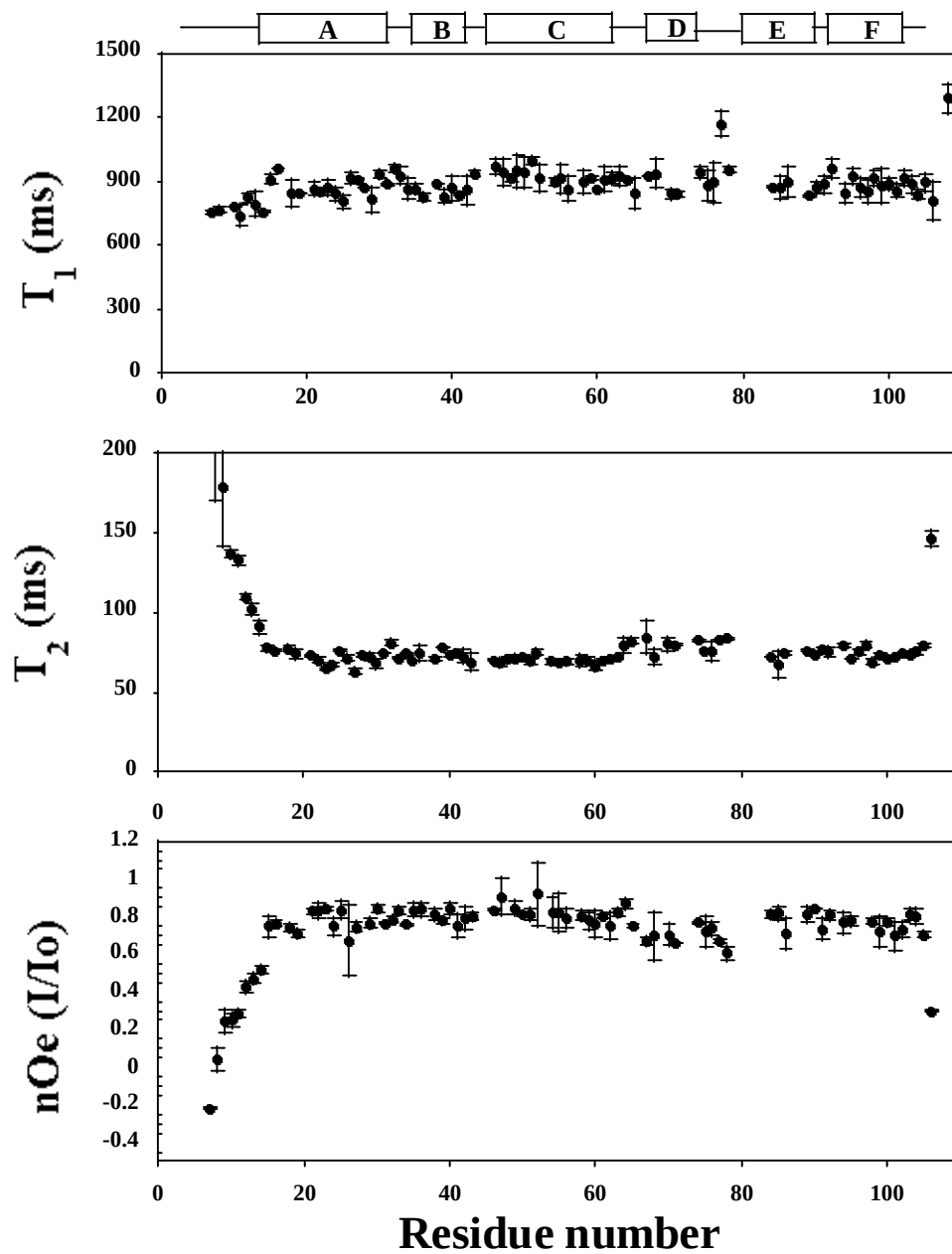


Figure 25. ^{15}N - T_1 , ^{15}N - T_2 And ^{15}N - $\{^1\text{H}\}$ Heteronuclear nOe Profiles Measured For Holo-A77V-TrpR Measured At 14.1 T.

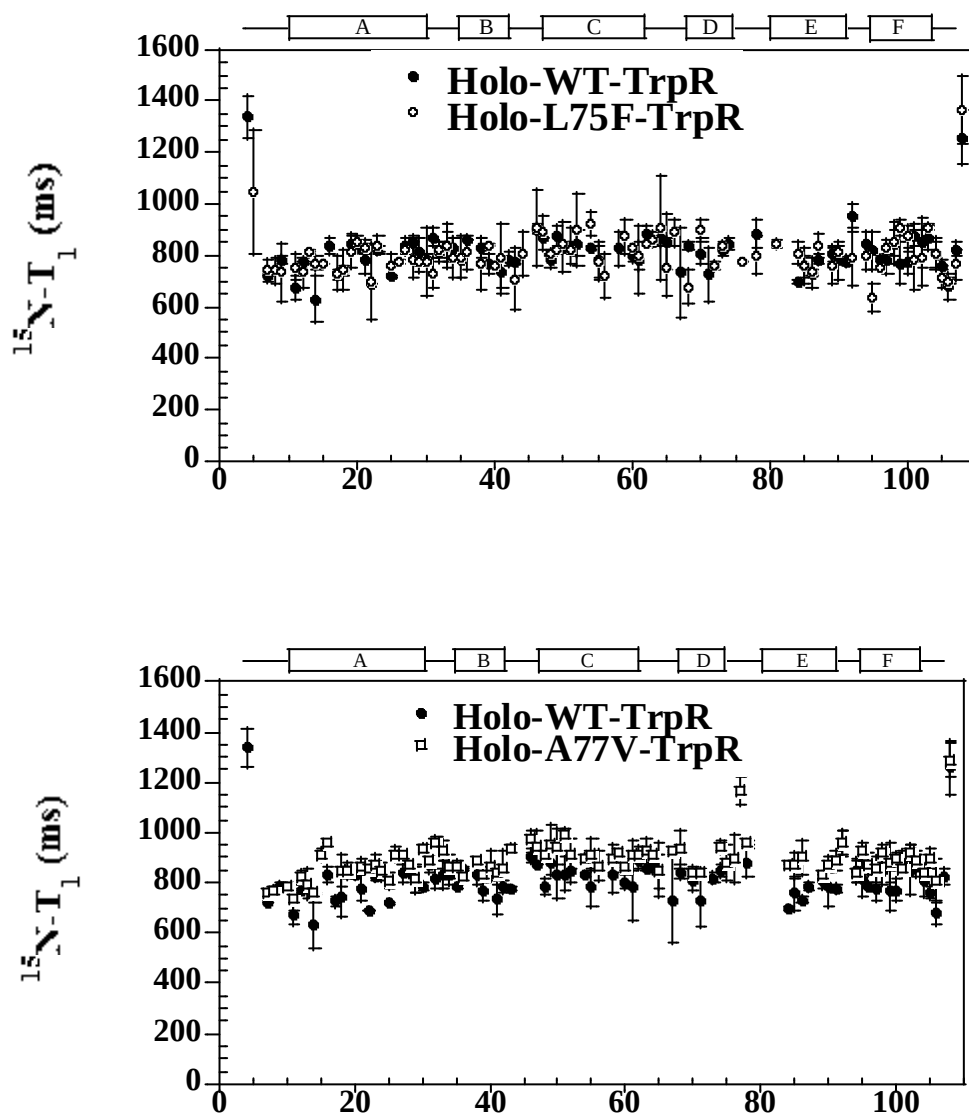


Figure 26. Comparison Between $^{15}\text{N}-T_1$ Profiles Of Holo-WT-TrpR And Holo-L75F-TrpR And Holo-WT-TrpR And Holo-A77V-TrpR. Black circles represent holo-WT-TrpR; open circles represent holo-L75F-TrpR; and open squares represent holo-A77V-TrpR.

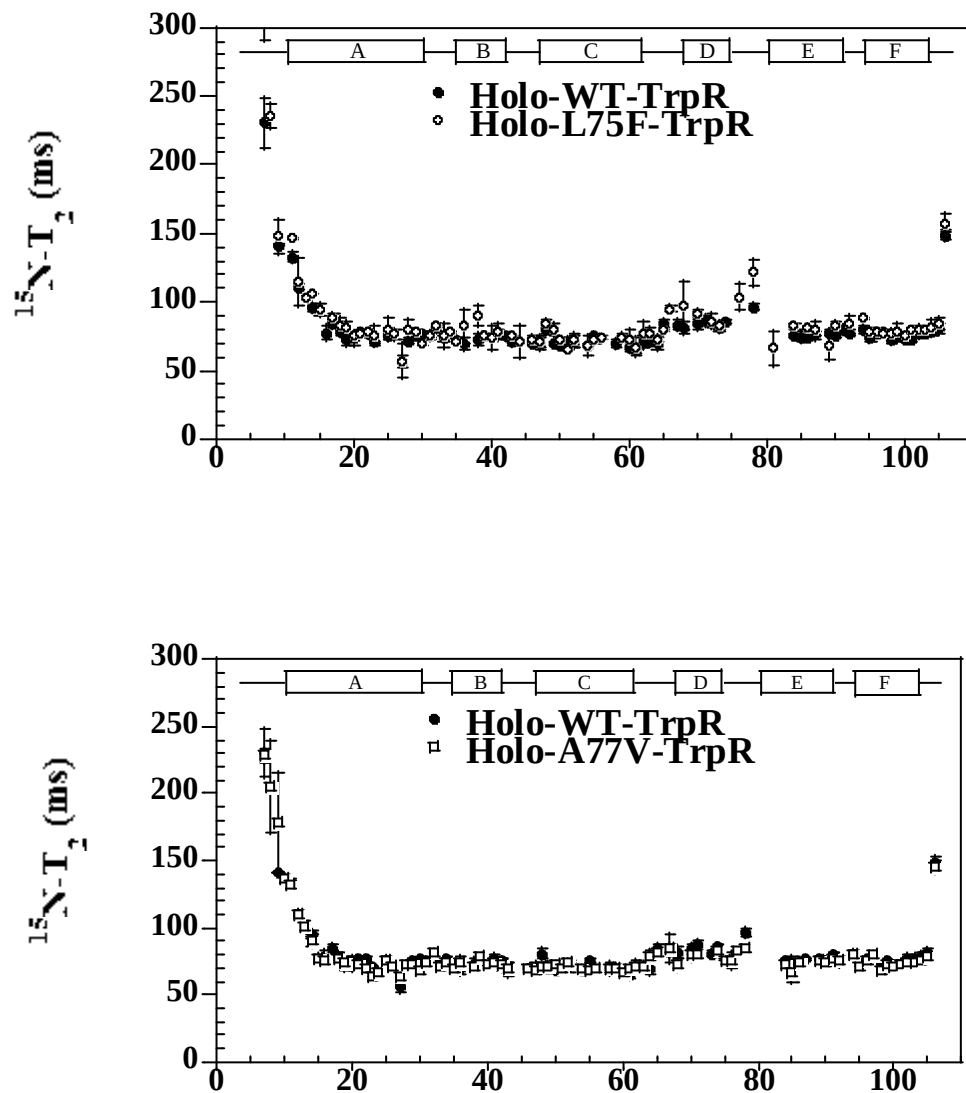


Figure 27. Comparison Between $^{15}\text{N}-T_2$ Profiles Of Holo-WT-TrpR And Holo-L75F-TrpR And Holo-WT-TrpR And Holo-A77V-TrpR. Black circles represent holo-WT-TrpR; open circles represent holo-L75F-TrpR; and open squares represent holo-A77V-TrpR.

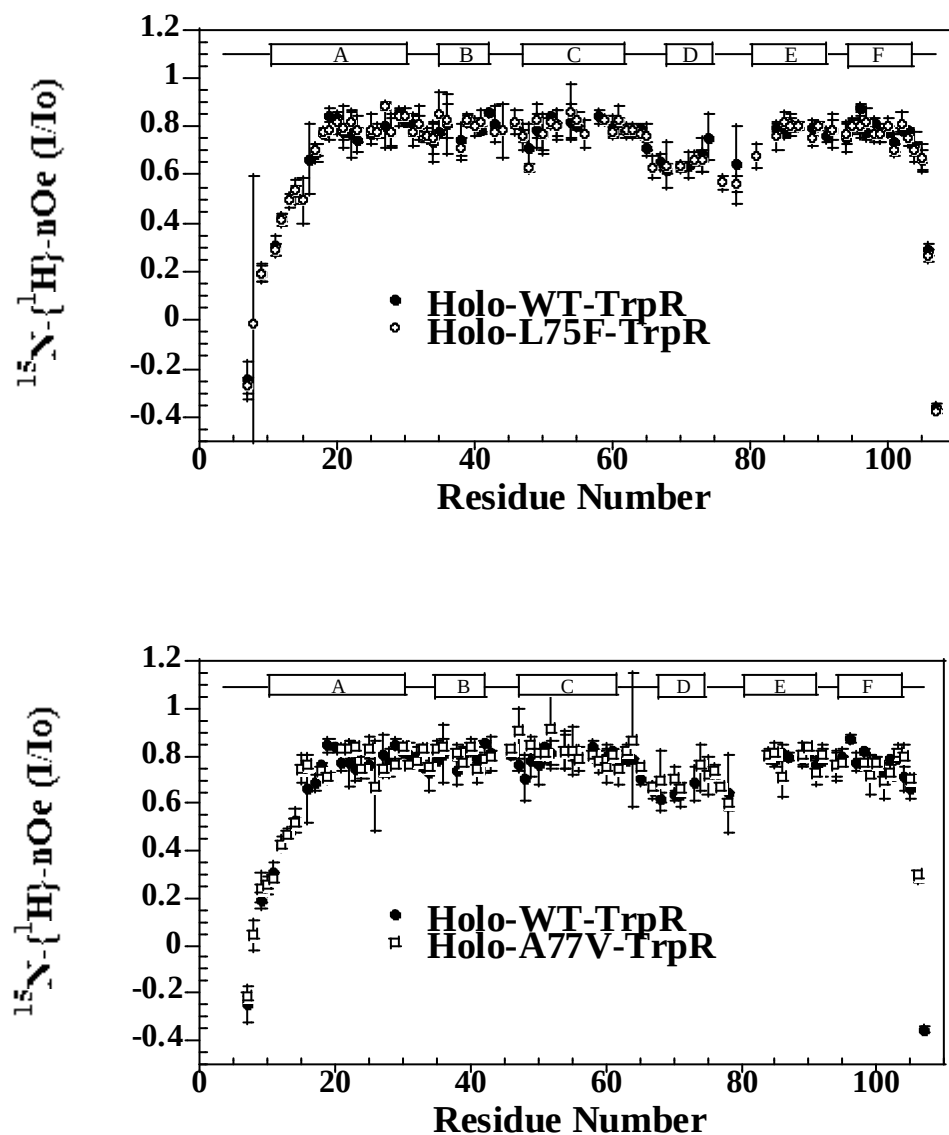


Figure 28. Comparison Between $^{15}\text{N}\{-^1\text{H}\}$ Heteronuclear nOe Profiles Of Holo-WT-TrpR And Holo-L75F-TrpR And Holo-WT-TrpR And Holo-A77V-TrpR. Black circles represent holo-WT-TrpR; open circles represent holo-L75F-TrpR; and open squares represent holo-A77V-TrpR.

apo-TrpR forms. The rotational correlation time (τ_m) for global reorientation of the holo-repressors in solution calculated are reported in Table 2. Both τ_m and $D_{\parallel}/D_{\perp}$ ratios are within experimental error of one another, indicating that all three holo-repressors possess overall similar global shape and tumbling properties in solution. Further, the observed values are in agreement with theoretical prediction of τ_m and $D_{\parallel}/D_{\perp}$ based on molecular weight, high-resolution structural analysis (20), hydrodynamic data and previously reported values (23). The generalized order parameter, S^2 , which reports on the amplitude of backbone N-H bond vector motions on the ps to ns timescale which occurring faster than the tumbling of the molecule ($\tau_m \sim 10$ ns for TrpR) were also calculated using model-free analysis (51) and are reported in Tables 23-25. The amides located on N- and C- terminal ends (residue 4-15 and 104-108) display low S^2 values (<0.5) indicating large amplitude motion. These values are consistent with solution structural studies of apo and holo TrpR which show this region disordered due to a lack of observed NOE constraints (20). The order parameter values for the remainder of the sequence are relatively high. Model selection accompanying the S^2 analysis indicates that the backbone dynamics of residues in the core α -helices of each holo-repressor were best described by a simple model of motion (model 1, S^2 only), indicative of very fast ps-ns internal N-H bond vector fluctuations ($\tau_e \leq 20$ ps) accompanied by small amplitudes of N-H backbone motions taking place. The uniformity of S^2 determined for backbone amides in this region

	Anisotropy $D_{\parallel}/D_{\perp}$	Tumbling Time τ_c (ns)
L75F-TrpR _{apo}	1.21 ± 0.03	10.45 ± 0.05
L75F-TrpR _{holo}	1.24 ± 0.04	10.06 ± 0.04
WT-TrpR _{apo}	1.28 ± 0.08	10.12 ± 0.10
WT-TrpR _{holo}	1.15 ± 0.05	10.30 ± 0.07
A77V-TrpR _{apo}	1.19 ± 0.03	10.30 ± 0.04
A77V-TrpR _{holo}	1.20 ± 0.04	10.50 ± 0.04

Table 2. Diffusion Properties As Measured For TrpR Apo And Holo Samples Using FAST ModelFree.

of the three holo-repressors also clearly indicate that there exists little difference in the equilibrium ps-ns fluctuations of the backbone N-H groups between the three proteins in their holo state. This situation is not much different from the S^2 values that were calculated for backbone amides residing in the core α -helices of the apo-repressors (69).

S^2 calculated from backbone amides located in helix E of the repressors' helix D-turn-helix E DNA binding domain are consistently high and are comparable to the core α -helices in holo-TrpR samples (Figure 33 on page 112). The ^{15}N relaxation parameters for helix E amides in each of the holo-repressors were also largely fit to the simplest model of motions (i.e. model 1), where ModelFree formalism is the most applicable (59). In contrast, S^2 values for backbone amides located in helix D (i.e. residue stretch 68-74) are

lower for holo-WT-TrpR, holo-L75F-TrpR, and holo-A77V-TrpR (Figure 33) indicating that these amides retain some degree of flexibility even when the L-Trp co-repressor is bound. These ^{15}N NMR relaxation data best fit to model 2 (S^2 and τ_e) for most amides and the more complex model 5 in some cases. Model 2 is characterized by S^2 and τ_e , where τ_e represents the correlation time for the fast internal motions occurring on a timescale greater than 20 ps. The fact that most of these residues (in the turn-helix-D-turn region) demonstrated $\tau_e \geq 50$ ps indicate that the entire region may be experiencing a cooperative fast time scale fluctuation. In general, the lowest S^2 values observed in this region were for the backbone amides in the turn region between helix D and helix E. For example G78 ($S^2 = 0.75$) for holo-WT-TrpR, G78 ($S^2 = 0.56$) for holo-L75F-TrpR and V77 ($S^2 = 0.64$) for holo-A77V-TrpR. The low S^2 (indicating larger ps-ns amplitude motions) is understandable considering that glycines provide a high degree of flexibility in a protein polypeptide chain.

Finally, backbone amides whose ^{15}N relaxation data invoked an R_{exch} parameter ($\geq 1.5 \text{ s}^{-1}$), which introduce a contribution from chemical exchange into data fitting (and is often indicative of conformational exchange on the μs to ms timescale, were sporadic and inconsistent between the three holo-repressors, thus limiting our ability to make conclusive observations about slower μs - ms timescale motions. More accurate observations of R_{exch} contributions to the internal dynamics would require collection of ^{15}N NMR relaxation data at a different magnetic field strength than 600 MHz (^1H Larmor frequency) which is beyond the scope of the current studies.

Reduced Spectral Density Analysis

Since there is a risk of over-fitting the relaxation data when using model-free analysis particularly with data collected at only one field strength, the ^{15}N NMR relaxation data was analyzed using reduced spectral density mapping (49, 50, 63). Analysis of ^{15}N - T_1 , ^{15}N - T_2 and ^{15}N - $\{^1\text{H}\}$ -nOe in terms of reduced spectral density functions ($J_{\text{eff}}(0)$, $J(\omega_{\text{N}})$ and $J(0.87\omega_{\text{H}})$) has the advantage that these functions are sensitive to frequencies of motions rather than amplitudes of motions (49). Plots of the three reduced spectral density function values per residue for the three holo proteins are shown in Figures 29 and 30 as comparison with each other and a complete tabulation of the results are available in the Tables 17-19. As foreshadowed by the trends in ^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOes, the reduced spectral density functions $J_{\text{eff}}(0)$, $J(\omega_{\text{N}})$ and $J(0.87\omega_{\text{H}})$ for NH bond vectors of the core α -helices (A, B, C, and F) as well as the E-helix of all three holo-repressors were found to be fairly uniform, indicating that core amides and E-helix have very similar motional properties. The $J(\omega_{\text{N}})$ functions were very similar throughout the sequence of all three proteins (Figures 29 and 30, middle panel). These uniform $J(\omega_{\text{N}})$ patterns are consistent with the uniform trends observed in the ^{15}N - T_1 data, and reflect the same insensitivity to residue position. The fact that ^{15}N - T_1 trends are consistent with $J(\omega_{\text{N}})$ profiles is not too surprising considering that $J(\omega_{\text{N}})$ is a measure of the spectral power of frequencies that contribute significantly to ^{15}N - T_1 relaxation (63). Based on the uniformity of the core values an apparent correlation time (τ_{m}) for overall reorientation of the molecules was calculated using the following equation (63):

$$\tau_{\text{m}} = \omega_{\text{N}}^{-1} [(J_{\text{eff}}(0) - J(\omega_{\text{N}})) / J(\omega_{\text{N}})]^{1/2}$$

yielding τ_m 's of 10.12 ns for holo-L75F-TrpR, 10.38 ns for holo-WT-TrpR and 11.03 ns for holo-A77VTrpR. These values are in good agreement with those calculated using model-free analysis and further support the similarity in structural shape.

In contrast to the core helices, small $J_{\text{eff}}(0)$ and large $J(0.87\omega_H)$ were observed for amides residing in the N- and C-terminal ends of the three proteins. These data are consistent with very rapid ps-ns motions. Differences in $J_{\text{eff}}(0)$ and large $J(0.87\omega_H)$ are also observed for backbone amides residing within the turn-helix D-turn region of the three holo-repressors. Elevated values of $J(0.87\omega_H)$ of ~ 3 ps/rad and corresponding decrease in $J_{\text{eff}}(0)$ of ~ 0.8 ns/rad of these amides compared to the core and helix E amides of the proteins indicate that these amides in the D-helix region are more flexible on a ps-ns timescale. Further, although subtle, one can see that the ps-ns fluctuations of the amides in the D-region of holo-A77V-TrpR are slightly less than holo-WT-TrpR.

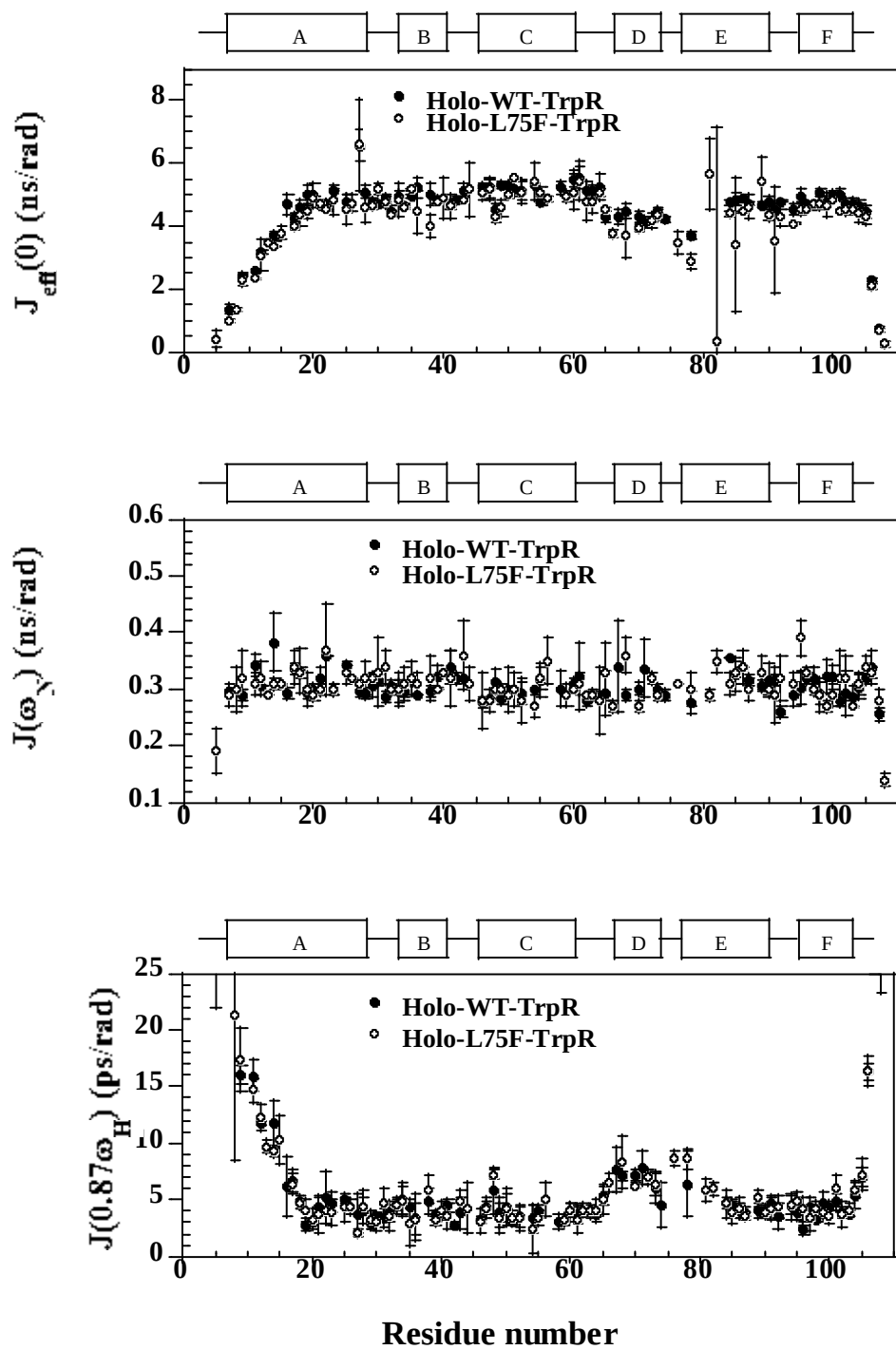


Figure 29. Comparison Of Reduced Spectral Density Functions Calculated For Holo-WT-TrpR (Filled Circles) And Holo-L75F (Open Crosses) Forms Of TrpR.

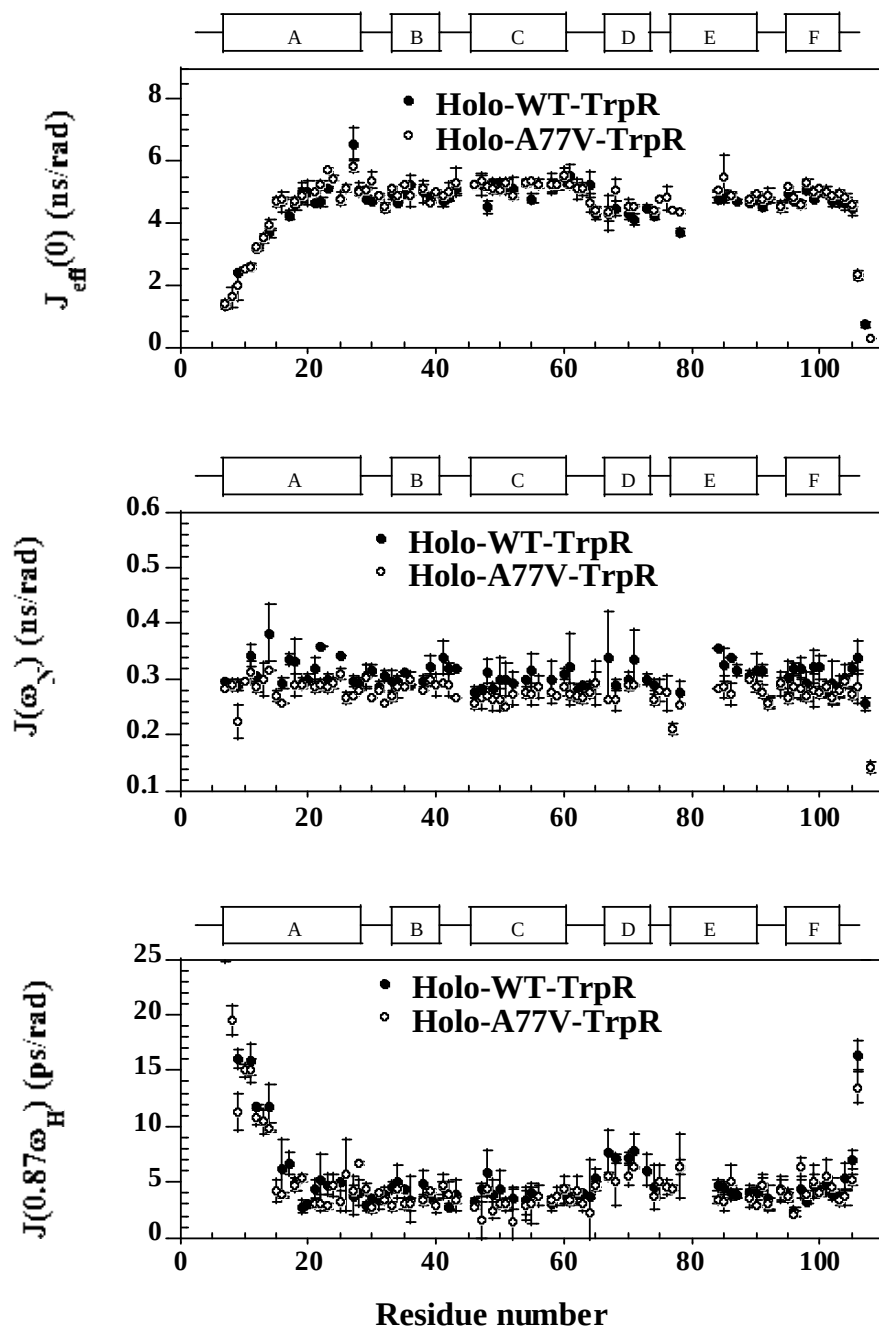


Figure 30. Comparison Of Reduced Spectral Density Functions Calculated For Holo-WT-TrpR (Filled Circles) And Holo-A77V (Open Crosses) Forms Of TrpR.

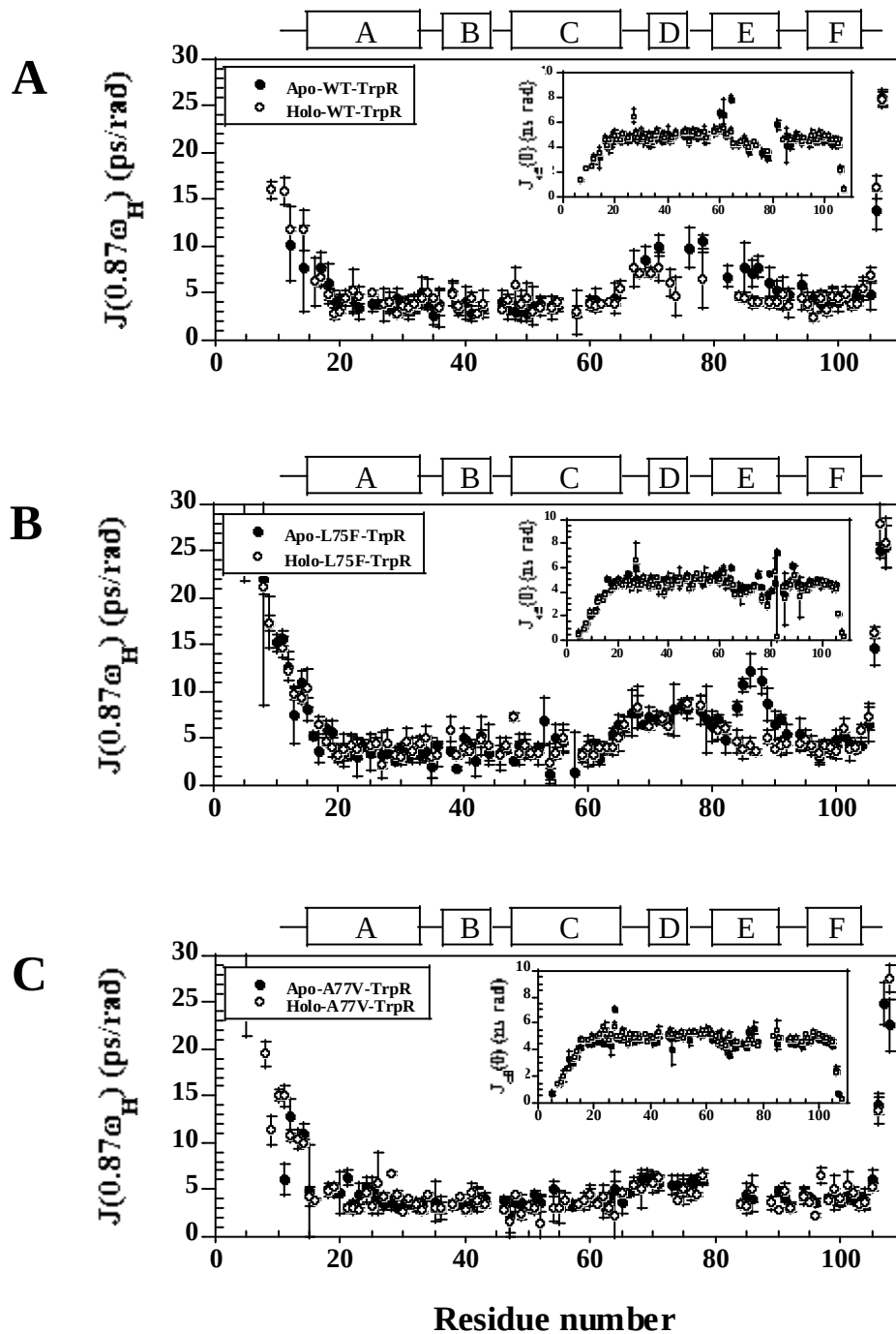


Figure 31. Comparison Of Reduced Spectral Density Functions $J(0.87\omega_H)$ And $J_{\text{eff}}(0)$ (Insets) Calculated For Apo (Filled Circles) And Holo (Open Circles) Forms Of The WT-TrpR (A), L75F-TrpR (B) and A77V-TrpR (C), With Schematics Of Secondary Structure Elements Of Trp Depicted Above The Plots.

Comparison Of Backbone Dynamics With Apo Forms

Comparison with apo-TrpR forms point out that the most notable backbone dynamics changes occurring upon L-Trp co-repressor binding and the effect that the L to F and A to V mutations at residue position 75 and 77, respectively, impart on the dynamics transition from apo- to holo-repressors. Although $J(\omega_N)$ profiles, when compared between the apo and holo forms of all three proteins revealed no significant differences, comparison of the $J(0.87\omega_H)$ profiles highlighted very protein specific differences (Figure 31). For WT-TrpR, the $J(0.87\omega_H)$ profiles between the apo and holo forms of the repressor were very similar for backbone amides located in the core α -helices of the protein, but were noticeably higher for amides located within the helix D-turn-helix E domain of apo-WT-TrpR. This difference was also observed in the S^2 order parameters calculated for these residues. Thus, it is clear that L-Trp co-repressor binding to apo-WT-TrpR induces dynamic changes in the helix D-turn-helix E amides. This “stiffening” of the helix D-turn-helix E DNA binding domain of WT-TrpR as observed in our ^{15}N NMR relaxations studies is consistent with previous data which reported a sequential stabilization of helix E and helix D upon L-Trp and DNA binding to the repressor (20, 25).

In the case of L75F-TrpR, backbone amides located within the core α -helices of the repressor again displayed similar $J(0.87\omega_H)$ profiles between the apo and the L-Trp-bound holo state. Helix D amides in holo-L75F-TrpR also displayed very similar $J(0.87\omega_H)$ values to what had been observed in apo-L75F-TrpR (Figure 31B), indicating that the rather rigid helix D in the apo-repressor (69) is not further stabilized or

motionally restrained by L-Trp binding. Backbone amides in helix E, however, displayed a drastic change in $J(0.87\omega_H)$ profiles between the apo and holo states of the protein. $J(0.87\omega_H)$ values measured for residues 84-89 were significantly lower in apo-L75F-TrpR. Upon formation of the holo-repressor, $J(0.87\omega_H)$ values for these residues decreased significantly converging to a uniform value of ~ 4 ps/rad, indicating that upon formation of holo-L75F-TrpR, ps-ns motions experienced by helix E amides are severely restricted, rendering these amides motionally restrained similar in magnitude to backbone amides of the core α -helices. Interestingly, the S^2 values observed between apo- and holo-forms in the D-E region of L75F-TrpR were similar. However, when one looks at the model selection best fit for helix-E, one can see that in the apo-form several residues were best fit with model 5 and 4, indicating very complicated motions, whereas in the holo-form many of these same residues were now best fit to model 1 or 2. This change in model is consistent with these amides becoming more motionally restrained. Thus, the data for both apo and holo-L75F-TrpR indicate that the Leu to Phe amino acid substitution at residue position 75 imparts greater flexibility in helix E of apo-L75F-TrpR compared to its wild-type counterpart, apo-WT-TrpR, and that upon L-Trp binding the flexibility seen in helix E of apo-L75F-TrpR is significantly reduced.

In contrast the $J(0.87\omega_H)$ and $J_{\text{eff}}(0)$ values measured for backbone amides of apo- and holo-A77V-TrpR were very similar (Figure 31, bottom panel) indicating that the dynamics profiles of the apo- and holo-repressors are very similar, even when considering backbone amides located in the helix D-turn-helix E domain of the A77V-TrpR mutant. This suggests that the apo state of A77V-TrpR already populates a set of

conformations that mimic the predominant conformation of the holo repressor. Further, the S^2 order parameters showed no obvious difference between the two forms (for comparable data points) and in general indicated a rigid core, slightly greater motion in the D-helix-turn region, and a rigid E-helix. Taken together these data suggest that the backbone dynamics profile of apo- and holo-A77V-TrpR are similar, and hence we conclude that the Ala to Val amino acid substitution at residue position 77 causes the resulting TrpR variant to adopt the backbone dynamics profile of the co-repressor bound (holo) form even in the absence of the co-repressor.

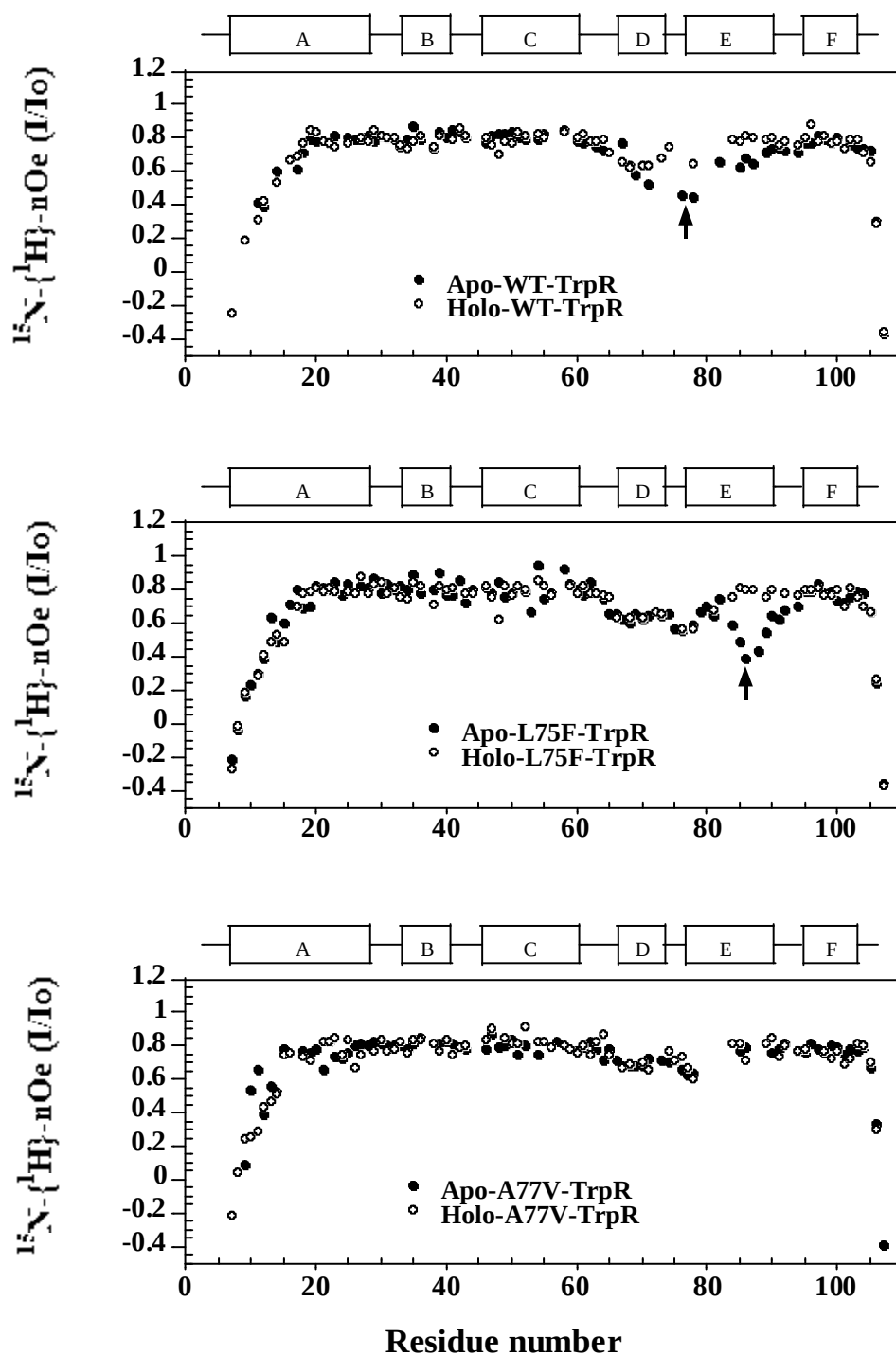


Figure 32. Comparison Plots Of $^{15}\text{N}\{-^1\text{H}\}\text{-nOe}$ Profiles Between Apo (Black Circles) And Holo (Open Circles) Forms Of The Three TrpR Forms.

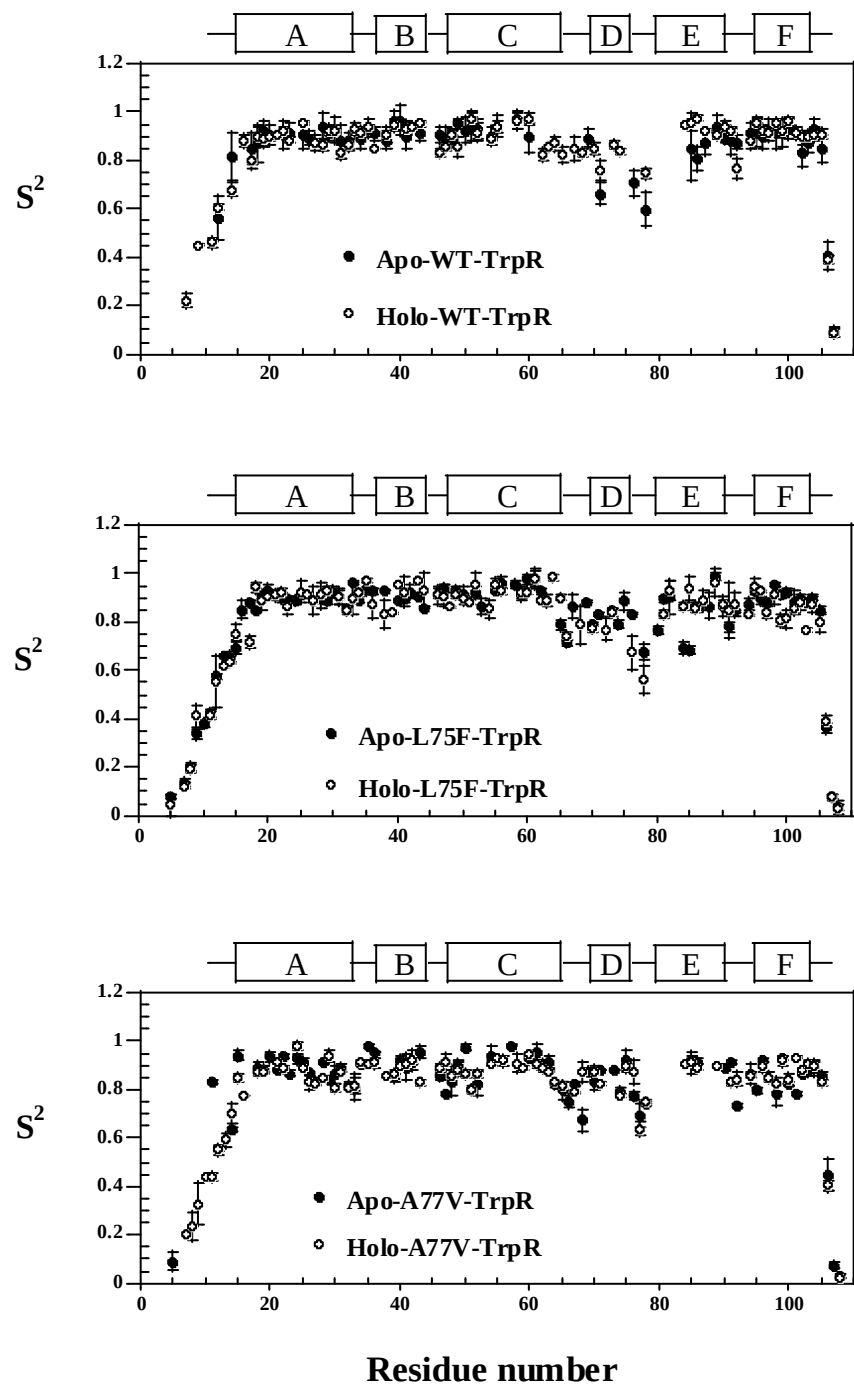


Figure 33. Comparison Plots Of The Calculated Order Parameter (S^2) Values Between Apo (Black Circles) And Holo (Open Circles) Forms Of The Three TrpR Forms.

MATERIALS AND METHODS

Sample Preparation

The ^{15}N NMR relaxation experiments were conducted on uniformly ^{15}N labeled samples of apo L75F-, A77V-, and WT-TrpR, prepared in a manner similar to previously published protocols (66). Briefly, uniformly ^{15}N labeled L75F and WT-trpR were isolated from *E. coli* strains CY15075 and CY15071 transformed with the overproducing plasmids pJPR2.L75F and pJPR2.WT, respectively. The strains were grown in M9 minimal media enriched with $[\text{}^{15}\text{N}]\text{NH}_4\text{Cl}$ (99% ^{15}N enriched, CIL, Cambridge, MA) as sole source of nitrogen.

A plasmid encoding for the A77V-TrpR mutant, pJPR2.AV77, was engineered using site- directed mutagenesis and pJPR2.WT as DNA template. Uniformly ^{15}N - or $^{15}\text{N}/^{13}\text{C}$ -enriched A77V-TrpR was isolated from CY15071 transformed with the overproducing plasmids pJPR2.A77V (35). $^{15}\text{N}/^{13}\text{C}$ apo-A77V-TrpR protein was used to obtain more complete resonance assignments than those currently available from published work (37). $^{15}\text{N}/^{13}\text{C}$ labeled apo-WT-TrpR was also expressed in CY15071 cells to obtain a complete list of $^{15}\text{N}/^1\text{H}$ backbone amide resonance assignments recorded under identical conditions as those used for the structure determination of apo-L75F-TrpR (66).

The CY15071 *E. coli* cell cultures were supplemented with 20 mL of a 0.2 M unlabeled threonine stock solution per liter to compensate for the fact that this cell strain cannot synthesize threonine *de novo*. As a result, TrpR samples produced from CY15071

bacterial cell growths lacked ^{15}N - or ^{15}N - and ^{13}C -labeled threonine residues, and signals from these residues were unobservable in ^{15}N -edited or ^{13}C -edited NMR experiments. CY15071 cells transfected with either pJR2.A77V or pJR2.WR were grown in M9 minimal medium supplemented with ^{15}N -labeled ammonium chloride and $^{13}\text{C}_6$ -labeled glucose as source nitrogen and carbon source, respectively. For ^{15}N NMR relaxation experiments, protein samples were concentrated to ~ 1.2 mM (protein protomer concentration) in an NMR buffer consisting of 500 mM NaCl, 50 mM NaH_2PO_4 , and 95% H_2O / 5% D_2O at pH 5.7.

Protein Purification

Mature cultures are placed on ice and poured into 250 ml centrifuge bottles and centrifuged at $8,500 \times g$ for 15 min. Each pellet is resuspended in 10 ml of 0.1 M Tris.HCl, pH=7.6 and 30 μl of 100mM PMSF. This results in about 90 ml of cell suspension for a 2 L culture. The cell suspension is then broken by one cycle of French press (external pressure 1300 psi). The lysate is then spun at $8,500 \times g$ for 25 min and the supernatant is collected. This solution is placed in a room temperature water bath and a 20% streptomycin sulfate solution is added to 1% with gentle stirring. The water bath is then slowly heated to 62°C on a hot plate and the solution is allowed to stand at 62°C for 5 min. The solution is then removed from the water bath and chilled on ice for 30 min. The thick white precipitate that forms is centrifuged at $8,500 \times g$ for 15 min and the supernatant is collected. The mixture is then placed in an ice bath and ammonium sulfate is added to 45% saturation with gentle stirring. After all the salt has been added the

mixture is allowed to stir at 0°C for 30 min and then centrifuged at 12,500 x g for 15 min. The resulting supernatant is collected and ammonium sulfate is added to 70% saturation, and after equilibration, spun at 12,500 x g for 15 min. The supernatant is discarded and the pellet is resuspended in 25 ml P11 buffer (10mM NaPO₄ , pH 7.6, 0.1mM EDTA, 0.1M NaCl). The protein solution is dialyzed against P11 buffer (4 L) overnight with three changes of buffer.

A 1.5 x 20 cm column of Whatman P-11 cellulose phosphate, equilibrated in P11 buffer, is constructed following the manufacturers protocol. The dialyzed protein solution is loaded onto the column and washed with 20 ml of P11 buffer. The column is developed using a gradient mixer with 120 ml P11 buffer containing a total concentration of 0.15 M NaCl in the mixing chamber, and 120 ml of P11 buffer containing a total concentration of 0.75 M NaCl in the gradient chamber. The column is run with a flow rate of 0.5ml/min collecting fractions every four minutes. Fractions containing protein, as determined by 280 nm absorbance (>0.1) are run on SDS-Page gels containing 15% polyacrylamide to determine purity. Contaminating protein bands are removed by generating a fresh P11 column and repeating the above procedure. Clean fractions containing L75F-TrpR are combined and dialyzed against NMR buffer (50 mM NaHPO₄, pH 5.7, 0.5 M NaCl) and concentrated by passing through Centricon YM12 membrane (Amicon), using a nitrogen pressure cell, to a final volume of 0.7 to 1.0ml.

NMR Spectroscopy

Sequential ^1H , ^{15}N , ^{13}C backbone chemical shift assignments of apo-WT-TrpR and apo-A77V-TrpR were conducted using uniformly ^{13}C , ^{15}N -labeled apo-TrpR proteins dissolved in NMR buffer. Triple resonance (^1H , ^{15}N , ^{13}C) multidimensional NMR experiments were recorded at 318 K (45°C) on a Bruker DRX600 spectrometer equipped with a triple-resonance probe and triple axis pulsed field gradients. Two-dimensional ^1H - ^{15}N -HSQC (70) spectra were acquired with spectral widths of 13 ppm in the ^1H (t_2) and 30 ppm in the ^{15}N (t_1) dimensions, with proton and nitrogen carrier frequencies set to 4.7 ppm and 116 ppm, respectively. The NMR data were collected with 1024 and 128 complex points in t_2 and t_1 , respectively, using a Waltz-16 (71) pulse sequence scheme for ^{15}N decoupling during data acquisition. Sequential ^1H , ^{15}N and ^{13}C backbone chemical shift assignments were extracted from a series of heteronuclear (^1H , ^{15}N , ^{13}C) 3D NMR experiments, including CBCACONH (72), HNCA (73), and HNCACB (74), acquired with spectral width of 12 ppm (in the ^1H , t_3), 67 ppm (in the ^{13}C , t_2), and 30 ppm (in ^{15}N , t_1 dimension). Data were collected with 512, 128, and 32 complex points in t_3 , t_2 , t_1 , respectively. All NMR spectra were processed using NMRPipe (75) and analyzed using the software program SPARKY (76). Similar apodization functions were used in all spectral dimensions, using shifted sine bell functions.

Backbone amide ^{15}N NMR relaxation experiments including ^{15}N - T_1 , ^{15}N - T_2 , and heteronuclear ^{15}N - $\{^1\text{H}\}$ -nOe were also conducted at 45 °C in triplicates for all three apo TrpR proteins using standard NMR pulse sequences (47, 77, 78). In addition to conducting experiments at 45 °C, a triplicate series of ^{15}N - $\{^1\text{H}\}$ -nOe measurements were

recorded on apo-L75F-TrpR at 37°C, the non-permissive temperature of the temperature-sensitive (ts) phenotype of the L75F-TrpR variant, to verify that the $^{15}\text{N}\{-^1\text{H}\}$ -nOe profiles observed at 45 °C (a temperature slightly above the 42 °C permissive temperature of the ts phenotype of L75F-trpR) are comparable to those recorded at 37 °C.

^{15}N - T_1 relaxation profiles were sampled at eight different relaxation delay time points of 40 ms, 96 ms, 200 ms, 400 ms, 600 ms, 1000 ms, and 1200 ms. ^{15}N - T_2 relaxation profiles were sampled at eight different relaxation delay periods of 8 ms, 16 ms, 32 ms, 40 ms, 64 ms, 80 ms, 104 ms, and 152 ms, with the delay between the series of ^{15}N 180 degree pulses applied in the CPMG sequence (79, 80) set to 0.5 ms. For both ^{15}N - T_1 and ^{15}N - T_2 measurements, the data were collected using 512 complex points in the ^1H acquisition time dimension (t_2) and 256 complex data points in the ^{15}N t_1 indirect time evolution dimension, using a WALTZ-16 ^{15}N -decoupling scheme during data acquisition. ^{15}N T_1 and T_2 values were calculated from the series of NMR spectra by nonlinear regression analysis of single exponential decays of resonance intensities. Errors in ^{15}N - T_1 and ^{15}N - T_2 values were estimated by Monte Carlo calculation (81).

$^{15}\text{N}\{-^1\text{H}\}$ -nOes were measured using a water-flip-back 2D nOe pulse sequence and the results corrected for the finite repetition delay according to the method of Grzesiek and Bax (78). Heteronuclear $^{15}\text{N}\{-^1\text{H}\}$ -nOe enhancements were established as the ratio of peak intensities (I/I_0) from NMR data sets acquired with (I) or without (I_0) solvent presaturation (78). $^{15}\text{N}\{-^1\text{H}\}$ -nOe spectra were recorded using 1024 and 256 complex data points in t_2 and t_1 , respectively, using 48 scans per t_1 increment. The $^{15}\text{N}\{-^1\text{H}\}$ -nOe experiments were recorded using a delay of 4.5 sec between scans to minimize

the introduction of systematic errors in measured nOe values that could be produced by incomplete signal recovery and solvent saturation (78). To minimize the impact of magnetic field drift, ^{15}N T_2 and $^{15}\text{N}\{-^1\text{H}\}$ -nOe data were collected in an interleaved manner, while 2D ^{15}N - T_1 datasets were acquired consecutively using a list of shuffled relaxation delay time points.

NMR Spectra Processing

All NMR spectra were processed using the NMRPipe/NMRDraw and SPARKY software (75, 76). Apodization was performed in both dimensions using a Lorentz-to-Gauss window function, which consists of a combination of an inverse exponential and a Gaussian line broadening function. The purpose of applying a Lorentz-to-Gauss lineshape function (rather than sine bell functions) is to replace the exponential decay of the original data with a Gaussian decay so that following Fourier transformation (FT), the NMR signals adopt Gaussian-like lineshapes rather than Lorentzian lineshapes. Application of this particular linebroadening function (specified by the GM command in NMRPipe and g_1 and g_2 input values) is necessary for accurate fitting of NMR signal intensities using the nonlinear least square nlinLS function of NMRPipe (75). Typical values of g_1 (the exponential term) and g_2 (the Gaussian linebroadening term) used for processing of the NMR relaxation data for TrpR consisted of g_1 and g_2 values of 10 Hz and 15Hz, respectively for processing of the t_2 (acquisition) dimension, and of g_1 and g_2 values of 6 Hz and 12 Hz, respectively, for processing of the t_1 (indirect) dimension of the 2D ^1H - ^{15}N NMR relaxation and $^{15}\text{N}\{-^1\text{H}\}$ -nOe experiments.

Data processing using NMR peak heights instead of peak volumes yielded comparable relaxation analysis and ^{15}N - T_1 and ^{15}N - T_2 profiles. The two spectral analysis approaches were used to establish that the final results (i.e. ^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOe enhancement values) were insensitive to whether the “raw data” consisted of integrated intensities or peak heights.

Errors associated with determination of ^{15}N - T_1 , ^{15}N - T_2 and ^{15}N - $\{^1\text{H}\}$ -nOe values represented $\pm$ one standard deviation between triplicate sets of measurements. These errors were consistent with those calculated by Monte Carlo simulations or spectral noise estimates (in the case of ^{15}N - $\{^1\text{H}\}$ -nOe measurements).

NMR Relaxation Analysis

Analyses of ^{15}N NMR relaxation parameters were carried out using FastModelFree (62). This approach was used to analyze ^{15}N NMR relaxation parameters in terms of internal motions of amide NH bond vectors in the presence anisotropic overall reorientation of the TrpR proteins as follows, and consisted of the following steps: (1) Acquisition and utilization of the molecular coordinates of the protein 3D structure (i.e. pdb coordinate files as determined by NMR); (2) Tabulation of ^{15}N - T_1 , ^{15}N - T_2 , and ^{15}N - $\{^1\text{H}\}$ -nOes as a function of backbone amide (NH bond vector) residue number; (3) Fitting of the principal components of the diffusion tensor of the molecule based on T_1/T_2 ratios and the protein molecular coordinates; (4) Determination of internal motional parameters including: Generalized order parameters S^2 , overall correlation time τ_m for global reorientation of the molecule, internal correlation times τ_e for N-H bond vector motions, and chemical exchange contributions (R_{ex}) to the relaxation based on Lipari-

Szabo formalism as implemented in FastModelFree and as described in Mandel et al. (53, 54, 59).

The NMR relaxation analysis consisted of first estimating the correlation time for overall molecular reorientation from the mean of 10% trimmed T_1/T_2 values and determining the global tumbling parameters for the proteins from best fit of these filtered T_1/T_2 ratios (59). All six L75F, A77V, and WT Trp repressors yielded ^{15}N - T_1/T_2 relaxation patterns consistent with an axially symmetric prolate model of diffusion, in agreement with previously reported values for WT-TrpR (23).

RELATING DYNAMICS DATA TO TRPR BIOLOGY

Sequence-specific protein-DNA interactions are responsible for the regulation of key biological functions in the cell. All of these regulatory pathways are built on the foundation that proteins are able to bind selectively to particular DNA site in the genome. TrpR binds to operator DNA in order to regulate the biosynthesis of L-Trp in *E. coli*. Besides, TrpR controls the expression of four other operons by binding specifically to them. Site-directed mutagenesis studies have identified two TrpR variants with mutations L75F and A77V which show deviation in L-Trp and DNA binding affinities from wild type TrpR. Highly meticulous studies involving biophysical and structural analysis have attempted to characterize the origin of these differences but could only achieve limited success. This dissertation presents the characterization and comparison of TrpR and two of its functionally opposite variants. The variants are particularly interesting because their mutation sites are located only one residue distance apart and they are both a result of single-site conservative amino acid mutation located on the same solvent exposed loop in TrpR.

Moreover, the TrpR system is particularly attractive to develop an understanding of such a sophisticated dynamic problem because so few components are used to mediate regulation in case of TrpR. and dynamics plays an equally important role as that of structure in modulating its DNA-binding properties and biological function (19, 20, 22-25). Despite a wealth of information about the biochemical and biophysical properties of TrpR and of functionally altered TrpR variants, our understanding of the mechanisms by which internal flexibility modulates TrpR function is incomplete. The characterization

and comparison of intrinsic disorder in the backbone of six different forms of TrpR- apo and holo forms- is our first step in exploring the hierarchy of ligand interactions and understand which features of the interaction impart variations in the phenotype of these three TrpR forms.

The functional site of TrpR includes a HTH motif and the turn sequence between helices H2 and H3 of this motif is well known to not tolerate any insertions or deletions as it has emerged to form more sophisticated motifs over evolution. Typically, the HTH region forms short α -helices when bound to DNA but appears to be disordered and exists as solvent exposed loops in other states. In TrpR, these helices go through a sequential ordering by first binding L-Trp corepressor and folding helix E and then interacting with its operator DNA to fold helix D. Given the significance associated with their location and the stepwise ordering during the binding events, it is easy to suspect that even slight changes in the sequence can disrupt the dynamics of this protein system. However, the fact that perturbations (subtle but significant) are observed in the chemical environments of the regions considerably far from the mutation site suggests that the nature of change that could bring about such functional tweaks is more dynamical than structural. This dissertation presents an exhaustive analysis of the microdynamic properties of TrpR with an aim to characterize the changes in dynamics that TrpR observes due to these mutations in both apo and holo forms of the TrpR.

Identification of Flexible Regions

Thorough analysis of the spectral density functions $J_{\text{eff}}(0)$ and $J(0.87\omega_H)$ reveals the presence of distinct dynamic subtleties specific to each apo-repressor. $J_{\text{eff}}(0)$, which reports variations in slower timescale motions, including overall molecular tumbling and conformational switches, identifies regions near the C-terminal region of helix C of apo-WT-TrpR to be undergoing slower μs -ms conformational exchange which appears to be absent in the TrpR mutants. In addition, low $J_{\text{eff}}(0)$ and high $J(0.87\omega_H)$ values for backbone amides in the HTH DNA binding domain of the apo-repressors confirm that helices D and E are highly flexible on fast ps-ns timescales with “flexibility hubs” located at different sites within the helix D-turn-helix E region of each apo-TrpR protein. Altogether, these results are consistent with previous observations of additional ^1H - ^1H nOe connectivities for apo-L75F-TrpR compared to apo-WT-TrpR which suggested that helix D in the L75F-TrpR protein was more ordered (and in all likelihood less flexible) than its counterpart in wild type TrpR (66).

Notably huge differences in ps-ns dynamics were also observed between the three apo-repressors for NH bond vectors located within helix E. Helix E amides were found to be motionally more restricted in apo-WT-TrpR than corresponding helix E amides of apo-L75F-TrpR. The ^{15}N NMR relaxation parameters and spectral density data analysis indicated that helix E amides of apo-L75F-TrpR have a significant increase in ps-ns motional flexibility compared to the corresponding amides of apo-WT-TrpR. ^{15}N - $\{^1\text{H}\}$ -nOe data collected at 37°C (non permissive temperature of the apo-L75F-TrpR ts mutant) displayed identical trends to those observed at 45°C (a temperature 3°C higher than the

42°C permissive temperature of the apo-L75F-TrpR ts mutant), enabling us to conclude that the flexibility of helix E of apo-L75F-TrpR is not altered by a switch from the permissive to the non-permissive temperature. Similarly, no changes in backbone dynamics of helix D amides were observed at the permissive temperature (37°C) compared to their dynamic profile observed at 45°C. These observations lead us to conclude that the ts phenotype of apo-L75F-TrpR is not manifested by a change in ps-ns backbone amide dynamics of the protein's DNA-binding domain at the two different temperatures.

The integral interdependence of structure, dynamics, and function is well established for TrpR, especially with respect to the importance of disorder-to-order transitions observed for structural elements within the helix D-turn-helix E domain upon L-Trp and DNA binding (24, 29). The lower $^{15}\text{N}\{-^1\text{H}\}$ -nOes and higher $J(0.87\omega_{\text{H}})$ values observed for backbone amides of helix D of apo-WT-TrpR support the notion that sequential ordering of helices E and D upon L-Trp and DNA binding is essential for proper TrpR function (25).

The Requirement Of A Flexible Helix E

The adaptability of helix E for DNA binding is related to the ability (and indeed the requirement, dictated by the DNA sequence) of TrpR to bind as a single dimer at the TrpR operator, but as tandem dimers at the other operators of the regulon. In the absence of L-Trp, TrpR cannot discriminate between operator and non-operator DNAs (binding affinities are equal). However, in the presence of L-Trp, operator DNA is bound ~200-fold more strongly but non-operator DNA is bound no more strongly than in absence of

L- Trp (82). Thus, it is clear that L-Trp enhances both the affinity and specificity of TrpR/DNA interactions. Both apo-L75F-TrpR and apo-A77V-TrpR display altered L-Trp binding properties that originate from dynamics differences between the TrpR mutants and WT-TrpR. Our studies suggest that modified dynamics should have a major impact on the DNA binding and binding specificity properties of the apo-L75F-TrpR and apo-A77V-TrpR.

It is important to re-emphasize here that helix E holds special significance to TrpR, as it serves as the recognition helix which “identifies” operator DNA sites and binds with the major groove of the DNA double helix in a “head-on” fashion. In addition, out of the five residues that hold the L-Trp molecule in its place in the L-Trp binding site of each protomer of the TrpR dimer (R54, T81, R84, L41’ and T44’), two (T81 and R84) are located on the N-terminus of helix E. From our comparison of $^{15}\text{N}\{-^1\text{H}\}$ -nOe data recorded at 45°C vs. 37°C, we infer that the ps-ns dynamics profile of apo-L75F-TrpR stays consistent from 45 °C to 42°C (permissive temperature). With this in mind, we propose that the disorder-to-order energy barrier must be too high in the ts TrpR mutant to be overcome by L-Trp binding at permissive temperatures. Failure of apo-L75F-TrpR to generate a well-ordered helix E that may form appropriate contacts in major groove provides a rationale for the L75F-TrpR mutant’s phenotype.

Helix D Is “Intrinsically” Dynamic

Comparison between the apo TrpR proteins suggests that the amides of helix D are the most flexible in apo-WT-TrpR, where as the mutations at position 75 and 77 result in restricted flexibility for apoL75F and apoA77V TrpRs. The apparent increase in

α -helicity observed in apo-L75F-TrpR can be rationalized by the formation of a more ordered, less flexible, helix D resulting from the leucine to phenylalanine amino acid substitution at residue position 75. Formation of a pre-ordered helix D in apo-L75F-TrpR

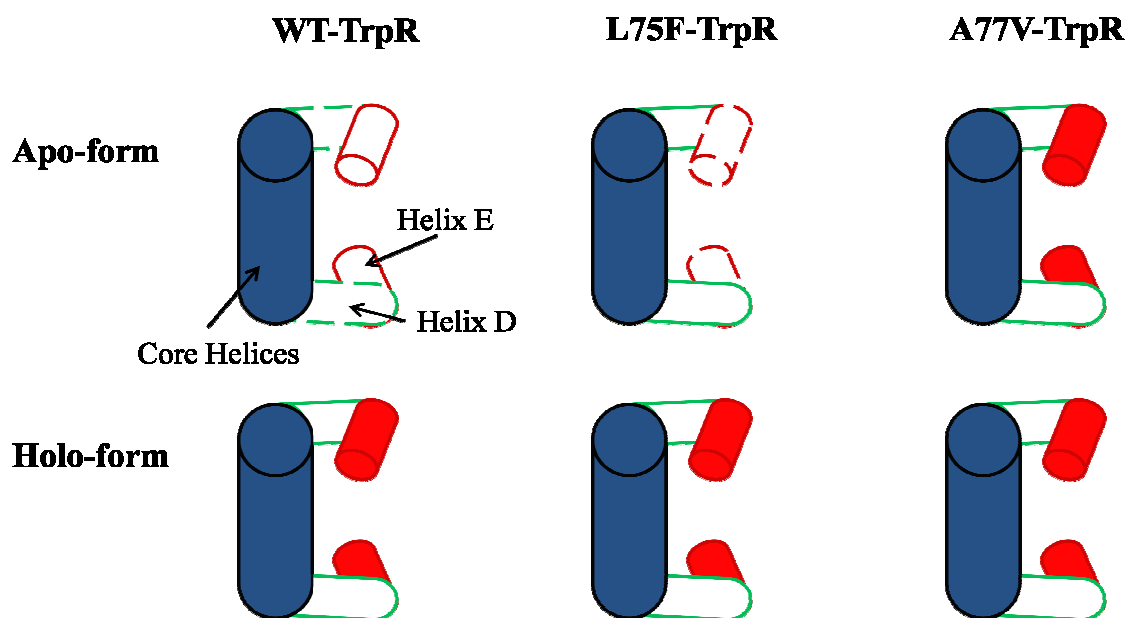


Figure 34. Schematic Of Flexibility Changes Observed By TrpR:

The TrpR core is shown as one big cylinder and the reading heads from each protomer are shown as protruding from the core in colors. Helix D is outlined as open green cylinders and helix E is outlined as short red cylinder (open and filled). Filled cylinders represent helices with restricted flexibility as that of the core region and broken lines represent helices observing high levels of flexibility on the picoseconds to nanosecond timescales. Top row indicates changes in the helix-turn-helix region that take place as a result of the (functionally-opposite) mutations and bottom row displays flexibility states of their Trp-bound holo-forms.

possibly adds to the energy barrier precluding the formation of a well-ordered helix E in L75F-TrpR upon L-Trp binding. In other words, the decrease in flexibility and preordering of helix D (even though this helix remains considerably more dynamic than the core helices) provides a small additional energy barrier to the sequential ordering of

helix E upon L-Trp co-repressor binding in apo-L75F-TrpR. Contrary to this and in the case of apo-WT-TrpR, helix E is the first to form into a well-defined α -helix upon formation of holo-WT-TrpR (21-24), which is later followed by formation of a well ordered helix D upon complex formation of holo-WT-TrpR with cognate DNA (25). Based on our results from studies reported here, we can therefore postulate that in apo-L75F-TrpR, the pre-ordering of helix D (as inferred from the dynamics studies here and the observation of additional interproton nOes in its structure determination (66) may interfere with the ordering of helix E upon holo-L75F-TrpR formation. The decreased flexibility of helix D amides could be a contributing factor to the 10x weaker L-Trp binding affinity of apo-L75F-TrpR compared to that of apo-WT-TrpR (30).

How Conserved Are Residues Leu75 And Ala77?

The functional variation at mutation sites L75 and A77 leads us to question how conserved these two residues are in other gram-negative bacteria, in addition to *E. coli*. BLAST searches conducted on the wild-type protein sequence and the two mutated sequences showed surprising results. Although L75F mutation does not exist in any sequence of TrpR from other organisms, and leucine 75 is highly conserved at this position of the HTH motif, the mutation A77V can be found in the native sequence of TrpR from several other gram-negative enterobacteria including *Citrobacter*, *Klebsiella*, *Shigella*, *Providencia* and *Xenophabdii*. The reason behind the super-repressor mutation in these species is unclear in the absence of structural elucidation and functional assays, but can be presumed to be a result of evolutionary selection from *E. coli* to other gram-negative bacteria, some of which include symbionts of higher order organisms like

Plautia stali and nematodes which might have evolved to have a more complex system of regulation of TrpR present in their cells.

Concluding Remarks

Consistent with the work of Jardetzky and co-workers (22), backbone amides located within the helix D-turn-helix E domain of apo-A77V-TrpR have been observed to be more restricted in terms of ps-ns motions than their counterparts in apo-WT-TrpR. Helix E amides of apo-A77V-TrpR, are also less flexible than the corresponding amides of apo-L75F-TrpR. The overall decrease in flexibility of the helix D-turn-helix E domain of apo-A77V-TrpR (i.e. decrease of internal motions on a wide range of timescales as observed in this work and that of Jardetzky and co-workers (21, 39) suggests that the ability of apo-A77V-TrpR to repress gene expression at low L-Trp concentration is made possible by pre-ordering of its DNA-binding domain. The reduced dynamics of the apo-A77V-TrpR thus mimics more closely molecular characteristics of the holo-WT-TrpR. Additional evidence for this comes from a close comparison of HSQCs of the two proteins with A77V in its apo form and WT in its holo form which shows that 98% of their NH resonance peaks in identical positions. Previous studies have also shown that the ability of apo-A77V-TrpR to discriminate between operator and non-operator DNA is impaired when compared to WT-TrpR (83). Our studies suggest that the reduced flexibility of both helix D and helix E amides of apo-A77V-TrpR is at the source of this impaired molecular recognition function.

A comparison of the tumbling parameters obtained for all six samples (apo and holo) indicates that the TrpR variants do not differ globally from WT-TrpR and possess

very similar structural folds and diffusion properties as the wild type protein. No striking changes were observed in the global diffusion properties on formation of holo samples from apo either, i.e. the reorientation time and the anisotropy in diffusion values were, within the error bars, same between the apo and holo forms. Analysis of the ^{15}N NMR relaxation data of the holo-repressors indicates that the backbone amide dynamics profiles of the three repressors in holo forms are very similar. Thus, the individual L75F and A77V amino acid substitutions do not impact the holo-repressor states as much as that of the apo-repressors. Backbone amides located within α -helices A–C and F that form the core of the TrpR dimer are characterized by limited picosecond to nanosecond N–H bond vector motions and high S^2 values indicating that picosecond to nanosecond internal motions of core amides are restricted significantly. Backbone amides located in helix E of the HTH DNA-binding domain of the holo-repressors exhibit very similar restrictions to picosecond to nanosecond motions and high S^2 values compared to those calculated for backbone amides in the core α -helices, demonstrating that the internal flexibility of helix E amides is as limited as that of core amides. Interestingly, helix D maintains some flexibility even in its L-Trp bound state in both WT and variant TrpR forms suggesting the requirement of a short flexible scaffold helix to orient helix E into the major groove of operator DNA and re-emphasizing the “intrinsically dynamic” nature of the flexible D helix in TrpR.

Our studies corroborate that stabilization of helix E is a necessity for TrpR to be able to bind DNA. These results also confirm that while TrpR forms a well-ordered structure in solution, it is highly dynamic. Based on these results we propose a model in

which apo-WT-TrpR samples a large distribution of ensemble populations, as a requirement for correct TrpR function and the two mutations very subtly affecting the energetics of the protein to cause a redistribution of ensemble populations. In case of Leu to Phe mutation at residue position 75, the distribution of populations is very slightly altered with no obvious modulation of phenotype until the temperature is raised. However, the redistribution is noticed to be significant enough to cause long range perturbations. On the other hand, the Ala to Val mutation at 77 narrows the population distribution of ensembles to mimic that of the L-Trp bound WT form. This change attenuates the requirement of L-Trp binding before DNA binding but in turn makes the apo-TrpR lose its adaptability to recognize multiple operator DNA sequences.

The change in dynamics brought about by single site mutations in the surface exposed loop described herein highlight the exquisite sensitivity of protein properties to changing only one side chain in the sequence. The present study also reinforces the importance of the intrinsic dynamic nature of helix D in TrpR WT and variants. We therefore conclude that TrpR can achieve proper function only if it is comprised of an optimal amount of flexibility (encompassing motions on a wide range of timescales) and minor dynamics perturbations especially in the helix D-turn-helix E DNA binding domain of the repressor can prove detrimental to the protein's function.

Future Work And Proposed Experiments

We have obtained the backbone dynamics profiles of TrpR and its functionally different mutants and have thoroughly analyzed and compared them to understand the

origins of their unusual binding properties. Now, we need to move our focus to the dynamics of side chains in order to get complete site-by-site mapping of internal motion details in the three different protein (mutant) forms. TrpR fulfils all structural requirements to display co-operative L-Trp binding. There is a possibility that upon binding of L-trp to one monomer subunit, the signal does get transferred to the other monomer. But instead of staying in the main chain dynamics, the signal dissipates out through the side chains of its residues. We should be able to characterize this type of dynamical change by characterizing side-chain of TrpR residues. The main chain dynamics reported here have successfully identified dynamic changes from the mutant forms and clearly reinforce that the direction of change in side chain dynamics can provide more information which should educate us about the difference in their functions thoroughly.

There are evidences that ligand-binding events may not necessarily be correlated to motional changes in backbone atoms. Keeping this issue in mind, characterization of TrpR and its mutants for their side-chain dynamics using NMR relaxation methods that report on the internal motions of sidechain deuterons or methyl group dynamics should be performed to reveal better insights. Methyl groups are particularly useful to characterize the dynamic properties of proteins because they occur in high-frequency, are often widely distributed throughout a protein's structural scaffold, and are often the best dynamics probes of motions taking place in the hydrophobic core of a protein. Amplitudes of methyl group motions are described by the order parameter corresponding

to the symmetry axis of the methyl group S^2_{axis} which provides a measure of the degree of reorientation of the -C-CH_3 carbon bond.(53, 84, 85)

Experiments To Study Side-chain Dynamics

We will utilize ^2H - and ^{13}C -based NMR relaxation approaches which require uniformly ^{13}C -labeled and random fractional deuteration at ~50%, to characterize the dynamic properties of methyl and methylene sidechains of L75F, WT, and A77V-TrpR. The NMR approach will involve examining the relaxation profiles of multi-spin coherences involving ^2H , ^{13}C , ^1H followed by obtaining pure ^2H - T_1 and T_2 relaxation rate constants. For methyl groups and following the generation of three-spin coherence ($I_z C_z D_z$ or $I_z C_z D_y$), relaxation time decays for individual methyl groups are extracted by monitoring the decrease in resonance intensities in 2D ^{13}C - ^1H correlation spectra recorded at increasing values of the variable relaxation delay (86). A third relaxation experiment is also recorded to measure the relaxation rate for the two-spin coherence $I_z C_z$. The latter is then subtracted from the relaxation rate for the three-spin coherence to yield pure ^2H longitudinal and transverse relaxation rates for the lone deuteron of the CH_2D methyl moiety (86).

Relaxation of sidechain deuterons is dominated by quadrupolar interactions denoted by $(e^2 q Q / \hbar)$ and best described by the following functional forms of the spectral density function $J(\omega)$ (87):

$$1/T_1(^2\text{H}) = 3/16 (e^2 q Q / \hbar)^2 [J(\omega(^2\text{H})) + 4J(2\omega(^2\text{H}))]$$

$$1/T_{1\rho}(^2\text{H}) = 1/32 (e^2 q Q / \hbar)^2 [J(0) + 15J(\omega(^2\text{H})) + 6J(2\omega(^2\text{H}))]$$

From these relationships, ^2H relaxation time constants are analyzed in terms of dynamical parameters using the model-free lipari-szabo formalism and yield the order parameters S^2 and internal correlation times τ_e (53). Full analysis of ^2H methyl relaxation data should be conducted according to published protocols (84, 86).

BACKBONE DYNAMICS OF E73, A HYPERTHERMOPHILIC PROTEIN

Introduction

One of the best represented and studied crenarchaeal viruses is the *Fuselloviridae* viral family. Fuselloviridae viruses have been isolated from acidic ($\text{pH} < 4$) hot (Temperature $> 70\text{ }^{\circ}\text{C}$) springs throughout the world. Their genetic and morphological diversity is reflected in their gene products, as only a small fraction of the open reading frames (ORFs) encoded by these viral genomes display significant sequence similarity to functionally well-characterized proteins. Best representatives of the *Fuselloviridae* include *Sulfolobus* spindle shaped virus-1 (SSV-1) and *Sulfolobus* spindle shaped virus-Ragged Hills (SSV-RH). The genome of SSV-RH codes for 37 ORFs that are closely similar to those encoded in SSV-1 genome. The names of the ORF in the genomic sequence and corresponding protein in these viruses are derived from one of 6 reading frames (A-F), and number of encoded amino acids. Despite extensive biological studies on *Sulfolobus* and SSV crenarchaeal viruses, little is known about the structures, dynamics and functions of these proteins contained within the open reading frames (ORFS) of the SSV genomes.

Toward this goal, we have undertaken study of 3D solution structure and backbone amide dynamics of E73, a 73-residue homodimeric protein encoded within SSV-RH's genome and demonstrate its dsDNA binding capabilities (this part of analysis mainly done by Casey Schlenker and Dr. Brian Tripet in our group). The overall three-dimensional fold of E73 consists of a tightly intertwined dimer with the N-terminal β -

strand of each E73 subunit interacting together to form a short antiparallel β -sheet. This antiparallel β -sheet packs together against helices H1 and H2 (and H1' and H2' of E73's second protomer) to form a canonical ribbon-helix-helix (RHH) domain (Figure 34). A sharp turn connects E73's β -strands to helix H1. The solvent exposed loop (L1) connects helices H1 and H2. In addition to this typical RHH fold, the structure of E73 presents a 3rd helix to the standard RHH fold which creates a structural cleft distal to the antiparallel β -sheet of E73's RHH domain. The loop (L2) bridging helices H2 and H3, creates the upper ridges of this shallow and V-shaped distal cleft of E73. Helix H3 wraps around helix H2' outside of, and opposite of, the face where H2 and H2' interact. This positioning essentially clamps H2' of one protomer between the H2 and H3 helices of the second protomer (and vice versa), resulting in a tightly packed and intertwined E73 dimer (Figure 34). The precise role of E73's nucleic acid binding function is still not fully understood. Superpositional docking of E73 on structurally homologous proteins suggests a DNA-binding function for E73 mediated through residue side-chains located majorly in the β -sheet region of E73. As part of our ongoing efforts to better understand the mechanisms of nucleic acid recognition in this system and to gain more insights into thermal adaptation of this protein, we have undertaken a detailed NMR study of the backbone dynamics of E73 in the absence of nucleic acid ligand. The ^{15}N relaxation studies reported below complement the solution structure analysis by providing evidence

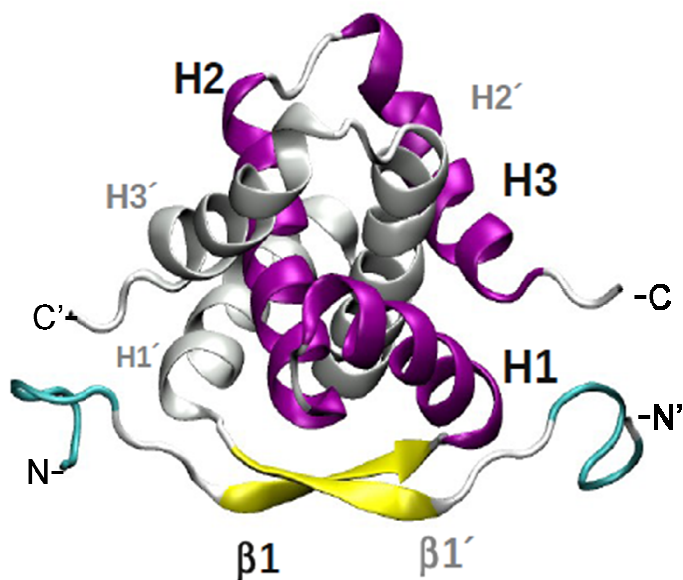


Figure 35: Solution Structure of E73: Ribbon representation of the first energy-minimized NMR structure. The two respective chains of the homodimer are colored gray and purple. The secondary structure elements are labeled $\beta 1$ (beta sheet; residues 11 to 16), H1 (α -helix 1; residues 18 to 32), H2 (α -helix 2; residues 35 to 53) and H3 (α -helix 3; residues 57 to 71). The symbol “ ’ ” denotes the second protomer chain. For the one protomer chain, α -helices are colored purple, the β -sheet is yellow, turn regions are white and the disordered region cyan.

for internal motions located at functionally important sites of unliganded E73, as discussed later in this dissertation.

^{15}N Relaxation Experiments

Backbone amide ^{15}N NMR relaxation experiments including ^{15}N -T₁, ^{15}N -T₂, and heteronuclear ^{15}N - $\{^1\text{H}\}$ -nOe were conducted in duplicates on a Bruker DRX 600 MHz spectrometer using standard NMR pulse sequences (47, 77, 78) at 312 K and a pH of 5.0.

^{15}N - T_1 relaxation profiles were sampled at eight different relaxation delay time points of 40 ms, 96 ms, 200 ms, 400 ms, 600 ms, 1000 ms, and 1200 ms. ^{15}N - T_2 relaxation profiles were sampled at eight different relaxation delay periods of 8 ms, 16 ms, 32 ms, 40 ms, 64 ms, 80 ms, 104 ms, and 152 ms, with the delay between the series of ^{15}N 180 degree pulses applied in the CPMG sequence set to 0.5 ms (79, 80). For both ^{15}N - T_1 and ^{15}N - T_2 measurements, the data were collected using 512 complex points in the ^1H acquisition time dimension (t_2) and 256 complex data points in the ^{15}N t_1 indirect time evolution dimension, using a WALTZ-16 ^{15}N -decoupling scheme during data acquisition. ^{15}N - T_1 and ^{15}N - T_2 were calculated from the series of NMR spectra using a two-parameter single exponential decay function of:

$$I(t) = I_0 \exp(-t/T_{1,2}) \quad (28)$$

where $I(t)$ is the peak height after a delay of time t and I_0 is the height at time $t = 0$.

Heteronuclear ^{15}N - $\{^1\text{H}\}$ -nOes were measured using a water-flip-back 2D heteronuclear nOe pulse sequence and the results corrected for the finite repetition delay according to the method of Grzesiek and Bax (78). For each residue amide, ^{15}N - $\{^1\text{H}\}$ -nOe was established as the ratio of peak intensities (I/I_0) from NMR data sets acquired with (I) or without (I_0) solvent presaturation (78). ^{15}N - $\{^1\text{H}\}$ -nOe spectra were recorded using 1024 and 256 complex data points in t_2 and t_1 , respectively, using 48 scans per t_1 increment. The ^{15}N - $\{^1\text{H}\}$ -nOe experiments were recorded using a delay of 4.5 sec between scans to minimize the introduction of systematic errors in measured ^{15}N - $\{^1\text{H}\}$ -nOes that could be produced by incomplete signal recovery and solvent saturation (78). To minimize the impact of magnetic field drift, ^{15}N T_2 and ^{15}N - $\{^1\text{H}\}$ -nOe data were

collected in an interleaved manner, while 2D ^{15}N - T_1 datasets were acquired consecutively using a list of shuffled relaxation delay time points.

NMR Relaxation Data Processing

All relaxation data were processed using the NMRPipe/NMRDraw (75) and SPARKY software (65). Apodization was performed in both dimensions using a Lorentz-to-Gauss window function, which consists of a combination of an inverse exponential and a Gaussian line broadening function. The purpose of applying a Lorentz-to-Gauss lineshape function (rather than sine bell functions) is to replace the exponential decay of the original data with a Gaussian decay so that following Fourier transformation (FT), the NMR signals adopt Gaussian-like line shapes rather than Lorentzian lineshapes. Application of this particular line broadening function (specified by the GM command in NMRPipe and g_1 and g_2 input parameters) is necessary for accurate fitting of NMR signal intensities. Typical values of g_1 (the exponential term) and g_2 (the Gaussian line broadening term) used for processing of the NMR relaxation data for E73 consisted of g_1 and g_2 values of 10 Hz and 15 Hz, respectively for processing of the t_2 (acquisition) dimension, and of g_1 and g_2 values of 5 Hz and 12 Hz, respectively, for processing of the t_1 (indirect) dimension of the 2D ^1H - ^{15}N NMR relaxation and ^{15}N - $\{^1\text{H}\}$ -nOe experiments. Errors associated with determination of ^{15}N - T_1 , ^{15}N - T_2 and ^{15}N - $\{^1\text{H}\}$ -nOes represented $\pm$ one standard deviation between duplicate sets of measurements using SPARKY (65). Typically, these error were on the order of 2.5% for ^{15}N - T_1 and ^{15}N - T_2 , and 4% for ^{15}N -

{¹H}-nOe. The relaxation parameters of E73 have been deposited to the BMRB under the Accession code 17069.

Derivation Of Dynamic Parameters

The characteristic timescales of the motion contributing to relaxation were assessed employing the reduced spectral density mapping (49, 52) according to the following relationships described by Bracken et al. (63):

$$\sigma_{\text{NH}} = R_1(n\text{Oe}-1)\gamma_{\text{N}}/\gamma_{\text{H}}$$

$$J(0.87\omega_{\text{H}}) = 4\sigma_{\text{NH}}/(5d^2)$$

$$J(\omega_{\text{N}}) = [4R_1 - 5\sigma_{\text{NH}}]/[3d^2 + 4c^2]$$

$$J_{\text{eff}}(0) = [6R_2 - 3R_1 - 2.72 \sigma_{\text{NH}}]/[3d^2 + 4c^2]$$

where $d = (\mu_0 h \gamma_{\text{N}} \gamma_{\text{H}} / 8\pi^2)(r^{-3})$ and $c = \omega_{\text{N}} \Delta\sigma / \sqrt{3}$. γ_{N} and γ_{H} are the gyromagnetic ratios of the ¹H and ¹⁵N nuclei, respectively, ω_{N} and ω_{H} are the Larmor frequencies, r is the internuclear ¹H-¹⁵N distance (1.02 Å), and $\Delta\sigma$ is the ¹⁵N CSA (-160 ppm). The subscript in $J_{\text{eff}}(0)$ refers to an “effective” $J(0)$, which is uncorrected for chemical exchange effects (63, 88) and R_1 and R_2 represent the relaxation rates calculated by inverting the respective relaxation time constants T_1 and T_2 . Reported error bars were calculated from propagation of experimental errors determined for the measured ¹⁵N- T_1 , ¹⁵N- T_2 , and ¹⁵N-{¹H}-nOe series.

The effective rotational correlation time at each residue, τ_{m} , was obtained from the spectral density functions using the following equation (63):

$$\tau_{\text{m}} = \omega_{\text{N}}^{-1} [(J_{\text{eff}}(0) - J(\omega_{\text{N}})) / J(\omega_{\text{N}})]^{1/2} \quad (29)$$

The ^{15}N NMR relaxation data were also analyzed using FastModelFree (62), an interface to ModelFree 4.1 (51), which is based on the model-free approach of Lipari-Szabo (53, 54), and analyzes ^{15}N NMR relaxation parameters in terms of internal motions of NH bond vectors in the presence of anisotropic overall diffusion of the proteins. An initial estimate of the correlation time for overall molecular reorientation, τ_m , was obtained from the mean of 10% trimmed ^{15}N - T_1/T_2 ratios (pre-screened using the method of Nicholson et al (58) and Pawley et al (57)). An axially symmetric model was chosen over isotropic and totally anisotropic diffusion models as indicated from the relative moments of inertia for E73 using pdbinertia (Art Palmer). All FastModelFree calculations were performed as described by Mandel et al. (53, 54, 59) to yield internal motional parameters, including S^2 generalized order parameters, internal correlation time for N-H bond vector motion, τ_e , and chemical exchange contribution to the relaxation, R_{ex} .

Backbone Dynamics Of E73

A complete set of ^{15}N Relaxation data (T_1 , T_2 and ^{15}N - $\{^1\text{H}\}$ -nOe – see Figure 35) was measured for a total of 58 out of 70 assigned non-proline residue amides (Table 26). Data for the remaining 12 residues was excluded from all calculations due to overlapped, very weak or missing correlation peaks. The 10% trimmed mean values of ^{15}N - T_1 , ^{15}N - T_2 and ^{15}N - $\{^1\text{H}\}$ -nOe were 741.0 ms, 75.4 ms and 0.71 respectively. The relaxation time constants of amides on the N- and C-termini were higher than rest of the protein which is typical for the terminal ends of the protein undergoing rapid motions. While the ^{15}N - T_1

times were about the same throughout the polypeptide sequence, two residues N32 and R33 show exceptionally high ^{15}N - T_1 values approaching those of the terminal amides in magnitude and were noticed to have the highest ^{15}N - T_1/T_2 ratios across the complete residue sequence of E73 (see Figure 35). High nOe values (> 0.7) were recorded for $\sim 70\%$ of all 58 residues indicating low flexibility of E73 amides on fast timescales (ps-ns). Relative moments of inertia for the E73 molecule were calculated to be 1:0.97:0.85 suggesting an axially symmetric type of diffusion. Based on the comparison of reduced χ^2 and F-statistic values, the axially symmetric model of E73 was identified to fit best with an oblate ellipsoid-like diffusion profile, yielding a $D_{\parallel}/D_{\perp}$ ratio and global rotational correlation time (τ_m) of 0.89 and 9.8 ns, respectively.

Data analysis was performed using the reduced spectral density mapping which provides an assessment of protein dynamics with no assumptions as to the nature of the molecule or its dynamic behavior. The reduced spectral density function $J_{\text{eff}}(0)$, $J(\omega_N)$ and $J(0.87\omega_H)$ were calculated for each residue in E73 using relaxation rates and nOe values measured at 14.1 T. Figure 36 shows the three spectral density functions plotted against the residues. The $J(\omega_N)$ function plot is fairly uniform for residues K11-F69 with rare spikes observed at the three loops connecting the secondary structure elements of E73. $J_{\text{eff}}(0)$ shows slight but significant difference between values in the H1 and H2-H3 regions. The N-terminal and $\beta 1$ residues have low values and the H1 residues have

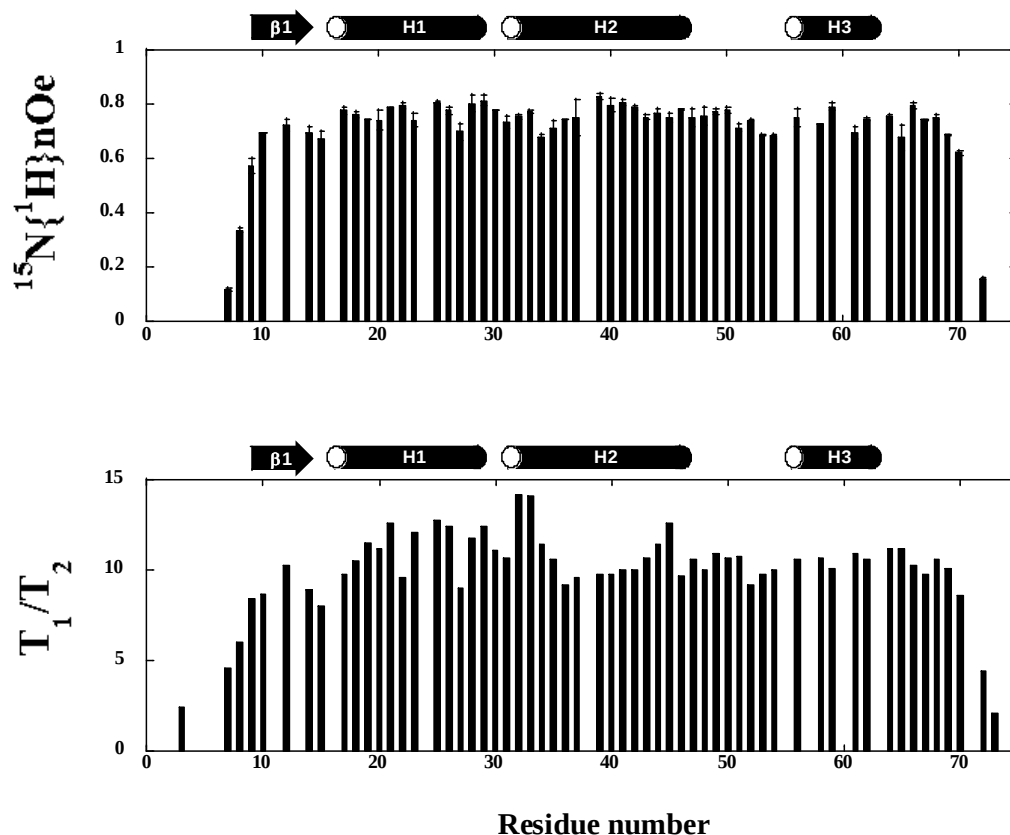


Figure 36. Relaxation Parameters- $^{15}\text{N}\{^1\text{H}\}\text{nOe}$ And $^{15}\text{N}-T_1/T_2$ Ratios Measured For E73 At A Magnetic Field Strength Of 14.1 T Plotted Against Residue Number. Secondary structure elements are depicted above each plot (cylinders = α -helices and arrow = β -strand). Error bars in (A) represent $\pm$ one standard deviation between duplicate sets of measurements.

slightly higher values of $J_{\text{eff}}(0)$ and the values decrease along the sequence. From residue D34 on, $J_{\text{eff}}(0)$ becomes constant until the last three residues when the values of $J_{\text{eff}}(0)$ drops again. Ten residues- Y21, T23, K25, L26, S28, V29, N32, R33, V44 and M45 show exceptionally large $J_{\text{eff}}(0)$ values recorded to be higher than one standard deviation from mean calculated for residues K11-F69 indicating significant conformational exchange motions occurring on slower timescales of μs -ms. Within the same region,

residues L14, A15 and L54 show elevated (greater than one standard deviation from mean) $J(0.87\omega_H)$ and reduced (smaller than one standard deviation from mean) $J_{eff}(0)$ values compared to the rest of the protein. In contrast, more residues, namely Y21, K25, L26, V29, N32 and R33 show higher $J_{eff}(0)$ and concomitant lower $J(0.87\omega_H)$ indicating that slower motions contribute significantly to relaxation in E73.

The calculated spectral density functions were also used to estimate the effective correlation time, τ_m , at each residue of the protein by using equation (29) and the average correlation time was calculated to be 9.7 ns which is in good agreement with the value obtained from FastModelfree (9.8 ns) and suggests that E73 exists as a dimer at solution conditions used in these studies.

E73 backbone dynamics were also analyzed by estimating the amplitude of motion(s) occurring at each residue amide site. In order to measure the motional intricacy at each of these sites, empirical models of motion were used to fit relaxation data to calculate the amplitude of motion experienced by the amide NH bond vector in the form of generalized order parameter, S^2 using FastModelfree (62). To achieve this, we considered five different dynamic models of motion using the methodology of Mandel et al (59) which defines the five models and their motional parameters as: (1) S^2 only, negligible effective NH bond correlation time, τ_e and conformational exchange rate, R_{ex} (or R_{exch}); (2) S^2 and τ_e only, negligible R_{ex} ; (3) S^2 and an R_{ex} term; (4) S^2 , τ_e and R_{ex} ; and (5) Separation of internal motions on two timescales differing from each other usually by an order of magnitude incorporating an additional order parameter S_f^2 for fast timescale

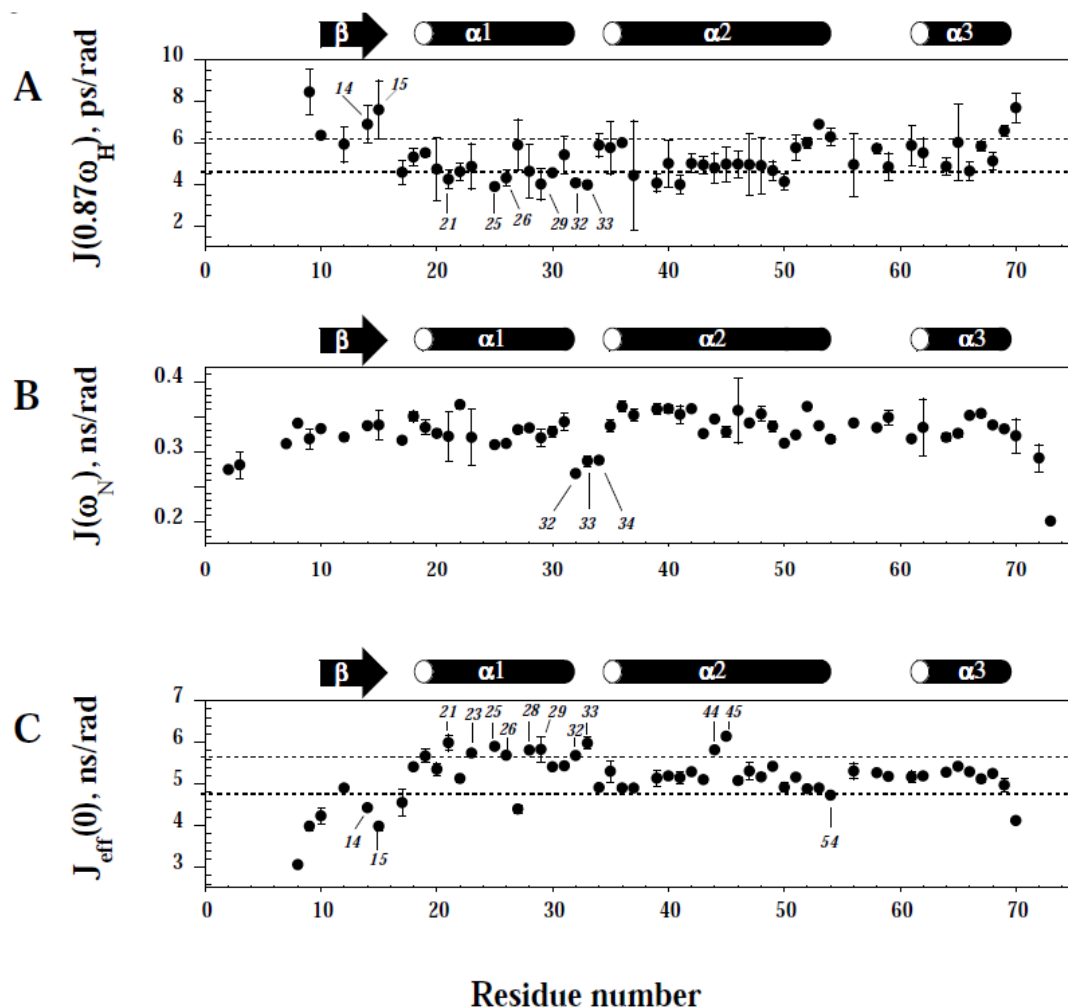


Figure 37: Spectral Density Functions At $0.87\omega_H$, ω_N And 0 Frequencies Obtained From ^{15}N Relaxation Data Measured For E73 At A Magnetic Field Of 14.1 T Plotted Against Residue Number (A, B and C). Dotted lines represent spectral density values corresponding to $\pm$ one standard deviation. Secondary structure elements are depicted above each plot (cylinders = α -helices and arrow = β -strand). Error bars indicate the uncertainties propagated from ^{15}N relaxation data.

and $S_s^2 (= S^2)$ for slow timescale, $\tau_e = \tau_s$. Although credibility of projections from this analysis could be somewhat weak due to the limitation of relaxation data obtained at single field strength, strong correlations can be drawn from the outcomes of the two essentially different approaches. About 40% residues fit with models 3 and 4

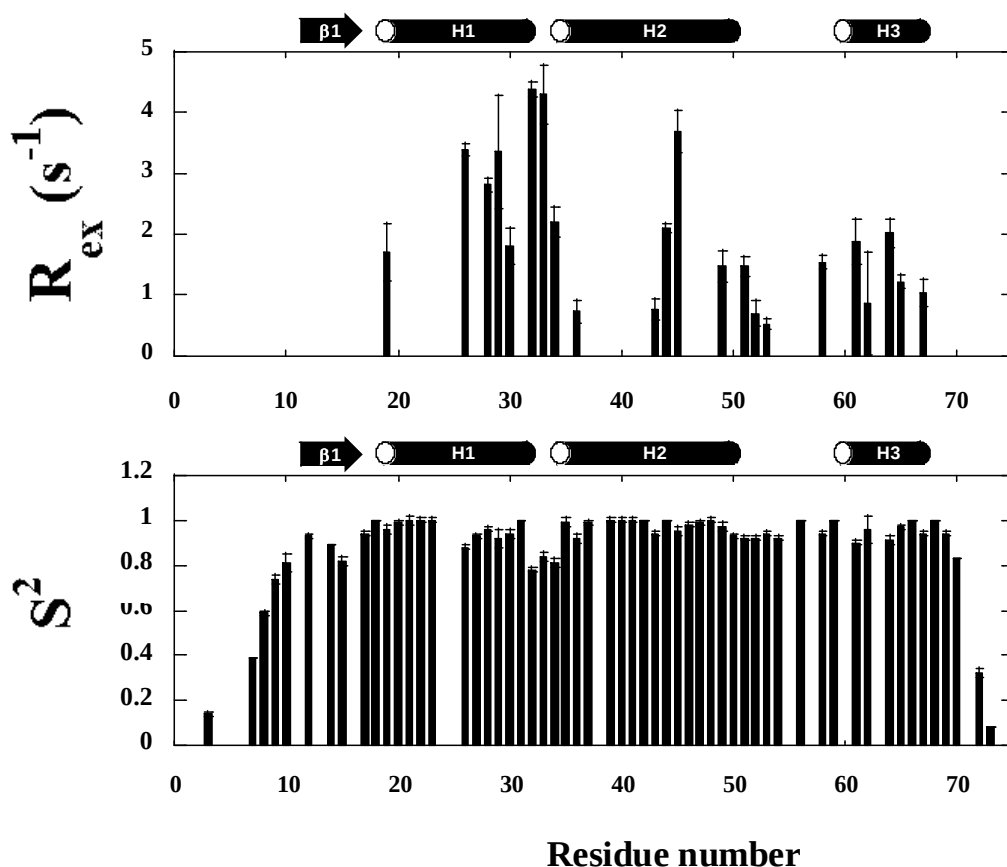


Figure 38. Conformational Exchange (R_{ex}) And Order (S^2) Lipari-Szabo Parameters Of E73 Plotted Against Residue Number. The dotted line in R_{ex} plot indicates mean R_{ex} value plus one standard deviation. Secondary structure elements are depicted above each plot (cylinders = α -helices and arrow = β -strand). Error bars indicate the uncertainties in ^{15}N relaxation data.

corroborating that E73 dynamics is governed by slower timescale motions and identifying residues S28, V29, N32 and R33 to be undergoing conformational exchange, as previously inferred from their spectral density functions too. Broadly, the dynamics profile of E73 resembles with that of a rigid protein with almost no residues fitting with model 5 in the residue stretch K11-F69 and exceptionally high S^2 values (> 0.92 , mean value 0.97) observed for all residues fit with simpler models 1 and 2.

Discussion

E73 belongs to the Ribbon-Helix-Helix (RHH) family of proteins that represent a diverse and large family of proteins used to typically regulate transcription of genes throughout all kingdoms of life including Prokarya, Archaea, and viruses. Transcriptional regulator proteins often need flexible domains to interact and maintain a temporal regulation. This typically requires a protein machinery to allow movements on different timescales on different locations of the protein. One of the major purposes of this flexibility is to control the diffusion rates of protein on and off the DNA and to make sure the transcription cycle duration is completed before cell multiplication.

The backbone dynamics of E73 as observed from the ^{15}N NMR relaxation experiments shows that E73 has an extremely rigid core. The motions in its core are highly restricted to fast timescale motions. This could suggest a fine-tuning step of reduction in the intrinsic motions of its core segments. A direct comparison of backbone dynamics at lower temperature has not been possible due to the lack of sequence matches for E73 outside the *Sulfolobus* kingdom which limits our search and hence the comparisons with any mesophilic counterparts of this protein. However, a close comparison with another study of TrpR reported in this text suggests that E73 experiences reduced flexibility overall. This could be a result of natural selection towards getting a rigid core to survive in the extreme conditions that SSV-RH inhabits. Altogether, the backbone amide dynamics provides evidence for fast ps-ns timescale NH bond vector motions in the proposed DNA-binding domain and motions on a slower μs to ms timescale for residues in loop connecting α -helices H1 and H2.

Flexible Regions Of E73

The analysis of the flexibility of backbone amides of E73 does reveal convincing results that indicate pockets of flexible regions located on the structure of E73. For convenience, let us consider the structure in three different segments. The first segment consists of the N-terminal β -sheet and is proposed to have a DNA binding function. The second segment consists of a hydrophobic core which holds the functional parts together and the third segment consists of the cleft and the ridges formed by the third helix wrapping on top of the rigid core of the protein. This segment is a putative interaction site for another partner. There are two flexible regions observed from the backbone dynamics studies that connect the three domains of E73.

With these backbone dynamics studies, we could identify flexible pockets located in the regions that could be helping the three domains co-ordinate with each other or could be responsible for enhancing the functional features of the domain themselves. Besides the N- and C-termini, the region that experiences high fast timescale (ps-ns) motions is the β -sheet which strengthens our prediction of the β -sheet functioning as the DNA binding site of E73. Two other regions that possess high fast timescale motions, although to a lesser extent are the short loop connecting H1 with H2 and the N-terminal side of the long loop connecting H2 with H3. The hypothesis in support of this result is that the three domains need to synchronize their movements in order to perform DNA binding. The only way they can do so is by having a fast timescale dynamics in the β -sheet which imparts it sufficient entropy to diffuse into the DNA major groove. On the other hand, the hydrophobic core requires only minimal amounts of flexibility which is

provided to it by slight fast timescale motions in loops connecting the helices potentially modulated by slow timescale motions in the C-terminus of H1 and the center of H2. These slow timescale motions particularly seem to be important optimize diffusion kinetics of the DNA binding β -sheet and the highly positively charged top cleft with their binding partners. A recent mutation study performed by Dr. Brian Tripet provides some support for this hypothesis. It was observed that K61E mutation in E73 significantly perturbs the chemical shifts of the β -sheet resonances. This suggests that any change in the chemical environment of the top cleft of E73 transmits long range perturbations to the β -sheet rearranging the proposed DNA binding site of E73. It also provides evidence for a model where E73 undergoes an activation step of binding with an interaction partner to be able to perform DNA binding function. Whether this activation is regulated by the host or the virus or both still remains a mystery to us and will need further studies to be unraveled.

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APPENDIX A

SUPPLEMENTAL INFORMATION

Table 3 – Chemical Shift Assignments (in ppm) Of Apo-WT-TrpR.

Residue	H ^N	¹⁵ N	C ^α	C ^β
Met1	-	-	-	-
Ala2	-	-	-	-
Gln3	-	-	56.41	30.28
Gln4	8.46	122.08	55.89	30.11
Ser5	8.36	118.28	56.17	63.75
Pro6	-	-	-	-
Tyr7	7.93	119.08	57.88	38.82
Ser8	7.91	116.98	58.54	64.07
Ala9	8.32	126.28	53.94	19.05
Ala10	8.09	121.78	54.22	19.23
Met11	7.99	118.38	56.86	32.72
Ala12	8.11	123.18	54.63	18.91
Glu13	8.2	119.38	58.52	29.69
Gln14	8.15	120.58	58.37	28.37
Arg15	8.29	118.28	58.32	30.52
His16	-	-	56.39	30
Gln17	8.36	118.28	59.25	28.32
Glu18	8.44	120.18	-	27.78
Trp19	8.11	122.08	59.37	29.57
Leu20	8.01	117.28	-	29.74
Arg21	-	-	-	-
Phe22	7.87	120.58	62.06	37.68
Val23	7.85	119.38	-	-
Asp24	8.09	121.38	57.83	41.66
Leu25	8.18	124.08	54.29	43
Leu26	8.26	119.38	57.52	40.51
Lys27	7.53	120.58	60.06	32.4
Asn28	7.75	116.68	56.12	39.17
Ala29	8.94	126.18	55.43	17.71
Tyr30	8.46	119.18	61.13	38.26
Gln31	7.45	117.28	58.2	28.73

Asn32	7.35	115.48	53.36	40.36
Asp33	7.94	117.38	56.42	39.56
Leu34	8.32	117.68	53.75	43.74
His35	9.03	120.68	59.57	28.76
Leu36	7.39	119.38	59.52	38.02
Pro37	-	-	-	-
Leu38	7.94	119.58	58.15	42.17
Leu39	8.9	120.48	58.58	42.05
Asn40	8.2	116.78	54.8	38.24
Leu41	7.53	119.28	57.47	43.05
Met42	8.1	113.58	56.12	33.55
Leu43	7.83	117.68	53.66	44.91
Thr44	-0.02	-0.12	2.52	2.52
Pro45	-0.02	-0.12	2.52	2.52
Asp46	8.07	114.68	57.08	40.46
Glu47	7.57	122.68	59.18	29.91
Arg48	7.87	118.08	60.4	30.69
Glu49	7.85	117.48	-	-
Ala50	7.95	122.08	55.56	17.93
Leu51	8.09	119.28	58.76	41.07
Gly52	8.24	104.78	47.47	-
Thr53	-	-	-	-
Arg54	8.56	121.08	60.4	30.59
Val55	7.86	119.08	67.14	31.57
Arg56	7.26	119.58	58.71	29.2
Ile57	-	-	-	-
Val58	8.08	119.08	-	-
Glu59	8.46	119.48	-	-
Glu60	8.25	116.18	58.54	29.1
Leu61	8.79	122.88	57.61	42.07
Leu62	8.3	119.68	57.66	42.47
Arg63	8.25	118.68	59.15	30.71
Gly64	7.77	104.48	46.71	-
Glu65	-	-	57.51	30.98
Met66	8.43	119.28	56.12	34.43
Ser67	8.48	117.38	57.96	65.14
Gln68	8.58	120.68	-	-
Arg69	8.11	118.88	58.59	-
Glu70	7.86	119.68	57.76	30.25

Leu71	8.21	121.38	56.91	42.54
Lys72	-	-	58.13	32.79
Asn73	8.02	117.58	54.96	39.02
Glu74	-	-	-	-
Leu75	-	-	-	-
Gly76	7.96	108.28	46.28	-
Ala77	7.51	121.98	51.92	20.21
Gly78	8.34	106.78	45.37	-
Ile79	-	-	-	-
Ala80	-	-	-	-
Thr81	-	-	-	-
Ile82	7.89	122.28	55.34	29.54
Thr83	-	-	-	-
Arg84	-	-	-	-
Gly85	8.2	108.48	46.75	
Ser86	8.35	116.18	60.99	63.58
Asn87	8.47	119.38	-	-
Ser88	-	-	-	-
Leu89	8.28	123.38	57.71	42.03
Lys90	7.84	118.38	58.89	32.55
Ala91	7.34	118.78	51.95	19.6
Ala92	7.4	122.98	50.48	17.84
Pro93	-	-	-	-
Val94	8.8	125.08	66.51	31.75
Glu95	9.67	119.08	60.08	28.56
Leu96	7.28	117.48	57.47	42.27
Arg97	7.99	119.88	59.84	32.57
Gln98	8.7	116.18	59.16	28.63
Trp99	7.51	121.28	61.92	28.88
Leu100	8.83	117.48	57.69	42.81
Glu101	-	-	-	-
Glu102	7.44	118.08	59.42	28.59
Val103	7.93	113.28	64.5	32.35
Leu104	8.32	117.48	56.17	42.93
Leu105	7.11	115.48	55.39	41.51
Lys106	7.22	119.38	56.44	32.84
Ser107	8.1	117.18	58.11	64.36
Asp108	7.94	127.68	55.83	42.2

Table 4 – Chemical Shift Assignments (in ppm) Of Apo-A77V-TrpR.

Residue	H ^N	¹⁵ N	C ^α	C ^β
Met1	-	-	-	-
Ala2	-	-	-	-
Gln3	8.29	119.88	56.19	29.77
Gln4	8.45	122.08	56.14	29.8
Ser5	8.33	118.48	-	-
Pro6	-	-	63.67	31.87
Tyr7	7.94	118.98	57.93	38.71
Ser8	7.92	117.18	58.42	63.96
Ala9	8.32	126.28	53.81	18.67
Ala10	8.09	121.68	53.95	18.86
Met11	7.99	118.38	56.79	32.54
Ala12	8.12	123.18	54.48	18.78
Glu13	8.2	119.38	58.57	29.46
Gln14	8.17	120.58	58.61	28.62
Arg15	8.31	118.18	58.43	30.38
His16	-	-	55.86	29.45
Gln17	8.36	118.28	59.01	28.82
Glu18	8.44	120.18	59.64	28.76
Trp19	8.13	122.38	59.58	29.21
Leu20	8.03	117.78	57.92	41.4
Arg21	7.97	119.38	59.53	30.44
Phe22	7.88	120.48	62.02	37.54
Val23	7.82	119.18	66.87	31.08
Asp24	8.05	121.28	57.31	41.49
Leu25	8.22	123.98	57.83	41.94
Leu26	8.23	119.18	57.41	41.3
Lys27	7.51	120.58	59.78	32.12
Asn28	7.75	116.68	55.88	38.97
Ala29	8.95	126.18	55.84	17.66
Tyr30	8.44	119.28	61.84	38.06
Gln31	7.44	117.18	58.07	28.41

Asn32	7.36	115.48	53.16	40.35
Asp33	7.93	117.28	56.22	39.93
Leu34	8.31	117.78	53.59	43.4
His35	9.03	120.78	59.48	28.62
Leu36	7.4	119.48	59.85	37.81
Pro37	-	-	65.37	30.6
Leu38	7.89	119.08	58.68	41.41
Leu39	8.91	120.68	58.73	41.62
Asn40	8.22	116.98	54.84	37.99
Leu41	7.57	119.48	57.1	42.82
Met42	8.08	113.88	55.88	33.18
Leu43	7.88	117.88	53.69	44.95
Thr44	-	-	-	-
Pro45	-	-	66.42	31.43
Asp46	8.09	114.58	56.75	40.29
Glu47	7.54	122.68	59.02	30.14
Arg48	7.85	118.18	60.2	30.31
Glu49	7.77	117.68	59.38	29.45
Ala50	7.93	121.98	55.34	17.77
Leu51	8.09	119.28	58.73	45.35
Gly52	8.24	104.98	47.54	-
Thr53	-	-	-	-
Arg54	8.6	121.38	60.59	30.58
Val55	7.89	119.08	67.19	31.24
Arg56	7.24	119.38	58.37	28.78
Ile57	8.2	117.98	66.42	37.84
Val58	7.98	118.18	-	-
Glu59	-	-	60.25	29.69
Glu60	8.32	114.88	57.67	29.1
Leu61	8.93	124.28	57.93	42.1
Leu62	8.27	119.58	57.46	42.5
Arg63	8.32	119.38	59.48	30.8
Gly64	7.62	102.48	-	-
Glu65	8.57	121.38	57.32	31.24
Met66	8.61	118.98	55.82	35.41
Ser67	8.78	118.58	57.39	65.06
Gln68	8.84	120.28	60.67	28.38
Arg69	8.16	117.68	59.26	30.11
Glu70	7.64	119.38	59.16	30.01

Leu71	8.4	121.28	58.04	42.6
Lys72	-	-	59.51	31.8
Asn73	7.71	117.28	55.62	38.76
Glu74	8.49	119.88	-	-
Leu75	8.01	114.28	54.92	43.19
Gly76	7.92	108.58	46.88	-
Val77	7.06	110.48	58.72	31.49
Gly78	8.24	106.68	44.9	-
Ile79	-	-	-	-
Ala80	-	-	-	-
Thr81	-	-	-	-
Ile82	-	-	-	-
Thr83	-	-	-	-
Arg84	-	-	59.7	29.49
Gly85	7.87	107.88	47.17	-
Ser86	8.51	116.68	61.51	63.07
Asn87	-	-	-	-
Ser88	-	-	-	-
Leu89	-	-	57.93	42.24
Lys90	7.89	118.38	59.16	32.42
Ala91	7.2	118.38	51.79	19.44
Ala92	7.35	123.18	50.57	17.78
Pro93	-	-	62.57	31.94
Val94	8.81	125.08	66.41	31.82
Glu95	9.66	118.98	59.94	28.56
Leu96	7.28	117.28	57.41	42.04
Arg97	7.89	119.48	61.24	29.98
Gln98	8.72	116.08	59.08	28.52
Trp99	7.51	121.38	61.68	28.41
Leu100	8.84	117.38	57.67	42.66
Glu101	8.02	117.38	60.15	29.42
Glu102	7.44	118.18	59.16	29.65
Val103	7.96	113.28	64.31	32.09
Leu104	8.36	117.38	52.61	42.65
Leu105	7.08	115.28	55	41.19
Lys106	7.22	119.18	56.53	32.68
Ser107	8.1	117.18	58.13	64.41
Asp108	7.94	127.78	55.84	42.4

Table 5. Chemical Shift Assignments (in ppm) For Holo-WT-TrpR.

<u>Residue</u>	<u>H^N</u>	<u>error</u>	<u>N</u>	<u>error</u>	<u>C^α</u>	<u>error</u>	<u>C^β</u>	<u>Error</u>
Met1	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	54.75	0.14	18.91	0.07
Gln3	8.19	0.01	119.38	0	57.09	1.06	29.94	0.18
Gln4	8.45	0.01	122.08	0	56.04	0.13	29.95	0.18
Ser5	8.35	0	117.98	0	56.66	0	63.7	0
Pro6	-	-	-	-	64.09	0.1	32.14	0.11
Tyr7	7.93	0	118.98	0	58.36	0.24	39.01	0.13
Ser8	7.92	0.01	117.28	0	58.72	0.35	64.32	0.06
Ala9	8.33	0.01	126.18	0	54.11	0.15	19.02	0.09
Ala10	8.09	0.01	121.38	0	54.15	0.1	19.02	0.07
Met11	7.99	0.01	118.38	0	56.95	0.21	32.67	0.09
Ala12	8.11	0	122.98	0	54.49	0	18.91	0
Glu13	8.21	0	119.28	0	58.88	0.05	29.91	0
Gln14	8.17	0.01	120.68	0	58.7	0.33	28.76	0.09
Arg15	8.36	0.01	118.48	0	58.71	0	30.27	0
His16	8.39	0	118.58	0	56.27	0.07	29.27	0.01
Gln17	8.35	0.01	118.08	0.05	59.2	0.29	28.81	0.19
Glu18	8.47	0.01	120.28	0	59.52	0.26	28.94	0.26
Trp19	8.11	0	122.28	0	59.56	0.11	29.32	0.1
Leu20	8.04	0.01	117.68	0	57.76	0.02	40.76	0.02
Arg21	7.95	0	119.68	0	59.87	0.21	30.25	0.08
Phe22	7.8	0.01	120.18	0	62.2	0.26	37.8	0.17
Val23	7.8	0	119.28	0	63.93	5.61	31.42	0.18
Asp24	8.08	0.01	121.28	0.02	57.64	0.13	41.71	0.13
Leu25	8.14	0.02	124.08	0	58.48	0	42.91	0.14
Leu26	8.26	0.01	119.28	0	57.39	0.29	40.58	0.12
Lys27	7.53	0	120.68	0	60.3	0.19	32.58	0.12
Asn28	7.74	0.01	116.58	0	56.25	0.18	39.28	0.08
Ala29	8.93	0	126.18	0	56.07	0.01	17.86	0.06
Tyr30	8.44	0.01	119.08	0	61.95	0.23	38.23	0.23
Gln31	7.44	0.01	117.08	0	58.3	0.21	28.71	0.16

Asn32	7.36	0.01	115.48	0	53.37	0.21	40.65	0.12
Asp33	7.94	0.01	117.18	0	56.53	0.14	40.13	0.12
Leu34	8.32	0.01	117.68	0	53.9	0.06	43.66	0.16
His35	9.08	0.01	120.88	0	59.78	0.19	29	0.13
Leu36	7.46	0	119.38	0	59.58	0	37.89	0
Pro37	-	-	-	-	65.74	0.09	31.19	0.08
Leu38	8.03	0.01	119.78	0	58.92	0.18	41.64	0.13
Leu39	8.92	0	120.78	0	54.52	7.11	41.89	0.04
Asn40	8.21	0.01	117.98	0	55.13	0.13	38.28	0.12
Leu41	7.77	0.01	119.08	0.02	58.22	0.01	42.45	0.16
Met42	8.68	0.01	114.28	0	56.31	0.28	32.36	0.06
Leu43	7.64	0.01	115.78	0	54.02	0	44.64	0
Thr44	-	-	-	-	-	-	-	-
Pro45	-	-	-	-	66	0.13	31.93	0.07
Asp46	7.99	0	114.68	0.05	56.82	0.17	40.5	0.1
Glu47	7.81	0.01	122.78	0	60.07	0.69	28.92	0.65
Arg48	8.15	0	117.88	0	60.51	0.72	30.56	0.22
Glu49	7.72	0	117.38	0	59.53	0.16	29.71	0.1
Ala50	8.05	0.01	121.88	0	55.48	0.1	17.77	0.17
Leu51	8.11	0.01	118.78	0	58.84	0	41.05	0
Gly52	8.04	0	133.98	0	-	-	-	-
Thr53	-	-	-	-	-	-	-	-
Arg54	8.68	0	121.58	0	61.2	0.41	31.14	0.52
Val55	7.8	0.01	118.38	0.51	67.39	0.27	31.74	0.03
Arg56	7.16	0.01	118.98	0	58.26	0	29.23	0
Ile57	-	-	-	-	-	-	-	-
Val58	8.49	0.02	118.78	0.04	68.23	0.22	31.8	0.08
Glu59	8.68	0	119.18	0	60.65	0.21	30.23	0.04
Glu60	8.28	0.01	115.08	0	57.97	0.29	29.47	0.07
Leu61	9.05	0.01	124.18	0	58.46	0.28	41.6	0
Leu62	8.29	0.02	119.38	0	58.11	0.87	42.75	0.1
Arg63	8.33	0.01	119.18	0	-	-	30.79	0
Gly64	7.66	0	132.78	0	46.31	0.87	-	-
Glu65	7.98	0.23	119.48	0.67	57.89	0.12	31.75	0.31
Met66	8.65	0	119.08	0	56.35	0.15	35.36	0.11
Ser67	8.73	0.01	118.18	0	60.22	3.78	62.81	3.57
Gln68	8.81	0.01	120.28	0	59.58	0.56	29.48	0.7
Arg69	7.6	0	117.38	0	59.81	0.69	30.25	0.25
Glu70	7.67	0	119.48	0	59.28	0.18	30.25	0.05

Leu71	8.4	0.01	121.18	0	58.31	0.08	42.68	0.09
Lys72	7.97	0	119.78	0.04	59.65	0.11	32.31	0.1
Asn73	7.87	0	117.38	0	55.73	0.1	39.05	0.12
Glu74	8.56	0	118.78	0	58.5	0.21	30.2	0.15
Leu75	8.02	0.01	114.78	0	55.23	0.18	43.28	0.16
Gly76	7.89	0	108.28	0	46.51	0.15	-	-
Ala77	7.04	0.01	121.38	0	50.9	0.15	21.08	0.05
Gly78	8.31	0	105.98	0	45.01	0	-	-
Ile79	-	-	-	-	-	-	-	-
Ala80	-	-	-	-	-	-	-	-
Thr81	-	-	-	-	-	-	-	-
Ile82	7.9	0	121.68	0	-	-	-	-
Thr83	-	-	-	-	-	-	-	-
Arg84	7.74	0.01	121.68	0	59.91	0.16	29.51	0.11
Gly85	7.65	0	109.38	0	47.34	0.2	-	-
Ser86	8.28	0	116.28	0	61.52	0	62.89	0
Asn87	8.45	0	119.08	0	-	-	-	-
Ser88	7.92	0	116.98	0	58.43	0.03	63.29	0.31
Leu89	8.35	0	123.88	0	57.99	0.32	42.5	0.13
Lys90	7.8	0.01	117.98	0.03	59.44	0.04	32.66	0.09
Ala91	7.1	0	118.18	0	52.1	0.07	19.5	0.2
Ala92	7.34	0	123.18	0	50.64	0	17.75	0
Pro93	-	-	-	-	63	0.09	32.65	0.05
Val94	8.82	0	125.08	0	66.62	0.12	32.11	0.02
Glu95	9.68	0	118.88	0	60.37	0.15	28.78	0.09
Leu96	7.28	0.01	117.28	0	57.64	0.12	42.37	0.19
Arg97	7.93	0.01	119.88	0.01	61.49	0.18	30.39	0.15
Gln98	8.74	0.01	116.08	0	59.29	0.09	28.72	0.11
Trp99	7.51	0.01	121.28	0	62.09	0.21	28.49	0.25
Leu100	8.87	0	117.38	0	58	0.12	43.01	0.14
Glu101	8.01	0	117.18	0	60.3	0.19	29.91	0.1
Glu102	7.43	0.01	118.08	0	59.4	0.11	29.93	0.07
Val103	7.96	0.01	113.28	0	64.56	0.15	32.4	0.12
Leu104	8.39	0	117.38	0	56.27	0.2	42.73	0.16
Leu105	7.06	0.01	114.98	0	55.36	0.13	41.41	0.09
Lys106	7.21	0.01	119.28	0	56.65	0.14	32.95	0.21
Ser107	8.11	0	117.28	0.01	58.29	0.21	64.54	0.18
Asp108	7.94	0	127.68	0.04	56.01	0	42.33	0

Table 6 – Chemical Shift Assignments (in ppm) For Holo-L75F-TrpR.

<u>Residue</u>	<u>H^N</u>	<u>error</u>	<u>N</u>	<u>error</u>	<u>C^α</u>	<u>error</u>	<u>C^β</u>	<u>Error</u>
Met1	-	-	-	-	-	-	-	-
Ala2	8.11	0	119.48	0	-	-	-	-
Gln3	8.3	0	118.88	0	57.38	0.18	30.66	0.02
Gln4	8.45	0.01	122.28	0.13	56.71	0.07	31.07	0.15
Ser5	8.04	0	116.78	0.04	56.99	0	64.71	0
Pro6	-	-	-	-	-	-	-	-
Tyr7	7.93	0	119.08	0	59.42	0.31	39.66	0.01
Ser8	7.91	0	116.88	0.01	59.64	0.25	64.97	0.13
Ala9	8.32	0.01	126.48	0.13	54.88	0.11	19.85	0.04
Ala10	8.09	0.01	121.48	0.13	54.86	0.09	19.65	0.11
Met11	7.98	0	118.18	0.04	57.67	0.14	33.53	0.13
Ala12	8.11	0	123.28	0.13	56.01	0.66	19.26	0.37
Glu13	8.28	0.11	119.28	0.08	59.5	0.07	30.54	0.13
Gln14	8.16	0.01	120.88	0.13	59.91	0	29.58	0.12
Arg15	8.32	0	118.18	0	56.89	0.09	30.85	0.05
His16	8.34	0.01	118.58	0.14	57.46	0.65	30.94	0.16
Gln17	8.35	0.01	118.28	0.13	59.99	0.33	29.33	0.07
Glu18	8.46	0	120.48	0.13	60.58	0.14	30.03	0.2
Trp19	8.1	0	122.38	0.12	60.36	0.19	30.26	0.21
Leu20	8.07	0.01	117.78	0.07	58.54	0.18	41.55	0.23
Arg21	7.96	0.01	119.68	0.02	60.78	0.23	30.84	0.08
Phe22	7.76	0.01	120.48	0.13	63.07	0.17	38.59	0.14
Val23	7.82	0	119.28	0.07	67.68	0.08	32.18	0.11
Asp24	8.1	0.01	121.58	0.11	58.27	0.05	42.38	0.03
Leu25	8.12	0.01	124.28	0.13	58.97	0.46	43.56	0.18
Leu26	8.28	0.05	119.28	0.08	58.36	0.17	41.6	0.18
Lys27	7.56	0	120.88	0.13	61.33	0.29	32.01	0.83
Asn28	7.74	0.01	116.78	0.15	57	0.02	39.88	0.07
Ala29	8.93	0	126.48	0.13	56.61	0.12	18.65	0.14
Tyr30	8.44	0	119.58	0.17	62.74	0.16	39.07	0.18
Gln31	7.43	0	117.28	0.13	59.13	0.08	29.57	0.21
Asn32	7.35	0.01	115.68	0.13	54.23	0.13	41.54	0.09

Asp33	7.93	0	117.08	0.09	57.31	0.02	40.95	0.02
Leu34	8.32	0	117.48	0.09	54.47	0.1	44.37	0.06
His35	9.07	0.01	121.08	0.13	60.55	0.15	29.7	0.14
Leu36	7.46	0.01	119.58	0.14	60.59	0	38.5	0
Pro37	-	-	-	-	64.69	0.22	32.82	0.01
Leu38	7.92	0.02	118.98	0.16	59.54	0.45	41.56	1.38
Leu39	8.9	0.01	120.78	0.13	59.5	0.01	43.06	0.39
Asn40	8.18	0.01	117.68	0.02	55.87	0.09	39.08	0.08
Leu41	7.72	0.02	119.18	0.11	59.25	0.07	43.28	0.1
Met42	8.62	0.01	114.18	0.07	57.37	0.32	33.31	0.09
Leu43	7.62	0.01	116.08	0.16	54.72	0	45.64	0
Thr44	9.54	0	116.68	0	-	-	-	-
Pro45	-	-	-	-	66.9	0	32.43	0.01
Asp46	7.99	0.01	114.88	0.16	57.48	0.06	41.37	0.11
Glu47	7.78	0.01	122.98	0.15	60	0	30.22	0.42
Arg48	8.16	0.02	118.38	0	59.88	0.07	30.83	0.09
Glu49	7.64	0.02	117.38	0	60.16	0.06	30.31	0.02
Ala50	8.03	0.01	122.08	0.02	56.23	0.06	18.5	0.06
Leu51	8.08	0	118.68	0.05	59.74	0.23	42.07	0.07
Gly52	8.07	0.01	103.78	0.15	46.78	0.62	-	-
Thr53	8.31	0.02	120.28	0.19	-	-	-	-
Arg54	8.66	0.01	121.48	0.03	61.58	0.18	31.51	0.18
Val55	7.7	0.01	117.88	0.09	68.14	0.2	32.6	0.16
Arg56	7.05	0.01	119.08	0.13	59.42	0	29.9	0
Ile57	-	-	-	-	-	-	-	-
Val58	-	-	-	-	69.02	0.1	32.56	0.1
Glu59	8.54	0	119.58	0.13	61.57	0.02	30.67	0.04
Glu60	8.22	0.01	115.78	0.1	59.12	0.1	30.05	0.17
Leu61	9	0.01	123.88	0.16	59.02	0.24	43.08	0.37
Leu62	8.27	0.03	119.28	0.03	58.51	0.14	43.22	0.1
Arg63	8.34	0.01	119.58	0.13	60.37	0.3	31.66	0.36
Gly64	7.68	0.02	103.18	0.14	47.67	0.11	-	-
Glu65	7.91	0.01	119.58	0.19	58.43	0.21	32.14	0.07
Met66	8.66	0.02	119.58	0.17	57.51	0.15	35.31	0.05
Ser67	8.63	0.01	117.98	0.15	58.84	0.27	66.01	0.2
Gln68	8.73	0.03	120.58	0.12	60.22	0.17	29.54	0.04
Arg69	8.15	0.01	118.78	0	59.56	0.22	31.07	0.14
Glu70	7.75	0.02	119.68	0.09	59.65	0.03	31.02	0.21
Leu71	8.34	0	121.18	0.09	58.74	0.17	43.47	0.02

Lys72	8.03	0.01	119.78	0.12	59.6	0.19	33.42	0.02
Asn73	8	0.01	117.58	0.02	-	-	41.58	2.01
Glu74	8.28	0.05	119.18	0.01	59.08	0.18	30.47	0.41
Leu75	7.99	0.19	116.18	2.61	58.66	0.16	40.89	0.2
Gly76	8.05	0.01	110.08	0.21	47.36	0.13	-	-
Val77	7.61	0.01	122.18	0.04	52.8	0.13	21.02	0.06
Gly78	8.33	0.01	106.98	0.12	45.98	0	-	-
Ile79	-	-	-	-	-	-	-	-
Ala80	7.76	0.01	128.88	0.15	55.61	0	19.59	0.01
Thr81	7.63	0.01	114.58	0.01	-	-	-	-
Ile82	7.84	0.01	120.78	0.01	-	-	-	-
Thr83	8.38	0	115.48	0	-	-	-	-
Arg84	7.78	0.02	121.58	0.15	60.33	0.03	30.18	0.06
Gly85	7.68	0.02	108.88	0.04	47.91	0.06	-	-
Ser86	8.29	0.01	116.08	0.02	62.41	0.13	63.87	0.08
Asn87	8.45	0.01	119.08	0.05	-	-	39.03	0
Ser88	7.98	0	116.68	0	60.08	0.03	64.11	0.05
Leu89	8.29	0	123.78	0.13	58.86	0.13	43.36	0.07
Lys90	7.85	0	118.18	0.14	60.24	0.2	33.46	0.18
Ala91	7.19	0.01	118.58	0.2	52.86	0.06	20.41	0.03
Ala92	7.35	0	123.28	0.13	51.26	0	18.71	0
Pro93	-	-	-	-	63.77	0.16	32.92	0.15
Val94	8.82	0	125.38	0.13	67.39	0.02	32.6	0.02
Glu95	9.69	0.02	118.88	0	61.07	0.15	29.64	0.24
Leu96	7.29	0.01	117.48	0.13	58.42	0.16	43.1	0.21
Arg97	7.92	0.02	119.68	0.01	62.31	0.09	31.28	0.3
Gln98	8.74	0.01	116.28	0.13	60.05	0.04	29.59	0.05
Trp99	7.49	0	121.58	0.13	62.77	0.13	29.57	0.36
Leu100	8.86	0	117.68	0.15	58.73	0.17	43.6	0.04
Glu101	8.01	0.01	117.18	0.01	61.04	0.2	30.62	0.02
Glu102	7.43	0	118.28	0.13	60.12	0.1	30.66	0.07
Val103	7.96	0	113.58	0.1	65.38	0.07	33.14	0.09
Leu104	8.38	0.01	117.58	0.1	57.24	0.14	43.53	0.15
Leu105	7.06	0.01	115.08	0.14	56.13	0.09	42.14	0.06
Lys106	7.2	0	119.38	0.14	57.44	0.14	33.78	0.09
Ser107	8.11	0.01	117.18	0.14	59.08	0.09	65.29	0.06
Asp108	7.94	0.01	127.88	0.14	56.79	0.04	43.15	0.01

Table 7 – Chemical Shift Assignments (in ppm) For Holo-A77V-TrpR.

<u>Residue</u>	<u>H^N</u>	<u>error</u>	<u>N</u>	<u>error</u>	<u>C^α</u>	<u>error</u>	<u>C^β</u>	<u>Error</u>
Met1	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	54.66	0.03	19.2	0.08
Gln3	8.28	0.01	119.78	0	56.57	0.71	29.88	0.13
Gln4	8.45	0	121.98	0	56.24	0.18	29.97	0.2
Ser5	8.36	0.01	118.08	0	56.14	0	63.76	0
Pro6	-	-	-	-	63.93	0.19	32.1	0.03
Tyr7	7.93	0	118.98	0	58.17	0.15	38.97	0.21
Ser8	7.92	0.01	117.18	0	58.79	0.2	64.2	0.18
Ala9	8.33	0	126.18	0	54.18	0.1	19.11	0.13
Ala10	8.09	0.01	121.38	0	54.04	0.13	18.94	0.05
Met11	7.99	0	118.28	0	56.92	0.17	32.79	0.06
Ala12	8.11	0	122.98	0	54.66	0.18	18.94	0.01
Glu13	8.19	0.01	119.18	0.03	52.84	11.5	29.66	0.06
Gln14	8.19	0.01	120.68	0	59.06	0.06	28.76	0.01
Arg15	8.37	0.01	117.88	0	59.46	0.65	31.59	0.94
His16	7.68	0.01	117.28	0.03	56.17	0.36	32.18	4.66
Gln17	8.37	0.01	118.18	0	58.93	0.21	29.35	0.65
Glu18	8.48	0.01	120.18	0	59.55	0.31	29.42	0.43
Trp19	8.13	0.01	122.18	0	59.51	0	29.31	0
Leu20	-	-	-	-	57.72	0.04	40.97	0
Arg21	7.95	0.01	119.68	0	59.95	0.27	30.21	0.12
Phe22	7.81	0.01	120.18	0	62.38	0.17	37.71	0.16
Val23	7.81	0.01	119.28	0	67	0.11	31.41	0.17
Asp24	8.06	0.01	121.28	0	57.54	0.18	41.87	0.22
Leu25	8.15	0.01	123.98	0	57.99	0	42.75	0
Leu26	8.22	0	119.28	0	57.61	0.1	40.79	0.09
Lys27	7.53	0	120.68	0	60.3	0.29	32.63	0.15
Asn28	7.74	0	116.58	0	56.09	0.22	39.2	0.09
Ala29	8.94	0.01	126.08	0	55.9	0.19	18.02	0.13
Tyr30	8.45	0.01	119.18	0	62.01	0.21	38.4	0.19
Gln31	7.43	0.01	116.98	0	58.29	0.19	28.77	0.14
Asn32	7.36	0.01	115.48	0	53.42	0.17	40.67	0.2

Asp33	7.94	0.01	116.98	0	56.43	0.31	39.92	0.4
Leu34	8.43	0.27	118.08	1.13	53.82	0.11	40.65	5.94
His35	9.09	0.01	120.88	0	59.85	0.15	39.17	14.64
Leu36	7.47	0.01	119.38	0	59.7	0	37.67	0
Pro37	-	-	-	-	65.74	0.15	31.36	0.12
Leu38	8.03	0	119.68	0	58.86	0.19	41.76	0.21
Leu39	8.92	0.01	120.78	0	58.64	0.21	41.95	0.14
Asn40	8.21	0.01	117.98	0	55.26	0.18	38.13	0.05
Leu41	7.77	0	118.98	0	58.63	0.14	39.86	4.37
Met42	8.7	0	114.38	0	56.48	0.2	32.65	0
Leu43	7.64	0.01	115.68	0	54.04	0	44.7	0
Thr44	-	-	-	-	-	-	-	-
Pro45	-	-	-	-	65.97	0.35	32.04	0.05
Asp46	7.99	0.01	114.58	0	56.75	0.22	40.48	0.16
Glu47	7.82	0.01	122.68	0	59.04	0.12	30	0.05
Arg48	7.6	0.01	117.28	0	60.8	0.12	30.49	0.06
Glu49	7.72	0.01	117.28	0	59.59	0.2	26.63	5.28
Ala50	8.06	0.01	121.98	0	55.34	0.04	17.81	0.06
Leu51	8.11	0.01	118.68	0	58.8	0	41.06	0
Gly52	8.04	0	133.98	0	-	-	-	-
Thr53	-	-	-	-	-	-	-	-
Arg54	8.68	0	121.68	0	60.94	0.12	30.75	0.17
Val55	7.8	0.01	117.98	0.03	67.5	0.23	31.86	0.2
Arg56	7.17	0.01	118.78	0	58.33	0	28.87	0
Ile57	-	-	-	-	-	-	-	-
Val58	8.47	0.01	118.58	0	68.19	0.27	31.89	0.07
Glu59	8.77	0.01	118.88	0	60.62	0.19	30.24	0.12
Glu60	8.3	0	114.78	0	57.83	0.28	29.47	0.23
Leu61	9.07	0.01	124.48	0	58.19	0.25	42.25	0.62
Leu62	8.26	0.01	119.18	0	53.91	6.47	42.55	0.02
Arg63	8.37	0	119.48	0	59.48	0	30.65	0
Gly64	7.64	0	132.18	0	45.02	0.07	-	-
Glu65	8.61	0	120.98	0	57.75	0.15	31.68	0.13
Met66	8.67	0	118.88	0	56.28	0.22	35.67	0.15
Ser67	8.82	0.01	118.58	0	57.45	0.06	65.24	0.18
Gln68	8.87	0.01	120.18	0	60.83	0	28.48	0
Arg69	-	-	-	-	59.73	0.12	30.54	0.04
Glu70	7.62	0.01	119.28	0	59.45	0.04	30.42	0.13
Leu71	8.45	0.01	121.28	0	58.19	0	42.65	0

Lys72	-	-	-	-	-	-	-	-
Asn73	-	-	-	-	55.78	0.11	38.93	0.17
Glu74	8.49	0.01	119.78	0	58.93	0.16	30.16	0.11
Leu75	8.07	0.01	113.88	0	55.12	0.16	43.75	0.22
Gly76	7.94	0.01	108.48	0	47.42	0.13	-	-
Val77	7.08	0.01	109.68	0	58.6	0.3	34.92	0.26
Gly78	8.28	0	105.98	0.04	44.9	0	-	-
Ile79	-	-	-	-	-	-	-	-
Ala80	-	-	-	-	-	-	-	-
Thr81	-	-	-	-	-	-	-	-
Ile82	-	-	-	-	-	-	-	-
Thr83	-	-	-	-	-	-	-	-
Arg84	7.81	0	121.68	0	59.75	0	29.41	0
Gly85	7.5	0.01	109.18	0	47.46	0.17	-	-
Ser86	8.32	0	116.18	0	61.66	0	63.03	0
Asn87	-	-	-	-	-	-	-	-
Ser88	-	-	-	-	-	-	62.75	0.11
Leu89	8.34	0	123.78	0	58.21	0.12	42.62	0.12
Lys90	7.82	0.01	117.98	0	59.41	0.13	32.55	0.01
Ala91	7.12	0.01	118.18	0	52.05	0.11	19.68	0.2
Ala92	7.34	0.01	123.08	0	50.66	0	17.78	0
Pro93	-	-	-	-	63.03	0.2	32.3	0.16
Val94	8.83	0.01	124.98	0	66.62	0.12	31.24	1.47
Glu95	9.7	0.01	118.88	0	60.2	0.16	28.86	0.14
Leu96	7.29	0.01	117.18	0	57.5	0	42.26	0
Arg97	8	0	119.38	0	61.6	0.14	30.37	0.13
Gln98	8.74	0	116.08	0	59.29	0.08	28.85	0.16
Trp99	7.5	0.01	121.28	0	62.08	0.29	28.77	0.21
Leu100	8.88	0	117.38	0	57.94	0.2	42.92	0.13
Glu101	8.02	0.01	117.18	0	60.32	0.2	29.83	0.08
Glu102	7.44	0.01	117.98	0	59.32	0.08	29.89	0.18
Val103	7.97	0.01	113.18	0	64.58	0.17	32.34	0.22
Leu104	8.4	0.01	117.28	0	56.45	0.17	42.76	0.19
Leu105	7.06	0.01	114.98	0	55.28	0.14	41.34	0.01
Lys106	7.21	0.01	119.18	0	56.67	0.11	32.75	0.02
Ser107	8.13	0.01	117.38	0	58.23	0.17	64.49	0.1
Asp108	7.95	0	127.68	0	55.89	0	42.31	0

Table 8 – ^{15}N -NMR Relaxation Parameters Measured For Apo-WT-TrpR At pH 5.7 And 318 K.

Residue	$^{15}\text{N}\{-^1\text{H}\}$ - nOe	$^{15}\text{N}\{-^1\text{H}\}$ -nOe error	$^{15}\text{N}\text{-T}_1$ (ms)	$^{15}\text{N}\text{-T}_1$ error (ms)	$^{15}\text{N}\text{-T}_2$ (ms)	$^{15}\text{N}\text{-T}_2$ error (ms)
Met1	-	-	-	-	-	-
Ala2	-	-	-	-	-	-
Gln3	-	-	-	-	-	-
Gln4	-2.15	0.83	1544.3	438.4	989.3	740
Ser5	-	-	-	-	-	-
Pro6	-	-	-	-	-	-
Tyr7	-0.24	0.1	790	28.8	234.3	93.5
Ser8	-	-	-	-	-	-
Ala9	-0.75	1.06	424.2	93.5	166.5	22.2
Ala10	-	-	-	-	-	-
Met11	0.41	0.12	437.6	427	117.8	23.7
Ala12	0.39	0.02	1101.3	580.6	116.7	14.5
Glu13	-	-	-	-	-	-
Gln14	0.6	0.24	823.2	94.8	119	45.3
Arg15	-	-	-	-	-	-
His16	-	-	-	-	-	-
Gln17	0.61	0.05	813.6	157.9	83.4	9.3
Glu18	0.71	0.1	765	95.6	86.6	10.1
Trp19	0.79	0.06	789.1	40.3	80.2	8.3
Leu20	0.78	0.02	818.5	77.5	76.2	4.6
Arg21	-	-	-	-	-	-
Phe22	0.77	0.06	904.6	272.1	77.1	5.8
Val23	0.82	0.07	812.4	47.8	75.7	6.3
Asp24	-	-	-	-	-	-
Leu25	0.8	0.04	825.2	90.1	77.6	5.7
Leu26	0.79	0.01	845.4	39.5	75.1	3.8
Lys27	0.79	0.08	843.5	172.4	56.1	2.6
Asn28	0.81	0.04	816.5	95.5	73.1	6.5
Ala29	0.78	0.05	782.8	48.4	78.4	3.5
Tyr30	0.81	0.04	829.1	73.5	72.6	6.7
Gln31	0.81	0.07	874.4	104.7	77.9	8.6

Asn32	0.79	0.06	859.4	48.7	79.7	5.8
Asp33	0.75	0.08	770.2	6.6	77.5	10.1
Leu34	0.79	0.04	808.5	46.3	81.7	7.2
His35	0.87	0.05	799	59.7	73.3	3.9
Leu36	0.8	0.07	816	38	76	4.3
Pro37	-	-	-	-	-	-
Leu38	0.73	0.06	854.4	124.8	79.1	3.2
Leu39	0.84	0.02	796.8	106.4	72.4	3.7
Asn40	0.8	0.07	821.5	191.3	71.3	6.5
Leu41	0.85	0.04	830.1	71.7	78.1	5.3
Met42	0.83	0.09	810	172.9	51.4	53.1
Leu43	0.81	0.03	887.4	88.5	71.3	3.6
Thr44	-	-	-	-	-	-
Pro45	-	-	-	-	-	-
Asp46	0.77	0.06	875.6	38.5	71.5	4.3
Glu47	0.81	0.06	917.4	69.7	71.6	5.2
Arg48	0.83	0.05	861.5	37.4	75.6	5.8
Glu49	0.82	0.07	849.7	85.2	70.3	1.6
Ala50	0.84	0.05	879	124	70.7	4.9
Leu51	0.8	0.11	886.7	140.3	72.2	5.5
Gly52	0.79	0.03	847.7	105.5	73.6	3.9
Thr53	-	-	-	-	-	-
Arg54	0.8	0.05	851.1	38.2	72.2	9.6
Val55	0.82	0.03	776.7	65.3	75.4	6.4
Arg56	-	-	-	-	-	-
Ile57	-	-	-	-	-	-
Val58	0.85	0.11	855.7	198.3	67.8	3.6
Glu59	-	-	-	-	-	-
Glu60	0.78	0.01	853.2	92.4	54.4	1.4
Leu61	0.77	0.05	866.2	141.4	56.5	11.5
Leu62	-	-	-	-	-	-
Arg63	0.75	0.05	841.1	46.1	73.2	4.4
Gly64	0.72	0.06	864.6	142.4	46.7	1.4
Glu65	-	-	-	-	-	-
Met66	-	-	-	-	-	-
Ser67	0.77	0.12	910.7	222.1	80.8	5.1
Gln68	0.63	0.06	702.2	124.4	90.6	10.9
Arg69	0.58	0.06	770.6	66.9	78.5	5.2
Glu70	-	-	-	-	-	-

Leu71	0.52	0.05	744.3	26.1	95.9	5.6
Lys72	-	-	-	-	-	-
Asn73	-	-	-	-	-	-
Glu74	-	-	-	-	-	-
Leu75	-	-	-	-	-	-
Gly76	0.46	0.03	882.2	164.1	100.8	8.2
Ala77	-	-	-	-	-	-
Gly78	0.45	0.02	823.1	37.9	109.2	9.9
Ile79	-	-	-	-	-	-
Ala80	-	-	-	-	-	-
Thr81	-	-	-	-	-	-
Ile82	0.66	0.01	806.1	147.9	62.3	4.6
Thr83	-	-	-	-	-	-
Arg84	-	-	-	-	-	-
Gly85	0.63	0.06	785.1	217.7	75.7	2.7
Ser86	0.68	0.05	713.1	86.3	82.5	4.4
Asn87	0.65	0.04	700.3	46.8	81.8	9.3
Ser88	-	-	-	-	-	-
Leu89	0.71	0.06	752.1	120.7	71.8	4.5
Lys90	0.74	0.04	774.1	88.7	76.8	1.5
Ala91	0.74	0.07	856.1	181.6	78.8	5.7
Ala92	0.72	0.01	901.7	118.3	77.3	2.5
Pro93	-	-	-	-	-	-
Val94	0.71	0.05	783	32.2	81.3	6
Glu95	0.77	0.01	864.2	192.5	75.9	8.3
Leu96	0.77	0.01	826.3	143.2	77.1	5.5
Arg97	0.81	0.03	798.2	73.3	76.2	5.5
Gln98	0.8	0.06	835.3	91.6	74.9	8.3
Trp99	0.77	0.04	806.4	93.3	77.2	6.4
Leu100	0.8	0.05	791	47.2	76.3	5.9
Glu101	-	-	-	-	-	-
Glu102	0.76	0.02	980	170	79.6	6.9
Val103	0.74	0.03	815	52.7	82.4	7.4
Leu104	0.73	0.04	767.2	37.9	81.9	7.4
Leu105	0.73	0.08	887.9	118.8	81	6.2
Lys106	0.3	0.05	802.8	92.2	150.5	15.8
Ser107	-0.37	0.02	827.9	20.7	387.4	35.6
Asp108	-1.21	0.01	1369.5	143.4	649.6	132.4

Table 9 – ^{15}N -NMR Relaxation Parameters Measured For Apo-L75F-TrpR At pH 5.7 And 318 K.

Residue	$^{15}\text{N}\{-^1\text{H}\}$ - nOe	$^{15}\text{N}\{-^1\text{H}\}$ - nOe error	$^{15}\text{N}\text{-T}_1$ (ms)	$^{15}\text{N}\text{-T}_1$ error (ms)	$^{15}\text{N}\text{-T}_2$ (ms)	$^{15}\text{N}\text{-T}_2$ error (ms)
Met1	-	-	-	-	-	-
Ala2	-	-	-	-	-	-
Gln3	-	-	-	-	-	-
Gln4	-1.38	0.06	1093	118.9	612.9	32.3
Ser5	-0.65	0.14	776.4	34.6	395.7	21.5
Pro6	-	-	-	-	-	-
Tyr7	-0.21	0.01	769.9	34.4	296.4	15.3
Ser8	-0.02	0.04	732.5	42.1	229	8.9
Ala9	0.15	0.03	746.5	24.4	164.5	6.5
Ala10	0.23	0.02	779.6	21.8	151.8	4.2
Met11	0.32	0.03	694.3	40.9	136.4	3.9
Ala12	0.39	0.03	753	60.1	108.6	1.4
Glu13	0.56	0.12	760.2	107.8	101.3	1.1
Gln14	0.51	0.03	726.1	55	98.2	2.3
Arg15	0.62	0.05	772.2	16.9	92.7	3.2
His16	0.77	0.07	857	33.6	71.4	1.2
Gln17	0.77	0.06	848.3	31.2	77.2	1.1
Glu18	0.74	0.06	797.7	94.1	80.4	0.7
Trp19	0.76	0.07	809.6	28.4	75.6	1.8
Leu20	0.82	0.02	829.6	36.9	72.6	2.3
Arg21	0.81	0.04	812.4	48.2	75.3	1.9
Phe22	0.78	0.04	795.7	169	76.2	1.9
Val23	0.85	0.06	837.1	6.8	73.1	1.3
Asp24	0.78	0.03	848.8	16.1	67.1	1
Leu25	0.81	0.06	765.6	69.5	75.1	0.3
Leu26	-	-	-	-	-	-
Lys27	0.82	0.08	844.1	55.9	61.6	3.3
Asn28	0.79	0.03	844	8.8	72	0.8
Ala29	0.85	0.04	783.4	39	78.1	1.8
Tyr30	0.79	0.02	864.2	42.9	73.1	1.6

Gln31	0.81	0.04	807.3	58.7	75.2	2.5
Asn32	0.8	0.03	867.6	72.1	79.7	1.2
Asp33	0.83	0.02	828.7	48	71.4	0.6
Leu34	0.78	0.05	848.3	31.2	77.2	1.1
His35	0.86	0.07	801.8	26.6	72.9	2.2
Leu36	0.76	0.03	808.8	14.1	73.1	1.4
Pro37	-	-	-	-	-	-
Leu38	0.78	0.04	797.3	15.9	73.2	0.2
Asn40	0.79	0.05	749.5	157.4	74.9	0.3
Leu41	0.79	0.04	806.7	43.8	75.8	1.4
Met42	0.82	0.08	830.1	43.1	72.3	3.9
Leu43	0.77	0.08	807.9	62.9	75.2	0.7
Thr44	0.8	0.02	893.1	73.4	77.5	0.6
Pro45	-	-	-	-	-	-
Asp46	0.82	0.06	886.7	65.9	72.1	1.6
Glu47	0.79	0.02	885.9	29.3	68.6	1.5
Arg48	0.82	0.04	903.2	79.5	73.8	0.9
Glu49	0.78	0.04	894.8	93.7	71.1	0.8
Ala50	0.77	0.04	895.1	77.7	71.4	3.3
Leu51	-	-	-	-	-	-
Gly52	0.81	0.04	831.9	45.3	72.3	2.1
Thr53	0.68	0.09	764.4	77.4	77.7	2.9
Arg54	0.89	0.07	833.2	62.3	67.3	0.5
Val55	0.77	0.04	817.5	210.1	70.6	0.4
Arg56	0.78	0.02	783.2	202	69.1	1.9
Ile57	-	-	-	-	-	-
Val58	0.79	0.03	856.1	70	68.2	1.1
Glu59	0.78	0.09	853.9	26.3	72.4	1.6
Glu60	0.8	0.09	875.3	45.5	65.6	1.8
Leu61	0.77	0.01	824.7	80.1	65	5.1
Leu62	0.82	0.06	857.1	28.7	70.6	1.1
Arg63	-	-	-	-	-	-
Gly64	0.74	0.05	754.8	81.3	60.3	1.5
Glu65	0.66	0.02	832	135.6	85.2	1.3
Met66	0.65	0.05	867.3	76.8	94.1	1.7
Ser67	0.62	0.12	781.2	97.1	81.2	4.9
Gln68	0.59	0.07	784	20.6	88.3	3.6
Arg69	0.64	0.02	829.1	4.8	82.4	4.5
Glu70	0.65	0.05	783.8	37	85.1	0.6

Leu71	0.66	0.03	786.7	41	81.8	0.7
Lys72	-	-	-	-	-	-
Asn73	0.67	0.03	826.1	39.6	80.3	0.3
Glu74	0.65	0.07	679.8	19.2	80.4	1.5
Phe75	0.6	0.05	796.3	48.2	67.2	2.2
Gly76	0.56	0.03	847.6	20.6	81.7	0.7
Ala77	-	-	-	-	-	-
Gly78	0.6	0.05	799.1	24.1	97.1	4.3
Ile79	0.67	0.1	734.8	64.8	66.4	1
Ala80	0.72	0.09	747.4	67.6	87.2	2
Thr81	0.63	0.02	765.6	21.4	76	1.1
Ile82	0.72	0.11	840.1	69.5	50.6	0.4
Thr83	-	-	-	-	-	-
Arg84	0.6	0.02	758.3	44.8	90.6	2.5
Gly85	0.5	0.04	747.5	49.6	94.7	1.3
Ser86	0.45	0.09	780.9	79.4	77.4	1.7
Asn87	-	-	-	-	-	-
Ser88	0.52	0.1	795.1	78.4	59.3	1.9
Leu89	0.62	0.1	809.3	36.8	68.5	1.6
Lys90	0.48	0.2	890.3	110.4	77.1	4.8
Ala91	0.64	0.02	822.1	16.8	81.7	1.7
Ala92	0.68	0.04	927.8	65.2	80.6	0.4
Pro93	-	-	-	-	-	-
Val94	0.71	0.06	854.2	44.2	78.9	2.8
Glu95	0.78	0.02	788.4	26.9	74.1	1.3
Leu96	0.79	0.02	830.9	77.5	76	2.1
Arg97	0.79	0.06	863	78.2	77.3	1.4
Gln98	0.79	0.03	868.6	69.8	71.9	0.5
Trp99	0.78	0.03	871.1	116.7	75.4	1.1
Leu100	0.76	0.04	856.3	59.6	74.1	1.3
Glu101	0.75	0.04	835.9	69	74.7	0.7
Glu102	0.72	0.11	882.7	50.4	76	0.5
Val103	0.76	0.03	880.7	64.5	77.7	0.5
Leu104	0.77	0.01	819.5	12.3	79	2.7
Leu105	0.7	0.05	828.1	140.1	79.6	1.5
Lys106	0.29	0.09	807.9	98.5	160.3	4.7
Ser107	-0.38	0.03	847.3	20.3	407.2	19.5
Asp108	-1.13	0.12	1352.8	112.7	751.1	135.1

Table 10 – ^{15}N -NMR Relaxation Parameters Measured For Apo-A77V-TrpR At pH 5.7 And 318 K.

Residue	^{15}N - $\{^1\text{H}\}$ - nOe	^{15}N - $\{^1\text{H}\}$ - nOe error	^{15}N - T_1 (ms)	^{15}N - T_1 error (ms)	^{15}N - T_2 (ms)	^{15}N - T_2 error (ms)
Met1	-	-	-	-	-	-
Ala2	-	-	-	-	-	-
Gln3	-	-	-	-	-	-
Gln4	-1.64	0.19	1279	60.8	777	36.8
Ser5	-0.52	0.23	889.5	136.8	413.7	71.8
Pro6	-	-	-	-	-	-
Tyr7	-	-	-	-	-	-
Ser8	-	-	-	-	-	-
Ala9	0.09	0.14	824.3	3.5	149.6	52.4
Ala10	0.54	0.28	824.7	1.1	98.2	23.8
Met11	0.66	0.09	868.8	10.2	108	16.9
Ala12	0.39	0.02	749.2	103.9	114.2	1
Glu13	0.56	0.11	821.8	19	97.72	8.5
Gln14	0.52	0.05	688	29.6	102.1	0.2
Arg15	0.78	0.22	707.3	18.3	85.4	3.1
His16	-	-	-	-	-	-
Gln17	-	-	-	-	-	-
Glu18	0.77	0.03	724.7	32.7	80.4	1.6
Trp19	0.76	0.02	800.4	18.3	81	1.8
Leu20	0.78	0.09	748.6	153.2	73.2	1.6
Arg21	0.66	0.04	837.4	40.7	78.5	0.5
Phe22	0.82	0.02	824	72.8	76.1	0.6
Val23	0.74	0.06	899.2	62.5	81.3	1.3
Asp24	0.73	0.05	809.9	17.4	66.2	6
Leu25	0.76	0.07	787.6	18.9	81	3.3
Leu26	0.8	0.02	833.4	4.2	85.8	13
Lys27	0.81	0.03	884.4	12.4	52.1	0.5
Asn28	0.81	0.04	825.6	3.7	74.8	1
Ala29	0.83	0.02	858.7	5.4	76.2	0.2
Tyr30	0.82	0.05	851	36.6	69.9	2.5

Gln31	0.81	0.02	852.2	45.5	75.5	1.5
Asn32	0.8	0.04	940.8	26.1	82.6	1.6
Asp33	0.81	0.04	891.5	57.4	71.3	2.1
Leu34	0.78	0.01	806.1	32.1	79.1	0.6
His35	0.81	0.11	828	96.8	70.7	0.6
Leu36	0.85	0.06	781.4	60.7	75.6	0.1
Pro37	-	-	-	-	-	-
Leu38	-	-	-	-	-	-
Leu39	0.82	0.01	835.4	26.2	73.7	1.4
Asn40	0.83	0.01	801.5	15.3	74.9	1.4
Leu41	0.81	0.02	862.6	120.1	79.5	5.1
Met42	0.79	0.05	773	27.8	78.7	0.9
Leu43	0.78	0.03	868	50.3	72.9	1.8
Thr44	-	-	-	-	-	-
Pro45	-	-	-	-	-	-
Asp46	0.78	0.03	913.6	18.5	72.9	2.5
Glu47	0.87	0.11	921.4	8.8	74.1	3.2
Arg48	0.8	0.06	867.8	65.4	93.9	23.6
Glu49	0.8	0.04	841.8	22.3	73.6	3.18
Ala50	0.83	0.02	843.2	93.9	70.9	1
Leu51	0.75	0.03	896.5	8.6	71.8	1.4
Gly52	0.8	0.05	880.9	53	73.1	0.7
Thr53	-	-	-	-	-	-
Arg54	0.75	0.04	770.4	41.5	77	5
Val55	-	-	-	-	-	-
Arg56	-	-	-	-	-	-
Ile57	0.82	0.02	850.4	78.4	70.4	0.2
Val58	-	-	-	-	-	-
Glu59	-	-	-	-	-	-
Glu60	0.77	0.04	812.1	3	67.2	3.2
Leu61	0.79	0.02	890.7	64.4	68.2	2.6
Leu62	0.83	0.07	804.4	55.2	76.7	0.8
Arg63	0.78	0.03	826.8	28.07	74.6	4.5
Gly64	0.72	0.04	854	7	70.2	1.7
Glu65	0.78	0.07	926.2	43.6	77.1	10.4
Met66	0.72	0.01	946.8	31.5	83.5	2.4
Ser67	0.69	0.05	903	27.5	86.2	0.9
Gln68	0.69	0.03	792.4	5.3	98.4	5.1
Arg69	0.68	0.03	792.6	67.8	79.3	1.1

Glu70	0.68	0.02	814.1	87.3	86.2	3.7
Leu71	0.72	0.04	779.6	26.4	81.4	0.3
Lys72	-	-	-	-	-	-
Asn73	0.72	0.04	806.7	93.1	79.9	0.1
Glu74	0.71	0.04	825.1	63.4	85.3	1.4
Leu75	0.71	0.04	878.4	69.3	69.1	4.8
Gly76	0.66	0.04	908	15.7	78.8	0.2
Val77	0.62	0.01	988.4	73	66.1	4.4
Gly78	0.64	0.04	925.9	2.3	80	2
Ile79	-	-	-	-	-	-
Ala80	-	-	-	-	-	-
Thr81	-	-	-	-	-	-
Ile82	-	-	-	-	-	-
Thr83	-	-	-	-	-	-
Arg84	-	-	-	-	-	-
Gly85	0.78	0.07	784.9	17.4	81.3	6.9
Ser86	0.79	0.07	818	9.6	74.4	2
Asn87	-	-	-	-	-	-
Ser88	-	-	-	-	-	-
Leu89	-	-	-	-	-	-
Lys90	0.75	0.02	798.4	32.9	80	1
Ala91	0.78	0.03	810.6	7.9	81.7	2.6
Ala92	0.81	0.03	977.7	8.2	81.3	0.1
Pro93	-	-	-	-	-	-
Val94	0.77	0.03	812.2	60	83.9	4.5
Glu95	0.76	0.01	897.4	10.3	75.7	0.3
Leu96	0.82	0.02	772.6	29.5	78.3	0.9
Arg97	0.78	0.02	700.1	270.6	72.2	6.2
Gln98	0.76	0.01	932.1	60.2	75	1.4
Trp99	0.8	0.06	798.3	10.5	72.6	1.7
Leu100	0.8	0.02	880.6	12.7	76.3	0.7
Glu101	0.75	0.03	922.9	6.9	77.1	0.8
Glu102	0.78	0.03	856.8	8.1	79.9	0.1
Val103	0.78	0.03	848.4	40.9	78.7	3.2
Leu104	0.8	0.05	801.3	63.2	80.1	3.2
Leu105	0.67	0.06	856.6	20.9	84.4	2.9
Lys106	0.34	0.05	729.4	1.2	141.4	14.3
Ser107	-0.4	0.04	878.4	73.3	431.5	18.2
Asp108	-1.26	0.03	1561.5	181.7	924.1	91.9

Table 11 – ^{15}N -Relaxation Parameters Measured For Holo-WT-TrpR At pH 5.7 And 318 K.

<u>Residue</u>	$\frac{^{15}\text{N}-\{^1\text{H}\}}{\text{nOe (I/Io)}}$	<u>error (I/Io)</u>	$\frac{^{15}\text{N}-\text{T}_1}{\text{(ms)}}$	$\frac{^{15}\text{N}-\text{T}_1}{\text{error (ms)}}$	$\frac{^{15}\text{N}-\text{T}_2}{\text{(ms)}}$	$\frac{^{15}\text{N}-\text{T}_2}{\text{error (ms)}}$
Met1	-	-	-	-	-	-
Ala2	-	-	-	-	-	-
Gln3	-	-	-	-	-	-
Gln4	-1.6	0.05	1338	77.78	656.9	133.93
Ser5	-	-	-	-	-	-
Pro6	-	-	-	-	-	-
Tyr7	-0.25	0.08	723.5	0.14	230.5	17.82
Ser8	-	-	-	-	-	-
Ala9	0.19	0.04	784.7	15.56	141.55	0.35
Ala10	-	-	-	-	-	-
Met11	0.31	0.04	677.2	45.82	132.75	3.75
Ala12	0.42	0.01	771.45	5.87	110.6	2.12
Glu13	-	-	-	-	-	-
Gln14	0.53	0.05	631.6	89.24	95.47	2.85
Arg15	-	-	-	-	-	-
His16	0.67	0.14	835.3	30.83	77.37	5.02
Gln17	0.69	0.04	726.4	17.4	84.29	2.93
Glu18	0.77	0.01	743.35	79.55	78.28	3.33
Trp19	0.85	0.03	846.15	30.05	73.27	5.01
Leu20	0.84	0.01	830.1	12.45	73.4	5.7
Arg21	0.78	0.04	779.25	50.13	77.69	0.81
Phe22	0.77	0.1	687.55	1.2	76.58	2.09
Val23	0.75	0.05	823.7	11.74	70.83	0.7
Asp24	0.77	0.01	718.95	1.77	75.59	2.87
Leu25	-	-	-	-	-	-
Leu26	-	-	-	-	-	-
Lys27	0.8	0.09	837.3	33.38	56.25	4.08
Asn28	0.78	0.06	850.85	22.7	71.79	2.5
Ala29	0.85	0.02	814.65	51.97	76.03	1.31
Tyr30	0.82	0.01	785.3	17.25	76.9	0.88
Gln31	0.81	0.05	872.15	32.46	74.98	1.33

Asn32	0.8	0.04	815.3	39.6	80.77	2.49
Asp33	0.76	0.02	835.1	55.86	73.45	2.59
Leu34	0.73	0.08	830.3	37.05	77.53	0.81
His35	0.78	0.01	785.75	5.59	73.26	0.86
Leu36	0.81	0.12	859.55	2.33	70.13	4.28
Pro37	-	-	-	-	-	-
Leu38	0.74	0.06	831	40.59	72.84	4.79
Leu39	0.82	0.02	769.6	38.18	75.02	1.9
Asn40	-	-	-	-	-	-
Leu41	0.79	0.02	733.9	56.43	77.12	2.62
Met42	0.86	0.01	784.4	27.15	75.03	1.83
Leu43	0.81	0.07	776.75	8.98	70.66	2.86
Thr44	-	-	-	-	-	-
Pro45	-	-	-	-	-	-
Asp46	0.81	0.02	902.1	19.52	70	0.8
Glu47	0.76	0.03	870.2	1.41	70.1	4.21
Arg48	0.71	0.1	784.5	35.36	80.26	3.7
Glu49	0.78	0.07	876.05	41.37	69.21	1.57
Ala50	0.77	0.08	832.65	97.65	68.69	1.4
Leu51	0.84	0.01	836	70.99	70.74	3.02
Gly52	0.81	0.05	848.75	47.45	71.1	4.09
Thr53	-	-	-	-	-	-
Arg54	0.82	0.07	830.75	6.86	68.77	0.78
Val55	0.8	0.02	782.05	73.89	75.62	2.02
Arg56	-	-	-	-	-	-
Ile57	-	-	-	-	-	-
Val58	0.84	0.03	829.55	66.12	69.93	2.99
Glu59	-	-	-	-	-	-
Glu60	0.8	0.04	802.15	27.93	66.31	2.72
Leu61	0.82	0.01	783.3	132.79	65.71	3.82
Leu62	0.78	0	885.65	30.62	71.73	4.21
Arg63	0.78	0	855.95	11.38	71.45	2.87
Gly64	0.79	0	869.75	30.9	70.16	5.18
Glu65	0.71	0.03	850.45	109.11	84.03	3.16
Met66	-	-	-	-	-	-
Ser67	0.65	0.04	732.9	171.54	83.51	4.23
Gln68	0.62	0.02	836.5	16.55	81.36	4.26
Arg69	-	-	-	-	-	-
Glu70	0.63	0.02	808.5	41.01	84	3.47

Leu71	0.64	0.05	727.95	104.58	86.82	3.56
Lys72	-	-	-	-	-	-
Asn73	0.68	0.07	818.2	20.22	80.69	1.73
Glu74	0.75	0.1	845.9	23.33	85.78	1.38
Leu75	-	-	-	-	-	-
Gly76	-	-	-	-	-	-
Val77	-	-	-	-	-	-
Gly78	0.64	0.16	882.7	55.72	96.62	2.74
Ile79	-	-	-	-	-	-
Ala80	-	-	-	-	-	-
Thr81	-	-	-	-	-	-
Ile82	-	-	-	-	-	-
Thr83	-	-	-	-	-	-
Arg84	0.79	0.02	699	7.78	75.91	1.22
Gly85	0.78	0.02	760.35	67.25	74.63	3.51
Ser86	0.81	0.03	731.95	12.23	73.97	1.15
Asn87	0.8	0.02	785.3	17.25	76.9	0.88
Ser88	-	-	-	-	-	-
Leu89	0.79	0.03	805.45	27.93	77.58	1.73
Lys90	0.8	0.02	782.05	73.89	75.62	2.02
Ala91	0.76	0.03	778.55	17.75	79.7	2.43
Ala92	0.78	0.07	954.65	49.14	76.39	0.23
Pro93	-	-	-	-	-	-
Val94	0.76	0.06	846.35	47.02	79.99	2.97
Glu95	0.8	0.03	819.75	72.05	73.45	2.86
Leu96	0.88	0.02	785.95	12.94	76.63	1.17
Arg97	0.78	0.04	779.25	50.13	77.69	0.81
Gln98	0.82	0.01	848.1	53.32	72.01	1.86
Trp99	0.77	0.04	769.5	80.61	75.84	0.57
Leu100	0.78	0.02	771.45	45.47	72.26	1.01
Glu101	0.73	0.04	879.2	45.26	73.25	2.4
Glu102	0.79	0.01	850.3	97.02	76.9	2.6
Val103	0.79	0.01	866.45	45.18	77.26	2.33
Leu104	0.72	0.06	809.05	61.17	78.15	3.09
Leu105	0.66	0.04	761.55	21.14	81.15	3.58
Lys106	0.29	0.02	680.55	49.99	148.95	4.31
Ser107	-0.36	0.01	824.75	29.63	389.15	34.15
Asp108	-1.23	0.02	1260	106.07	807.1	27.86

Table 12 – ^{15}N -Relaxation parameters measured for holo-L75F-TrpR at pH 5.7 and 318 K.

<u>Res</u>	<u>$^{15}\text{N}\{-^1\text{H}\}$ nOe (I/Io)</u>	<u>error (I/Io)</u>	<u>$^{15}\text{N-T}_1$ (ms)</u>	<u>$^{15}\text{N-T}_1$ error (ms)</u>	<u>$^{15}\text{N-T}_2$ (ms)</u>	<u>$^{15}\text{N-T}_2$ error (ms)</u>
Met1	-	-	-	-	-	-
Ala2	-	-	-	-	-	-
Gln3	-	-	-	-	-	-
Gln4	-	-	-	-	-	-
Ser5	-0.97	0.28	1046.8	242.11	654.9	278.74
Pro6	-	-	-	-	-	-
Tyr7	-0.27	0.03	741.85	42.78	306.15	14.78
Ser8	-0.01	0.61	740.2	51.9	235.15	8.84
Ala9	0.19	0.04	734.3	114.55	147.55	11.67
Ala10	0.29	0.02	755.6	49.78	146.6	0.57
Met11	-	-	-	-	-	-
Ala12	0.42	0.03	735.1	58.55	114.95	17.47
Glu13	0.49	0.03	817.1	0.85	102.75	1.2
Gln14	0.54	0.02	770	29.27	105.9	0.28
Arg15	0.49	0.1	768.65	2.47	94.38	4.87
His16	-	-	-	-	-	-
Gln17	0.7	0.03	730.7	63.92	89.07	2.2
Glu18	0.78	0.02	740.4	17.68	82.97	5.74
Trp19	0.79	0.05	817.35	65.41	81.11	4.2
Leu20	0.82	0.05	854.95	21.85	74.82	0.88
Arg21	0.8	0.09	833.05	28.64	76.67	0.17
Phe22	0.82	0.04	697.35	148.14	78.97	1.24
Val23	0.79	0.06	840.4	36.77	75.63	6.99
Asp24	-	-	-	-	-	-
Leu25	0.79	0.06	761.85	47.31	80.27	7.98
Leu26	0.78	0.03	777.55	8.56	76.65	3.89
Lys27	0.89	0.02	819.45	10.68	57.22	11.89
Asn28	0.78	0.07	780.25	69.51	79.45	7.06
Ala29	0.84	0.04	771.25	32.46	77.78	1.39
Tyr30	0.85	0.03	777.05	130.32	70.06	1.93

Gln31	0.78	0.06	730.3	59.68	75.31	3.08
Asn32	0.81	0.07	823.15	32.46	82.62	1.84
Asp33	0.76	0.03	836.35	87.47	75.72	8.32
Leu34	0.75	0.06	791.5	80.19	78.94	0.13
His35	0.85	0.1	792.45	82.66	70.57	0.51
Leu36	0.83	0.07	812.45	65.97	82.21	11.84
Pro37	-	-	-	-	-	-
Leu38	0.71	0.05	767.2	101.12	89.9	6.94
Leu39	0.83	0.02	838.65	4.31	75.94	0.86
Asn40	0.8	0.04	759.95	0.64	74.69	8.85
Leu41	0.82	0.05	787.35	134.42	78.22	6.17
Met42	-	-	-	-	-	-
Leu43	0.78	0.01	706.4	119.36	74.87	0.11
Thr44	0.78	0.11	804.7	86.27	71.3	11.55
Pro45	-	-	-	-	-	-
Asp46	0.82	0.05	908.55	147.71	72.03	3.19
Glu47	0.76	0.05	889.4	67.6	71.05	4.39
Arg48	0.63	0.02	802.3	3.82	84.22	3.13
Glu49	0.82	0.07	819.4	11.31	79.51	4.53
Ala50	0.77	0.07	843.15	6.86	72.69	2.1
Leu51	0.82	0.03	818.05	9.97	65.96	0.1
Gly52	0.81	0.06	898.75	138.81	71.96	4.72
Thr53	-	-	-	-	-	-
Arg54	0.86	0.12	923.45	47.73	68.02	7.21
Val55	0.83	0.03	777.7	61.8	71.92	2.91
Arg56	0.77	0.06	719.45	85.21	74.2	1.14
Ile57	-	-	-	-	-	-
Val58	-	-	-	-	-	-
Glu59	0.82	0.02	872.7	66.04	73.85	2.38
Glu60	0.78	0.03	830.05	7.57	72.22	7.42
Leu61	0.83	0.06	799.8	53.46	67.46	7.01
Leu62	0.78	0.03	841.65	2.47	76.36	9.44
Arg63	0.78	0.03	862.4	26.87	76.61	5
Gly64	0.77	0.02	905.3	200.39	72.42	1.55
Glu65	0.76	0.05	754.95	111.09	79.76	0.77
Met66	0.63	0.04	889.2	50.35	95.09	2.79
Ser67	-	-	-	-	-	-
Gln68	0.64	0.09	677.85	63.57	96.91	17.53

Arg69	-	-	-	-	-	-
Glu70	0.64	0.01	901.6	35.64	91.97	2.84
Leu71	-	-	-	-	-	-
Lys72	0.66	0.03	758	14.71	86.39	4.45
Asn73	0.66	0.05	835.35	15.49	82.62	3.13
Glu74	-	-	-	-	-	-
Phe75	-	-	-	-	-	-
Gly76	0.57	0.03	776.15	0.92	103.29	9.49
Ala77	-	-	-	-	-	-
Gly78	0.56	0.03	801.05	72.05	121.55	9.4
Ile79	-	-	-	-	-	-
Ala80	-	-	-	-	-	-
Thr81	0.68	0.05	848.5	15.42	66.2	12.46
Ile82	-	-	-	-	-	-
Thr83	-	-	-	-	-	-
Arg84	0.76	0.06	808.25	44.05	82.2	1.77
Gly85	0.81	0.05	756.1	44.83	-	-
Ser86	0.8	0.04	735.35	58.34	80.84	0.28
Asn87	0.81	0.01	835.85	46.03	79.25	6.07
Ser88	-	-	-	-	-	-
Leu89	0.75	0.03	761.4	69.16	68.22	10.06
Lys90	0.8	0.01	817.4	21.78	83.03	3.03
Ala91	-	-	-	-	-	-
Ala92	0.78	0.04	789.8	105.36	84.46	5.03
Pro93	-	-	-	-	-	-
Val94	0.77	0.04	795.25	50.42	87.93	0.5
Glu95	0.8	0.03	633.35	55.23	78.44	2.91
Leu96	0.8	0.05	749.95	8.84	79.13	0.18
Arg97	0.82	0.06	833.05	28.64	76.67	0.17
Gln98	0.77	0.03	851.05	82.8	77.06	3.51
Trp99	0.77	0.03	907.6	30.12	78.15	6.6
Leu100	0.8	0.04	874	42.29	75.35	0.03
Glu101	0.7	0.03	784.1	115.4	80.35	0.69
Glu102	0.81	0.05	791.2	105.36	79.63	3.27
Val103	0.76	0.03	907.35	11.38	79.99	0.21
Leu104	0.7	0.03	802.95	49.85	81.94	5.08
Leu105	0.67	0.06	714.6	43.13	83.95	3.93

Lys106	0.27	0.03	700.8	12.59	157.7	6.93
Ser107	-0.37	0.01	764.55	58.9	388.05	11.38
Asp108	-1.24	0.02	1365	134.35	887.9	188.23

Table 13 – ^{15}N -Relaxation Parameters Measured For Holo-A77V-TrpR At pH 5.7 And 318 K.

<u>Residue</u>	$\frac{^{15}\text{N}-\{^1\text{H}\}}{\text{nOe (I/Io)}}$	$\frac{^{15}\text{N}-\{^1\text{H}\}}{\text{nOe error (I/Io)}}$	$\frac{^{15}\text{N}-\text{T}_1}{\text{(ms)}}$	$\frac{^{15}\text{N}-\text{T}_1}{\text{error (ms)}}$	$\frac{^{15}\text{N}-\text{T}_2}{\text{(ms)}}$	$\frac{^{15}\text{N}-\text{T}_2}{\text{error (ms)}}$
Met1	-	-	-	-	-	-
Ala2	-	-	-	-	-	-
Gln3	-	-	-	-	-	-
Gln4	-	-	-	-	-	-
Ser5	-	-	-	-	-	-
Pro6	-	-	-	-	-	-
Tyr7	-0.22	0.01	758.3	10.47	228.3	4.67
Ser8	0.04	0.06	768.1	14.85	204.65	34.3
Ala9	0.25	0.06	-	-	178.4	37.05
Ala10	0.25	0.03	784	0.71	136.55	2.47
Met11	0.29	0.02	740.95	50.84	132.55	3.32
Ala12	0.43	0.03	824.15	21.57	109.3	1.98
Glu13	0.47	0.03	795.45	61.45	101.54	3.48
Gln14	0.52	0.02	757.5	6.51	90.75	4.29
Arg15	0.75	0.06	910.4	23.62	77.71	2.18
His16	0.76	0.01	960.85	1.48	76.22	0.44
Gln17	-	-	-	-	-	-
Glu18	0.74	0.02	847.1	63.36	77.37	1.35
Trp19	0.71	0.02	846.35	2.05	74.11	2.96
Leu20	-	-	-	-	-	-
Arg21	0.83	0.02	867.3	31.25	73.07	0.71
Phe22	0.83	0.04	852.7	12.3	70.07	1.9
Val23	0.84	0.01	873.5	35.92	64.34	0.55
Asp24	0.75	0.05	844.25	32.03	67.14	1.28
Leu25	0.83	0.05	807.2	30.41	76.23	0.09

Leu26	0.67	0.19	912.95	28.64	71.57	1.46
Lys27	0.75	0.02	909.95	3.18	63.24	1.75
Asn28	-	-	873.4	2.26	73.35	0.9
Ala29	0.77	0.02	815.15	57.91	72.23	2.15
Tyr30	0.84	0.02	935.05	18.17	68.27	3.2
Gln31	0.77	0.01	885.65	6.43	74.47	0.16
Asn32	0.79	0.01	960.55	19.87	81.02	1.69
Asp33	0.83	0.02	928.95	41.79	71.44	0.62
Leu34	0.76	0.01	864.45	51.12	74.23	0.55
His35	0.83	0.03	866.65	18.17	69.72	0.01
Leu36	0.84	0.03	828.15	18.17	74.63	4.67
Pro37	-	-	-	-	-	-
Leu38	0.81	0.03	886.25	1.06	71.13	1.39
Leu39	0.78	0.01	830	31.82	78.03	0.13
Asn40	0.84	0.03	867.85	59.89	73.29	0.81
Leu41	0.75	0.06	839.7	7.5	74.35	0.71
Met42	0.79	0.06	859.4	68.45	72.69	3.87
Leu43	0.8	0.02	937.8	12.45	69.11	5.73
Thr44	-	-	-	-	-	-
Pro45	-	-	-	-	-	-
Asp46	0.83	0.01	973.55	36.7	70.33	0.84
Glu47	0.9	0.1	943.15	61.02	68.41	2.67
Arg48	-	-	912.1	12.3	70.61	1.61
Glu49	0.85	0.04	949.1	79.05	71.55	2.33
Ala50	0.81	0.01	944.15	71.49	72.78	1.09
Leu51	0.81	0.04	994.55	19.02	69.46	1.53
Gly52	0.92	0.17	915.65	62.01	74.9	1.46
Thr53	-	-	-	-	-	-
Arg54	0.83	0.08	897.95	17.04	69.36	1.51
Val55	0.82	0.1	912.5	64.77	68.04	0.34
Arg56	0.79	0.05	867.4	61.94	69.42	1.76
Ile57	-	-	-	-	-	-
Val58	0.81	0.02	895.65	54.52	69.73	4.01
Glu59	0.78	0.05	917.65	9.26	70.13	1.94
Glu60	0.76	0.07	864.95	2.62	66.46	2.99
Leu61	0.8	0.01	909.55	58.2	70.04	1.14
Leu62	0.75	0.07	912.95	28.64	71.5	0.93
Arg63	0.82	0.03	925.45	47.31	71.78	0.52
Gly64	0.87	0.28	911.65	4.88	78.95	4.74

Glu65	0.75	0.01	844.7	71.14	81.88	1.87
Met66	-	-	-	-	-	-
Ser67	0.67	0.02	924.4	1.27	84.68	10.44
Gln68	0.7	0.12	937.45	67.95	72.38	4.55
Arg69	-	-	-	-	-	-
Glu70	0.7	0.05	841.75	25.39	79.91	4.33
Leu71	0.66	0.01	843.05	14.21	79.62	0.51
Lys72	-	-	-	-	-	-
Asn73	-	-	-	-	-	-
Glu74	0.77	0.01	940.75	24.82	82.74	0.64
Leu75	0.72	0.08	878.25	72.76	76.04	0.98
Gly76	0.74	0.03	895.35	96.1	75.9	5.7
Val77	0.67	0.01	1168.5	55.86	82.94	0.07
Gly78	0.61	0.04	956.65	8.98	83.9	0.87
Ile79	-	-	-	-	-	-
Ala80	-	-	-	-	-	-
Thr81	-	-	-	-	-	-
Ile82	-	-	-	-	-	-
Thr83	-	-	-	-	-	-
Arg84	0.81	0.02	874.1	4.81	72.3	0.33
Gly85	0.82	0.04	870.95	52.4	66.97	8.09
Ser86	0.71	0.08	900.05	71.21	74.43	0.98
Asn87	-	-	-	-	-	-
Ser88	-	-	-	-	-	-
Leu89	0.81	0.04	834.6	4.38	75.98	0.28
Lys90	0.84	0.01	872.75	28.78	73.92	0.74
Ala91	0.73	0.05	886.45	39.24	76.8	1.2
Ala92	0.81	0.03	961.45	46.17	75.07	3.3
Pro93						
Val94	0.77	0.05	843.85	41.08	79.59	1.13
Glu95	0.78	0.02	924.2	34.22	70.98	0.23
Leu96	-	-	874.75	44.62	75.12	1.98
Arg97	-	-	855.4	52.19	79.44	1.91
Gln98	0.77	0.02	918	36.91	68.95	1.48
Trp99	0.72	0.08	881.3	78.49	73.18	1.43
Leu100	0.77	0.02	892.25	27.93	71.47	0.01
Glu101	0.7	0.08	857.35	27.22	72.62	0.18
Glu102	0.73	0.04	916.8	32.67	74.55	0.33
Val103	0.81	0.03	884.95	38.96	74.02	1.46

Leu104	0.8	0.04	837.7	21.78	75.74	1.73
Leu105	0.7	0.02	897.6	41.01	79.07	1.57
Lys106	0.3	0.01	808.1	85.7	146	4.24
Ser107	-	-	-	-	-	-
Asp108	-1.26	0.02	1288	69.3	876.75	3.18

Table 14. Spectral Density Values For Apo-WT-TrpR At 14.1 T.

Residue	$J_{\text{eff}}(0)$, ns/rad			$J(\omega_N)$, ns/rad			$J(0.87\omega_H)$, ps/rad		
Met1	-			-			-		
Ala2	-			-			-		
Gln3	-			-			-		
Gln4	-			-			-		
Ser5	-			-			-		
Pro6	-			-			-		
Tyr7	-			-			-		
Ser8	-			-			-		
Ala9	-			-			-		
Ala10	-			-			-		
Met11	-			-			-		
Ala12	3.09	±	0.39	0.25	±	0.1	10.2	±	3.97
Glu13	-			-			-		
Gln14	3.23	±	0.88	0.3	±	0.04	7.67	±	4.58
Arg15	-			-			-		
His16	-			-			-		
Gln17	4.37	±	0.5	0.31	±	0.06	7.66	±	1.72
Glu18	4.19	±	0.52	0.32	±	0.04	5.97	±	2.17
Trp19	4.55	±	0.48	0.31	±	0.02	4.16	±	1.19
Leu20	4.78	±	0.3	0.3	±	0.03	4.22	±	0.55
Arg21	-			-			-		
Phe22	4.74	±	0.36	0.29	±	0.07	4.2	±	1.49
Val23	4.83	±	0.41	0.31	±	0.02	3.45	±	1.33
Asp24	-			-			-		
Leu25	4.7	±	0.36	0.3	±	0.03	3.81	±	0.87

Leu26	4.86	±	0.25	0.29	±	0.01	3.87	±	0.26
Lys27	-			-			-		
Asn28	5.01	±	0.46	0.31	±	0.03	3.67	±	0.86
Ala29	4.63	±	0.21	0.32	±	0.02	4.39	±	1.07
Tyr30	5.06	±	0.47	0.3	±	0.03	3.59	±	0.82
Gln31	4.72	±	0.53	0.29	±	0.03	3.44	±	1.29
Asn32	4.58	±	0.38	0.29	±	0.02	3.83	±	1.11
Asp33	4.73	±	0.61	0.32	±	0.01	5.07	±	1.6
Leu34	4.46	±	0.4	0.31	±	0.02	4.06	±	0.82
His35	4.98	±	0.27	0.32	±	0.02	2.55	±	0.94
Leu36	4.8	±	0.27	0.31	±	0.02	3.84	±	1.34
Pro37	-			-			-		
Leu38	4.6	±	0.2	0.29	±	0.04	5.01	±	1.33
Leu39	5.04	±	0.28	0.32	±	0.04	3.17	±	0.58
Asn40	5.14	±	0.55	0.31	±	0.07	3.93	±	1.68
Leu41	4.67	±	0.35	0.3	±	0.02	2.83	±	0.79
Met42	-			-			-		
Leu43	5.14	±	0.28	0.28	±	0.03	3.38	±	0.65
Thr44	-			-			-		
Pro45	-			-			-		
Asp46	5.13	±	0.32	0.28	±	0.01	4.09	±	1.07
Glu47	5.14	±	0.38	0.27	±	0.02	3.26	±	1.08
Arg48	4.85	±	0.38	0.29	±	0.01	3.08	±	0.91
Glu49	5.2	±	0.13	0.3	±	0.03	3.34	±	1.38
Ala50	5.19	±	0.35	0.29	±	0.05	2.9	±	0.96
Leu51	5.09	±	0.38	0.29	±	0.04	3.59	±	2.08
Gly52	4.97	±	0.28	0.3	±	0.03	3.9	±	0.67
Thr53	-			-			-		
Arg54	5.13	±	0.7	0.29	±	0.01	3.68	±	0.93
Val55	4.84	±	0.43	0.32	±	0.03	3.62	±	0.69
Arg56	-			-			-		
Ile57	-			-			-		
Val58	5.4	±	0.29	0.3	±	0.07	2.83	±	2.36
Glu59	-			-			-		
Glu60	6.78	±	0.18	0.29	±	0.03	4.05	±	0.49
Leu61	6.72	±	1.17	0.29	±	0.05	4.23	±	1.24
Leu62	-			-			-		
Arg63	-			-			-		
Gly64	7.98	±	0.24	0.25	±	0.08	4.45	±	1.72

Glu65	-	-	-
Met66	-	-	-
Ser67	-	-	-
Gln68	-	-	-
Arg69	4.62 ± 0.34	0.31 ± 0.03	8.52 ± 1.34
Glu70	-	-	-
Leu71	3.72 ± 0.23	0.32 ± 0.01	10 ± 1.11
Lys72	-	-	-
Asn73	-	-	-
Glu74	-	-	-
Leu75	-	-	-
Gly76	3.57 ± 0.31	0.28 ± 0.06	9.85 ± 2.1
Ala77	-	-	-
Gly78	3.27 ± 0.3	0.29 ± 0.01	10.5 ± 0.67
Ile79	-	-	-
Ala80	-	-	-
Thr81	-	-	-
Ile82	5.88 ± 0.36	0.31 ± 0.05	6.74 ± 1.11
Thr83	-	-	-
Arg84	-	-	-
Gly85	4.17 ± 1.39	0.33 ± 0.1	7.79 ± 2.67
Ser86	4.36 ± 0.25	0.35 ± 0.04	7.09 ± 1.4
Asn87	4.43 ± 0.53	0.35 ± 0.02	7.81 ± 1.04
Ser88	-	-	-
Leu89	5.06 ± 0.31	0.33 ± 0.05	6.11 ± 1.69
Lys90	4.72 ± 0.1	0.32 ± 0.03	5.27 ± 0.99
Ala91	4.62 ± 0.34	0.3 ± 0.06	4.91 ± 1.69
Ala92	4.72 ± 0.16	0.28 ± 0.03	4.89 ± 0.59
Pro93	-	-	-
Val94	4.46 ± 0.34	0.31 ± 0.01	5.79 ± 1.05
Glu95	4.85 ± 0.53	0.29 ± 0.05	4.27 ± 0.81
Leu96	4.73 ± 0.36	0.3 ± 0.05	4.41 ± 0.68
Arg97	4.78 ± 0.36	0.31 ± 0.03	3.73 ± 0.67
Gln98	4.91 ± 0.57	0.3 ± 0.03	3.77 ± 1.22
Trp99	4.72 ± 0.42	0.31 ± 0.04	4.48 ± 0.95
Leu100	4.77 ± 0.38	0.32 ± 0.02	3.96 ± 1.02
Glu101	-	-	-
Glu102	4.62 ± 0.43	0.26 ± 0.04	3.89 ± 0.69
Val103	4.42 ± 0.41	0.3 ± 0.02	4.99 ± 0.65

Leu104	4.43	±	0.41	0.32	±	0.01	5.52	±	0.84
Leu105	4.51	±	0.37	0.28	±	0.04	4.8	±	1.51
Lys106	2.3	±	0.25	0.29	±	0.04	13.8	±	2.08
Ser107	0.73	±	0.09	0.25	±	0.01	25.9	±	0.74
Asp108	-			-			-		

Table 15. Spectral Density Values For Apo-L75F-TrpR At 14.1 T.

Residue	$J_{\text{eff}}(0)$, ns/rad			$J(\omega_N)$, ns/rad			$J(0.87\omega_H)$, ps/rad		
Met1	-			-			-		
Ala2	-			-			-		
Gln3	-			-			-		
Gln4	-			-			-		
Ser5	0.68	±	0.06	0.26	±	0.01	35.4	±	1.84
Pro6	-			-			-		
Tyr7	1.01	±	0.07	0.28	±	0.01	24.5	±	1.1
Ser8	1.38	±	0.06	0.3	±	0.02	22	±	1.71
Ala9	2.05	±	0.09	0.31	±	0.01	17.3	±	0.87
Ala10	2.25	±	0.07	0.29	±	0.01	15.2	±	0.95
Met11	2.51	±	0.08	0.33	±	0.02	15.7	±	0.9
Ala12	3.25	±	0.05	0.31	±	0.03	12.7	±	1.54
Glu13	3.5	±	0.05	0.33	±	0.05	7.52	±	3.07
Gln14	3.61	±	0.1	0.33	±	0.03	11	±	1.14
Arg15	3.86	±	0.14	0.31	±	0.01	8.11	±	1.22
His16	5.12	±	0.09	0.29	±	0.01	5.29	±	0.45
Gln17	4.71	±	0.07	0.29	±	0.01	3.68	±	1.27
Glu18	4.5	±	0.05	0.31	±	0.03	6.14	±	0.68
Trp19	4.81	±	0.12	0.3	±	0.01	5.61	±	1.18
Leu20	5.03	±	0.17	0.3	±	0.02	3.21	±	0.59
Arg21	4.83	±	0.13	0.31	±	0.02	3.45	±	0.94
Phe22	4.76	±	0.13	0.32	±	0.06	4.06	±	1.17
Val23	4.99	±	0.1	0.3	±	0.01	2.97	±	1.94
Asp24	5.45	±	0.08	0.29	±	0.01	4.23	±	0.52
Leu25	4.83	±	0.03	0.33	±	0.03	3.47	±	1.91
Leu26	-			-			-		

Lys27	5.98	±	0.35	0.3	±	0.02	3.16	±	2.45
Asn28	5.07	±	0.06	0.3	±	0.01	3.34	±	0.19
Ala29	4.64	±	0.11	0.32	±	0.01	2.6	±	0.43
Tyr30	4.99	±	0.11	0.29	±	0.02	3.98	±	0.4
Gln31	4.84	±	0.17	0.31	±	0.02	3.09	±	0.45
Asn32	4.56	±	0.08	0.29	±	0.02	3.62	±	0.77
Asp33	5.11	±	0.04	0.3	±	0.02	3.21	±	0.75
Leu34	4.71	±	0.07	0.29	±	0.01	3.68	±	1.09
His35	5	±	0.16	0.31	±	0.01	1.95	±	1.21
Leu36	4.98	±	0.11	0.31	±	0.01	4.26	±	0.2
Pro37	-			-			-		
Leu38	4.97	±	0.01	0.31	±	0.01	3.7	±	0.37
Leu39	5.08	±	0.05	0.3	±	0.02	1.68	±	0.21
Asn40	4.82	±	0.06	0.34	±	0.07	4.95	±	1.08
Leu41	4.79	±	0.09	0.31	±	0.02	4.26	±	0.65
Met42	5.05	±	0.28	0.3	±	0.02	2.64	±	1.73
Leu43	4.84	±	0.05	0.31	±	0.02	5.22	±	2.07
Thr44	4.71	±	0.04	0.28	±	0.02	3.35	±	0.78
Pro45	-			-			-		
Asp46	5.08	±	0.11	0.28	±	0.02	3.35	±	1.68
Glu47	5.34	±	0.12	0.28	±	0.01	3.88	±	0.55
Arg48	4.95	±	0.06	0.28	±	0.02	2.6	±	0.22
Glu49	5.14	±	0.07	0.28	±	0.03	4.23	±	1.13
Ala50	5.13	±	0.24	0.28	±	0.02	3.84	±	1.05
Leu51	-			-			-		
Gly52	5.05	±	0.15	0.3	±	0.02	3.93	±	0.45
Thr53	4.66	±	0.18	0.32	±	0.03	6.79	±	2.52
Arg54	5.43	±	0.04	0.31	±	0.02	1.13	±	0.57
Val55	5.16	±	0.06	0.31	±	0.06	4.95	±	1.62
Arg56	5.26	±	0.17	0.33	±	0.08	4.56	±	1.21
Ile57	-			-			-		
Val58	5.37	±	0.09	0.3	±	0.02	1.46	±	4.27
Glu59	5.04	±	0.12	0.29	±	0.01	3.1	±	0.74
Glu60	5.6	±	0.16	0.28	±	0.02	3.56	±	1.68
Leu61	5.66	±	0.47	0.3	±	0.03	4.38	±	0.5
Leu62	5.18	±	0.08	0.29	±	0.01	2.74	±	0.56
Arg63	-			-			-		
Gly64	6.07	±	0.16	0.33	±	0.04	5.43	±	0.61
Glu65	4.24	±	0.07	0.3	±	0.04	6.47	±	0.94

Met66	3.83	±	0.07	0.28	±	0.02	6.33	±	0.98
Ser67	4.45	±	0.28	0.32	±	0.05	7.71	±	2.5
Gln68	4.07	±	0.17	0.31	±	0.01	7.79	±	1.71
Arg69	4.4	±	0.25	0.29	±	0.01	6.42	±	0.2
Glu70	4.23	±	0.03	0.31	±	0.01	7.39	±	0.86
Leu71	4.42	±	0.04	0.31	±	0.01	7.13	±	0.69
Lys72	-			-			-		
Asn73	4.52	±	0.02	0.29	±	0.01	6.79	±	0.68
Glu74	4.45	±	0.09	0.36	±	0.01	8.03	±	2.72
Phe75	5.43	±	0.19	0.3	±	0.02	8.45	±	0.66
Gly76	4.44	±	0.04	0.28	±	0.01	8.1	±	0.95
Ala77	-			-			-		
Gly78	3.69	±	0.18	0.3	±	0.01	7.99	±	1.57
Ile79	5.48	±	0.09	0.33	±	0.03	7.05	±	3.56
Ala80	4.11	±	0.1	0.33	±	0.03	6.32	±	1.64
Thr81	4.77	±	0.07	0.32	±	0.01	7.15	±	0.31
Ile82	7.31	±	0.06	0.3	±	0.02	4.87	±	1.39
Thr83	-			-			-		
Arg84	3.95	±	0.12	0.32	±	0.02	8.24	±	0.79
Gly85	3.76	±	0.06	0.32	±	0.02	10.7	±	0.75
Ser86	4.67	±	0.11	0.3	±	0.03	12.3	±	1.83
Asn87	-			-			-		
Ser88	6.18	±	0.2	0.3	±	0.03	11.1	±	1.27
Leu89	5.32	±	0.12	0.3	±	0.01	8.7	±	1.76
Lys90	4.73	±	0.33	0.28	±	0.03	6.4	±	1.58
Ala91	4.43	±	0.09	0.3	±	0.01	7.04	±	0.41
Ala92	4.52	±	0.03	0.26	±	0.02	5.39	±	1.08
Pro93	-			-			-		
Val94	4.61	±	0.18	0.29	±	0.02	5.47	±	1.67
Glu95	4.9	±	0.09	0.31	±	0.01	4.16	±	0.24
Leu96	4.79	±	0.14	0.3	±	0.03	3.96	±	0.68
Arg97	4.71	±	0.09	0.29	±	0.03	2.92	±	0.61
Gln98	5.08	±	0.04	0.29	±	0.02	3.98	±	0.47
Trp99	4.84	±	0.08	0.29	±	0.04	3.8	±	0.74
Leu100	4.92	±	0.09	0.29	±	0.02	4.93	±	1
Glu101	4.87	±	0.05	0.29	±	0.02	5.24	±	0.47
Glu102	4.8	±	0.04	0.28	±	0.02	4.45	±	0.28
Val103	4.69	±	0.03	0.28	±	0.02	3.73	±	0.31
Leu104	4.6	±	0.17	0.3	±	0.01	4.19	±	0.07

Leu105	4.55	±	0.11	0.3	±	0.06	6.38	±	1.84
Lys106	2.13	±	0.08	0.29	±	0.03	14.6	±	1.72
Ser107	0.69	±	0.05	0.25	±	0.01	25	±	0.8
Asp108	0.35	±	0.09	0.14	±	0.01	25.5	±	2.18

Table 16. Spectral Density Values For Apo-A77V-TrpR At 14.1 T.

Residue	$J_{\text{eff}}(0)$, ns/rad			$J(\omega_N)$, ns/rad			$J(0.87\omega_H)$, ps/rad		
Met1	-			-			-		
Ala2	-			-			-		
Gln3	-			-			-		
Gln4	-			-			-		
Ser5	0.69	±	0.16	0.23	±	0.03	27	±	5.64
Pro6	-			-			-		
Tyr7	-			-			-		
Ser8	-			-			-		
Ala9	-			-			-		
Ala10	-			-			-		
Met11	3.35	±	0.57	0.28	±	0.01	6.1	±	1.6
Ala12	3.07	±	0.05	0.32	±	0.04	12.8	±	1.81
Glu13	-			-			-		
Gln14	3.45	±	0.01	0.35	±	0.01	10.9	±	1.18
Arg15	4.2	±	0.16	0.35	±	0.01	4.84	±	4.82
His16	-			-			-		
Gln17	-			-			-		
Glu18	4.48	±	0.09	0.34	±	0.01	4.95	±	0.65
Trp19	4.46	±	0.11	0.31	±	0.01	4.68	±	0.4
Leu20	4.95	±	0.12	0.34	±	0.07	4.67	±	2.23
Arg21	4.63	±	0.03	0.29	±	0.01	6.36	±	0.8
Phe22	4.78	±	0.04	0.3	±	0.03	3.43	±	0.47
Val23	4.48	±	0.07	0.27	±	0.02	4.5	±	1.16
Asp24	5.54	±	0.54	0.31	±	0.01	5.22	±	0.96
Leu25	4.47	±	0.19	0.31	±	0.01	4.75	±	1.45
Leu26	4.26	±	0.68	0.3	±	0.01	3.74	±	0.38

Lys27	7.11	±	0.07	0.28	±	0.01	3.35	±	0.53
Asn28	4.87	±	0.07	0.3	±	0.01	3.59	±	0.74
Ala29	4.78	±	0.01	0.29	±	0.01	3.08	±	0.37
Tyr30	5.23	±	0.19	0.29	±	0.01	3.31	±	0.94
Gln31	4.82	±	0.11	0.29	±	0.01	3.5	±	0.39
Asn32	4.41	±	0.08	0.26	±	0.01	3.31	±	0.69
Asp33	5.13	±	0.16	0.28	±	0.02	3.32	±	0.75
Leu34	4.58	±	0.04	0.31	±	0.01	4.26	±	0.25
His35	5.16	±	0.06	0.3	±	0.03	3.62	±	2.15
Leu36	4.8	±	0.02	0.32	±	0.02	2.99	±	1.19
Pro37	-			-			-		
Leu38	-			-			-		
Leu39	4.95	±	0.09	0.3	±	0.01	3.37	±	0.22
Asn40	4.85	±	0.09	0.31	±	0.01	3.31	±	0.06
Leu41	4.58	±	0.32	0.29	±	0.04	3.47	±	0.58
Met42	4.6	±	0.06	0.32	±	0.01	4.23	±	1.01
Leu43	5.01	±	0.14	0.28	±	0.02	3.95	±	0.62
Thr44	-			-			-		
Pro45	-			-			-		
Asp46	5.03	±	0.18	0.27	±	0.01	3.74	±	0.51
Glu47	4.94	±	0.21	0.27	±	0.01	2.21	±	1.8
Arg48	3.97	±	1.04	0.29	±	0.02	3.62	±	1.07
Glu49	4.96	±	0.22	0.3	±	0.01	3.71	±	0.75
Ala50	5.15	±	0.08	0.3	±	0.03	3.16	±	0.48
Leu51	5.1	±	0.1	0.28	±	0.01	4.37	±	0.52
Gly52	5	±	0.05	0.28	±	0.02	3.56	±	0.94
Thr53	-			-			-		
Arg54	4.71	±	0.32	0.32	±	0.02	5.07	±	0.81
Val55	-			-			-		
Arg56	-			-			-		
Ile57	5.2	±	0.03	0.29	±	0.03	3.31	±	0.49
Val58	-			-			-		
Glu59	-			-			-		
Glu60	5.44	±	0.28	0.3	±	0.01	4.41	±	0.73
Leu61	5.38	±	0.21	0.28	±	0.02	3.7	±	0.41
Leu62	4.73	±	0.05	0.31	±	0.02	3.31	±	1.39
Arg63	4.89	±	0.31	0.3	±	0.01	4.15	±	0.59
Gly64	5.21	±	0.13	0.29	±	0.01	5.11	±	0.74
Glu65	4.78	±	0.66	0.27	±	0.01	3.71	±	1.24

Met66	4.36	±	0.13	0.26	±	0.01	4.63	±	0.23
Ser67	4.21	±	0.05	0.27	±	0.01	5.37	±	0.86
Gln68	3.64	±	0.2	0.31	±	0.01	6.09	±	0.62
Arg69	4.56	±	0.06	0.31	±	0.03	6.34	±	0.84
Glu70	4.19	±	0.2	0.3	±	0.03	6.19	±	0.8
Leu71	4.44	±	0.02	0.31	±	0.01	5.59	±	0.81
Lys72	-			-			-		
Asn73	4.53	±	0.03	0.31	±	0.04	5.46	±	1.02
Glu74	4.24	±	0.07	0.3	±	0.02	5.52	±	0.9
Leu75	5.31	±	0.37	0.28	±	0.02	5.16	±	0.98
Gly76	4.62	±	0.01	0.27	±	0.01	5.83	±	0.68
Val77	5.58	±	0.41	0.24	±	0.02	5.99	±	0.53
Gly78	4.56	±	0.12	0.26	±	0.01	6.06	±	0.67
Ile79	-			-			-		
Ala80	-			-			-		
Thr81	-			-			-		
Ile82	-			-			-		
Thr83	-			-			-		
Arg84	-			-			-		
Gly85	4.46	±	0.39	0.31	±	0.01	4.36	±	1.39
Ser86	4.89	±	0.13	0.3	±	0.01	4	±	1.31
Asn87	-			-			-		
Ser88	-			-			-		
Leu89	-			-			-		
Lys90	4.52	±	0.06	0.31	±	0.01	4.87	±	0.43
Ala91	4.43	±	0.15	0.3	±	0.01	4.22	±	0.55
Ala92	4.49	±	0.1	0.25	±	0.01	3.02	±	0.47
Pro93	-			-			-		
Val94	4.32	±	0.25	0.3	±	0.02	4.41	±	0.69
Glu95	4.83	±	0.02	0.27	±	0.01	4.16	±	0.17
Leu96	4.62	±	0.05	0.32	±	0.01	3.65	±	0.43
Arg97	-			-			-		
Gln98	4.87	±	0.09	0.27	±	0.02	4.04	±	0.33
Trp99	5.01	±	0.13	0.31	±	0.01	3.9	±	1.19
Leu100	4.78	±	0.05	0.28	±	0.01	3.56	±	0.36
Glu101	4.74	±	0.05	0.27	±	0.01	4.21	±	0.51
Glu102	4.55	±	0.01	0.29	±	0.01	4.01	±	0.55
Val103	4.62	±	0.19	0.29	±	0.02	4.05	±	0.62
Leu104	4.52	±	0.2	0.31	±	0.02	3.9	±	1.09

Leu105	4.29	±	0.15	0.29	±	0.01	6.02	±	1.09
Lys106	2.44	±	0.26	0.32	±	0.01	14.1	±	1.08
Ser107	0.64	±	0.04	0.24	±	0.02	24.9	±	2.27
Asp108	0.27	±	0.05	0.12	±	0.01	22.6	±	2.78

Table 17. Spectral Density Values For Holo-WT-TrpR At 14.1 T.

<u>Residue</u>	<u>$J_{\text{eff}}(0)$, ns/rad</u>			<u>$J(\omega_N)$, ns/rad</u>			<u>$J(0.87\omega_H)$, ps/rad</u>		
Met1	-			-			-		
Ala2	-			-			-		
Gln3	-			-			-		
Gln4	-			-			-		
Ser5	0.42	±	0.26	0.19	±	0.04	30.1	±	8.18
Pro6	-			-			-		
Tyr7	0.96	±	0.06	0.29	±	0.02	26.7	±	1.65
Ser8	1.34	±	0.07	0.3	±	0.04	21.3	±	12.8
Ala9	2.31	±	0.21	0.32	±	0.05	17.4	±	2.81
Ala10	-			-			-		
Met11	2.33	±	0.02	0.31	±	0.02	14.7	±	1.09
Ala12	3.08	±	0.51	0.32	±	0.03	12.3	±	1.2
Glu13	3.47	±	0.04	0.29	±	0	9.71	±	0.57
Gln14	3.34	±	0.01	0.31	±	0.01	9.33	±	0.55
Arg15	3.79	±	0.21	0.31	±	0	10.3	±	2.07
His16	-			-			-		
Gln17	4.02	±	0.11	0.34	±	0.03	6.41	±	0.88
Glu18	4.35	±	0.33	0.33	±	0.01	4.63	±	0.44
Trp19	4.47	±	0.24	0.3	±	0.03	4.03	±	1.02
Leu20	4.87	±	0.06	0.29	±	0.01	3.28	±	0.92
Arg21	4.74	±	0.01	0.3	±	0.01	3.74	±	1.67
Phe22	4.55	±	0.1	0.37	±	0.08	4.13	±	1.25
Val23	4.83	±	0.48	0.3	±	0.01	3.9	±	1.13
Asp24	-			-			-		

Leu25	4.52	±	0.48	0.33	±	0.02	4.32	±	1.23
Leu26	4.73	±	0.25	0.32	±	0	4.43	±	0.63
Lys27	6.58	±	1.43	0.31	±	0	2.09	±	0.39
Asn28	4.57	±	0.42	0.32	±	0.03	4.43	±	1.45
Ala29	4.65	±	0.09	0.32	±	0.01	3.24	±	0.84
Tyr30	5.19	±	0.16	0.33	±	0.06	3.07	±	0.82
Gln31	4.8	±	0.2	0.34	±	0.03	4.7	±	1.34
Asn32	4.38	±	0.1	0.3	±	0.01	3.62	±	1.33
Asp33	4.84	±	0.55	0.3	±	0.03	4.49	±	0.71
Leu34	4.59	±	0.03	0.31	±	0.03	4.95	±	1.28
His35	5.16	±	0.05	0.32	±	0.03	2.97	±	2.12
Leu36	4.45	±	0.69	0.31	±	0.02	3.26	±	1.37
Pro37	-			-			-		
Leu38	4	±	0.33	0.32	±	0.04	5.93	±	1.3
Leu39	4.79	±	0.06	0.3	±	0	3.16	±	0.38
Asn40	4.89	±	0.63	0.33	±	0	4.12	±	0.82
Leu41	4.64	±	0.38	0.32	±	0.05	3.62	±	1.15
Met42	-			-			-		
Leu43	4.82	±	0.05	0.36	±	0.06	4.94	±	0.84
Thr44	5.18	±	0.86	0.31	±	0.03	4.29	±	2.16
Pro45	-			-			-		
Asp46	5.09	±	0.25	0.28	±	0.05	3.14	±	1.01
Glu47	5.16	±	0.34	0.28	±	0.02	4.23	±	0.9
Arg48	4.29	±	0.17	0.3	±	0	7.21	±	0.41
Glu49	4.57	±	0.28	0.3	±	0.01	3.43	±	1.3
Ala50	5.02	±	0.15	0.29	±	0	4.27	±	1.27
Leu51	5.55	±	0.01	0.3	±	0	3.43	±	0.56
Gly52	5.09	±	0.35	0.28	±	0.04	3.35	±	1.1
Thr53	-			-			-		
Arg54	5.43	±	0.59	0.27	±	0.02	2.36	±	2.15
Val55	5.06	±	0.21	0.32	±	0.02	3.42	±	0.66
Arg56	4.87	±	0.08	0.35	±	0.04	5.02	±	1.46
Ile57	-			-			-		
Val58	-			-			-		
Glu59	4.95	±	0.17	0.29	±	0.02	3.23	±	0.43
Glu60	5.08	±	0.55	0.3	±	0	4.12	±	0.58
Leu61	5.45	±	0.6	0.31	±	0.02	3.31	±	1.16
Leu62	4.8	±	0.62	0.29	±	0	4.08	±	0.59
Arg63	4.76	±	0.33	0.29	±	0.01	3.98	±	0.54

Gly64	5.05	±	0.12	0.28	±	0.06	4.05	±	0.94
Glu65	4.52	±	0.06	0.33	±	0.05	5.02	±	1.33
Met66	3.79	±	0.12	0.27	±	0.01	6.52	±	0.8
Ser67	-			-			-		
Gln68	3.71	±	0.71	0.36	±	0.03	8.31	±	2.27
Arg69	-			-			-		
Glu70	3.93	±	0.13	0.27	±	0.01	6.23	±	0.28
Leu71	-			-			-		
Lys72	4.16	±	0.23	0.32	±	0.01	7	±	0.62
Asn73	4.38	±	0.18	0.29	±	0.01	6.36	±	0.91
Glu74	-			-			-		
Leu75	-			-			-		
Gly76	3.45	±	0.36	0.31	±	0	8.65	±	0.63
Ala77	-			-			-		
Gly78	2.9	±	0.24	0.3	±	0.03	8.58	±	0.94
Ile79	-			-			-		
Ala80	-			-			-		
Thr81	5.64	±	1.12	0.29	±	0.01	5.89	±	0.98
Ile82	0.36	±	6.79	0.35	±	0.02	5.98	±	0.61
Thr83	-			-			-		
Arg84	4.4	±	0.1	0.31	±	0.02	4.64	±	1.2
Gly85	3.39	±	2.13	0.33	±	0.02	3.91	±	1.05
Ser86	4.45	±	0.03	0.34	±	0.03	4.24	±	0.9
Asn87	4.6	±	0.38	0.3	±	0.02	3.56	±	0.27
Ser88	-			-			-		
Leu89	5.4	±	0.78	0.33	±	0.03	5.15	±	0.76
Lys90	4.36	±	0.17	0.3	±	0.01	3.81	±	0.21
Ala91	3.56	±	1.68	0.29	±	0.05	4.2	±	0.93
Ala92	4.28	±	0.27	0.32	±	0.04	4.39	±	1
Pro93	-			-			-		
Val94	4.09	±	0.03	0.31	±	0.02	4.52	±	0.82
Glu95	4.56	±	0.18	0.39	±	0.03	4.93	±	0.84
Leu96	4.56	±	0.01	0.33	±	0.01	4.15	±	1.04
Arg97	4.74	±	0.01	0.3	±	0.01	3.37	±	1.18
Gln98	4.73	±	0.21	0.29	±	0.03	4.23	±	0.64
Trp99	4.68	±	0.4	0.27	±	0.01	3.95	±	0.53
Leu100	4.84	±	0.01	0.29	±	0.02	3.59	±	0.74
Glu101	4.5	±	0.06	0.32	±	0.05	6.04	±	1.08
Glu102	4.55	±	0.2	0.32	±	0.04	3.79	±	1.16

Val103	4.55	±	0.01	0.27	±	0	4.12	±	0.53
Leu104	4.42	±	0.29	0.31	±	0.02	5.85	±	0.7
Leu105	4.27	±	0.21	0.34	±	0.02	7.21	±	1.42
Lys106	2.13	±	0.11	0.33	±	0.01	16.3	±	0.71
Ser107	0.7	±	0.04	0.28	±	0.02	28	±	2.02
Asp108	0.27	±	0.09	0.14	±	0.01	25.9	±	2.57

Table 18. Spectral Density Values For Holo-L75F-TrpR At 14.1 T.

<u>Residue</u>	<u>$J_{\text{eff}}(0)$, ns/rad</u>			<u>$J(\omega_N)$, ns/rad</u>			<u>$J(0.87\omega_H)$, ps/rad</u>		
Met1	-			-			-		
Ala2	-			-			-		
Gln3	-			-			-		
Gln4	-			-			-		
Ser5	-			-			-		
Pro6	-			-			-		
Tyr7	1.37	±	0.12	0.3	±	0	26.9	±	1.78
Ser8	-			-			-		
Ala9	2.43	±	0.01	0.29	±	0.01	16	±	0.85
Ala10	-			-			-		
Met11	2.58	±	0.08	0.34	±	0.02	15.9	±	1.41
Ala12	3.19	±	0.06	0.31	±	0	11.8	±	0.22
Glu13	-			-			-		
Gln14	3.68	±	0.13	0.38	±	0.05	11.7	±	2.12
Arg15	-			-			-		
His16	4.7	±	0.33	0.29	±	0.01	6.18	±	2.57
Gln17	4.26	±	0.16	0.34	±	0.01	6.67	±	0.94
Glu18	4.62	±	0.21	0.33	±	0.04	4.84	±	0.56
Trp19	4.99	±	0.34	0.29	±	0.01	2.76	±	0.58
Leu20	4.98	±	0.4	0.3	±	0.01	2.99	±	0.19
Arg21	4.66	±	0.05	0.32	±	0.02	4.43	±	0.81
Phe22	4.7	±	0.14	0.36	±	0	5.2	±	2.26

Val23	5.15	± 0.05	0.3	± 0.01	4.72	± 0.91
Asp24	-		-		-	
Leu25	4.78	± 0.2	0.34	± 0	4.99	± 0.22
Leu26	-		-		-	
Lys27	6.57	± 0.52	0.3	± 0.01	3.74	± 1.73
Asn28	5.09	± 0.2	0.29	± 0.01	4.05	± 1.09
Ala29	4.78	± 0.09	0.31	± 0.02	2.88	± 0.42
Tyr30	4.72	± 0.06	0.32	± 0.01	3.57	± 0.2
Gln31	4.87	± 0.09	0.29	± 0.01	3.41	± 0.85
Asn32	4.49	± 0.15	0.31	± 0.01	3.84	± 0.78
Asp33	4.96	± 0.18	0.3	± 0.02	4.49	± 0.5
Leu34	4.68	± 0.05	0.3	± 0.01	5.1	± 1.48
His35	4.96	± 0.06	0.31	± 0	4.36	± 0.2
Leu36	5.23	± 0.34	0.29	± 0	3.44	± 2.08
Pro37	-		-		-	
Leu38	5.01	± 0.35	0.3	± 0.01	4.87	± 1.19
Leu39	4.83	± 0.13	0.32	± 0.02	3.65	± 0.42
Asn40	-		-		-	
Leu41	4.69	± 0.17	0.34	± 0.03	4.49	± 0.55
Met42	4.84	± 0.13	0.32	± 0.01	2.8	± 0.23
Leu43	5.15	± 0.22	0.32	± 0	3.82	± 1.45
Thr44	-		-		-	
Pro45	-		-		-	
Asp46	5.24	± 0.06	0.28	± 0.01	3.29	± 0.35
Glu47	5.23	± 0.32	0.28	± 0	4.31	± 0.53
Arg48	4.51	± 0.22	0.31	± 0.02	5.79	± 1.99
Glu49	5.29	± 0.12	0.28	± 0.01	3.91	± 1.29
Ala50	5.32	± 0.12	0.3	± 0.04	4.34	± 1.65
Leu51	5.17	± 0.24	0.3	± 0.03	2.99	± 0.32
Gly52	5.15	± 0.31	0.29	± 0.02	3.5	± 1
Thr53	-		-		-	
Arg54	5.31	± 0.06	0.3	± 0	3.37	± 1.24
Val55	4.8	± 0.14	0.32	± 0.03	3.99	± 0.53
Arg56	-		-		-	
Ile57	-		-		-	
Val58	5.22	± 0.23	0.3	± 0.03	3.02	± 0.63
Glu59	-		-		-	
Glu60	5.51	± 0.24	0.31	± 0.01	3.9	± 0.79
Leu61	5.56	± 0.35	0.32	± 0.06	3.65	± 0.67

Leu62	5.11	± 0.32	0.28	± 0.01	3.88	± 0.14
Arg63	5.12	± 0.21	0.29	± 0	4.01	± 0.07
Gly64	5.23	± 0.4	0.29	± 0.01	3.77	± 0.13
Glu65	4.31	± 0.18	0.29	± 0.04	5.38	± 0.88
Met66	-		-		-	
Ser67	4.3	± 0.24	0.34	± 0.08	7.64	± 1.99
Gln68	4.46	± 0.24	0.29	± 0.01	7.11	± 0.37
Arg69	-		-		-	
Glu70	4.3	± 0.18	0.3	± 0.01	7.16	± 0.5
Leu71	4.12	± 0.19	0.34	± 0.05	7.81	± 1.52
Lys72	-		-		-	
Asn73	4.49	± 0.1	0.3	± 0.01	6.09	± 1.36
Glu74	4.22	± 0.07	0.29	± 0.01	4.6	± 2
Phe75	-		-		-	
Gly76	-		-		-	
Ala77	-		-		-	
Gly78	3.72	± 0.11	0.28	± 0.02	6.4	± 2.91
Ile79	-		-		-	
Ala80	-		-		-	
Thr81	-		-		-	
Ile82	-		-		-	
Thr83	-		-		-	
Arg84	4.75	± 0.08	0.35	± 0	4.68	± 0.47
Gly85	4.86	± 0.23	0.33	± 0.03	4.53	± 0.56
Ser86	4.89	± 0.08	0.34	± 0	4.06	± 0.63
Asn87	4.72	± 0.06	0.32	± 0.01	3.96	± 0.4
Ser88	-		-		-	
Leu89	4.68	± 0.11	0.31	± 0.01	4.06	± 0.58
Lys90	4.8	± 0.14	0.32	± 0.03	3.99	± 0.56
Ala91	4.54	± 0.15	0.32	± 0.01	4.79	± 0.58
Ala92	4.79	± 0.02	0.26	± 0.01	3.6	± 1.15
Pro93	-		-		-	
Val94	4.54	± 0.18	0.29	± 0.02	4.42	± 1.11
Glu95	4.96	± 0.2	0.3	± 0.03	3.81	± 0.62
Leu96	4.73	± 0.07	0.32	± 0.01	2.38	± 0.38
Arg97	4.66	± 0.05	0.32	± 0.02	4.43	± 0.82
Gln98	5.07	± 0.14	0.29	± 0.02	3.31	± 0.28
Trp99	4.78	± 0.05	0.32	± 0.03	4.7	± 0.92
Leu100	5.03	± 0.07	0.32	± 0.02	4.46	± 0.47

Glu101	4.99	±	0.17	0.28	±	0.01	4.8	±	0.78
Glu102	4.73	±	0.18	0.29	±	0.04	3.87	±	0.5
Val103	4.71	±	0.16	0.29	±	0.02	3.8	±	0.2
Leu104	4.64	±	0.2	0.3	±	0.02	5.42	±	1.24
Leu105	4.45	±	0.2	0.32	±	0.01	6.95	±	0.81
Lys106	2.27	±	0.08	0.34	±	0.03	16.3	±	1.31
Ser107	0.73	±	0.08	0.26	±	0.01	25.7	±	0.85
Asp108	-			-			-		

Table 19. Spectral Density Values For Holo-A77V-TrpR At 14.1 T.

<u>Residue</u>	<u>$J_{\text{eff}}(0)$, ns/rad</u>			<u>$J(\omega_N)$, ns/rad</u>			<u>$J(0.87\omega_H)$, ps/rad</u>		
Met1	-			-			-		
Ala2	-			-			-		
Gln3	-			-			-		
Gln4	-			-			-		
Ser5	-			-			-		
Pro6	-			-			-		
Tyr7	1.39	±	0.04	0.28	±	0	25.1	±	0.4
Ser8	1.62	±	0.31	0.29	±	0.01	19.5	±	1.28
Ala9	1.99	±	0.47	0.22	±	0.03	11.3	±	1.61
Ala10	2.53	±	0.05	0.3	±	0	15	±	0.61
Met11	2.61	±	0.08	0.31	±	0.02	15	±	1.07
Ala12	3.25	±	0.06	0.29	±	0.01	10.8	±	0.64
Glu13	3.51	±	0.14	0.3	±	0.03	10.4	±	1.07
Gln14	3.95	±	0.2	0.32	±	0	9.88	±	0.42
Arg15	4.7	±	0.14	0.27	±	0.01	4.29	±	0.99
His16	4.8	±	0.03	0.26	±	0	3.89	±	0.16
Gln17	-			-			-		
Glu18	4.7	±	0.09	0.29	±	0.02	4.79	±	0.53
Trp19	4.92	±	0.2	0.29	±	0	5.34	±	0.37
Leu20	-			-			-		
Arg21	5	±	0.05	0.29	±	0.01	3.05	±	0.37
Phe22	5.22	±	0.15	0.29	±	0.01	3.1	±	0.73
Val23	5.71	±	0.05	0.29	±	0.01	2.87	±	0.22

Asp24	5.45	±	0.11	0.29	±	0.01	4.64	±	0.98
Leu25	4.76	±	0.01	0.31	±	0.01	3.29	±	0.96
Leu26	5.12	±	0.11	0.27	±	0.01	5.66	±	3.18
Lys27	5.82	±	0.17	0.27	±	0	4.29	±	0.35
Asn28	4.98	±	0.06	0.28	±	0	6.64	±	0.17
Ala29	5.04	±	0.17	0.3	±	0.02	4.41	±	0.49
Tyr30	5.39	±	0.28	0.27	±	0	2.67	±	0.35
Gln31	4.9	±	0.01	0.28	±	0	4.05	±	0.04
Asn32	4.51	±	0.1	0.26	±	0	3.41	±	0.18
Asp33	5.13	±	0.05	0.27	±	0.01	2.86	±	0.38
Leu34	4.91	±	0.04	0.29	±	0.02	4.34	±	0.27
His35	5.25	±	0	0.29	±	0	3.05	±	0.54
Leu36	4.89	±	0.33	0.3	±	0.01	3.02	±	0.56
Pro37	-			-			-		
Leu38	5.14	±	0.11	0.28	±	0	3.35	±	0.51
Leu39	4.66	±	0.01	0.3	±	0.01	4.15	±	0.25
Asn40	4.98	±	0.06	0.29	±	0.02	2.9	±	0.58
Leu41	4.9	±	0.05	0.29	±	0	4.64	±	1.11
Met42	5.03	±	0.29	0.29	±	0.02	3.83	±	1.19
Leu43	5.33	±	0.45	0.27	±	0	3.34	±	0.32
Thr44	-			-			-		
Pro45	-			-			-		
Asp46	5.23	±	0.07	0.26	±	0.01	2.73	±	0.1
Glu47	5.38	±	0.21	0.27	±	0.02	1.65	±	1.64
Arg48	5.19	±	0.12	0.27	±	0	4.46	±	0.04
Glu49	5.13	±	0.17	0.26	±	0.02	2.48	±	0.67
Ala50	5.04	±	0.08	0.26	±	0.02	3.14	±	0.3
Leu51	5.3	±	0.13	0.25	±	0	2.99	±	0.63
Gly52	4.88	±	0.09	0.27	±	0.02	1.36	±	2.94
Thr53	-			-			-		
Arg54	5.29	±	0.12	0.28	±	0.01	2.94	±	1.35
Val55	5.39	±	0.03	0.27	±	0.02	3.09	±	1.78
Arg56	5.27	±	0.14	0.29	±	0.02	3.8	±	0.93
Ile57	-			-			-		
Val58	5.26	±	0.32	0.28	±	0.02	3.32	±	0.41
Glu59	5.23	±	0.14	0.27	±	0	3.74	±	0.86
Glu60	5.52	±	0.25	0.29	±	0	4.34	±	1.2
Leu61	5.23	±	0.08	0.27	±	0.02	3.43	±	0.28
Leu62	5.12	±	0.07	0.27	±	0.01	4.29	±	1.19

Arg63	5.1	±	0.04	0.27	±	0.01	3.03	±	0.54
Gly64	4.63	±	0.3	0.28	±	0.01	2.23	±	4.73
Glu65	4.43	±	0.12	0.29	±	0.02	4.64	±	0.42
Met66	-			-			-		
Ser67	4.33	±	0.56	0.26	±	0	5.56	±	0.33
Gln68	5.07	±	0.33	0.26	±	0.02	5.01	±	2.07
Arg69	-			-			-		
Glu70	4.55	±	0.25	0.29	±	0.01	5.57	±	0.91
Leu71	4.56	±	0.03	0.29	±	0	6.31	±	0.11
Lys72	-			-			-		
Asn73	-			-			-		
Glu74	4.41	±	0.04	0.26	±	0.01	3.8	±	0.11
Leu75	4.79	±	0.07	0.28	±	0.02	4.98	±	1.47
Gly76	4.82	±	0.38	0.28	±	0.03	4.54	±	0.68
Val77	4.43	±	0.01	0.21	±	0.01	4.43	±	0.24
Gly78	4.34	±	0.05	0.25	±	0	6.39	±	0.67
Ile79	-			-			-		
Ala80	-			-			-		
Thr81	-			-			-		
Ile82	-			-			-		
Thr83	-			-			-		
Arg84	5.05	±	0.02	0.28	±	0	3.38	±	0.35
Gly85	5.51	±	0.69	0.29	±	0.02	3.23	±	0.75
Ser86	4.91	±	0.07	0.27	±	0.02	5.02	±	1.44
Asn87	-			-			-		
Ser88	-			-			-		
Leu89	4.79	±	0.02	0.3	±	0	3.56	±	0.76
Lys90	4.94	±	0.05	0.29	±	0.01	2.87	±	0.1
Ala91	4.75	±	0.08	0.28	±	0.01	4.76	±	0.89
Ala92	4.89	±	0.22	0.26	±	0.01	3.08	±	0.51
Pro93	-			-			-		
Val94	4.56	±	0.07	0.29	±	0.01	4.27	±	0.94
Glu95	5.17	±	0.02	0.27	±	0.01	3.71	±	0.35
Leu96	4.86	±	0.13	0.29	±	0.02	2.13	±	0.21
Arg97	4.57	±	0.12	0.28	±	0.02	6.39	±	0.81
Gln98	5.32	±	0.12	0.27	±	0.01	3.91	±	0.37
Trp99	4.99	±	0.1	0.28	±	0.03	4.98	±	1.47
Leu100	5.12	±	0.01	0.28	±	0.01	4.02	±	0.37
Glu101	5.02	±	0.01	0.29	±	0.01	5.47	±	1.52

Glu102	4.91	±	0.02	0.27	±	0.01	4.59	±	0.71
Val103	4.93	±	0.11	0.28	±	0.01	3.35	±	0.52
Leu104	4.81	±	0.12	0.3	±	0.01	3.71	±	0.74
Leu105	4.61	±	0.1	0.27	±	0.01	5.24	±	0.45
Lys106	2.36	±	0.08	0.29	±	0.03	13.5	±	1.4
Ser107	-			-			-		
Asp108	0.26	±	0.01	0.14	±	0.01	27.5	±	1.48

Table 20 – Motional Parameters Computed For Apo-WT-TrpR Using Model-free analysis

Res	Model	S^2	err	S^2_f	err	S^2_s	err	τ_e (ns)	err (ns)	R_{ex} (s ⁻¹)	err (s ⁻¹)
Met1	-	-	-	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	-	-	-	-	-	-	-
Gln3	-	-	-	-	-	-	-	-	-	-	-
Gln4	-	-	-	-	-	-	-	-	-	-	-
Ser5	-	-	-	-	-	-	-	-	-	-	-
Pro6	-	-	-	-	-	-	-	-	-	-	-
Tyr7	-	-	-	-	-	-	-	-	-	-	-
Ser8	-	-	-	-	-	-	-	-	-	-	-
Ala9	-	-	-	-	-	-	-	-	-	-	-
Ala10	-	-	-	-	-	-	-	-	-	-	-
Met11	-	-	-	-	-	-	-	-	-	-	-
Ala12	2	0.56	0.09	-	-	0.56	0.09	1200.1	376.9	-	-
Glu13	-	-	-	-	-	-	-	-	-	-	-
Gln14	1	0.82	0.1	-	-	0.82	0.1	-	-	-	-
Arg15	-	-	-	-	-	-	-	-	-	-	-
His16	-	-	-	-	-	-	-	-	-	-	-
Gln17	2	0.85	0.07	-	-	0.85	0.07	70.4	476.3	-	-
Glu18	1	0.87	0.08	-	-	0.87	0.08	-	-	-	-
Trp19	1	0.92	0.04	-	-	0.92	0.04	-	-	-	-
Leu20	1	0.91	0.04	-	-	0.91	0.04	-	-	-	-
Arg21	-	-	-	-	-	-	-	-	-	-	-
Phe22	1	0.91	0.06	-	-	0.91	0.06	-	-	-	-
Val23	1	0.91	0.04	-	-	0.91	0.04	-	-	-	-

[illegible]

[illegible]

Glu102	2	0.83	0.06	-	-	0.83	0.06	16.5	302.4	-	-
Val103	2	0.87	0.04	-	-	0.87	0.04	34.2	141.9	-	-
Leu104	1	0.93	0.04	-	-	0.93	0.04	-	-	-	-
Leu105	1	0.85	0.06	-	-	0.85	0.06	-	-	-	-
Lys106	5	0.41	0.06	0.76	0.07	0.54	0.08	1064	152.2	-	-
Ser107	5	0.09	0.02	0.7	0.02	0.13	0.02	827.9	16.6	-	-
Asp108	-	-	-	-	-	-	-	-	-	-	-

Table 21 – Motional Parameters Computed For Apo-L75F-TrpR Using Model- free Analysis

Res	Model	S^2	Err	S^2_f	err	S^2_s	err	τ_e (ns)	err (ns)	R_{ex} (s^{-1})	err (s^{-1})
Met1	-	-	-	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	-	-	-	-	-	-	-
Gln3	-	-	-	-	-	-	-	-	-	-	-
Gln4	-	-	-	-	-	-	-	-	-	-	-
Ser5	5	0.08	0.01	0.81	0.03	0.1	0.02	670.1	17.7	-	-
Pro6	-	-	-	-	-	-	-	-	-	-	-
Tyr7	5	0.14	0.02	0.74	0.03	0.19	0.02	889.9	12.9	-	-
Ser8	5	0.2	0.01	0.77	0.04	0.26	0.03	975	46.7	-	-
Ala9	5	0.34	0.02	0.79	0.02	0.43	0.02	1024.5	42.2	-	-
Ala10	5	0.38	0.02	0.77	0.02	0.5	0.02	1033.6	55.4	-	-
Met11	5	0.42	0.02	0.85	0.03	0.49	0.03	1135.2	42.6	-	-
Ala12	5	0.58	0.01	0.87	0.04	0.66	0.04	1010	130.9	-	-
Glu13	1	0.66	0.01	-	-	0.66	0.01	-	-	-	-
Gln14	5	0.65	0.02	0.9	0.04	0.72	0.04	1092.6	157.4	-	-
Arg15	5	0.7	0.03	0.87	0.03	0.8	0.03	1237	368.9	-	-
His16	4	0.85	0.04	-	-	0.85	0.04	37.4	18.2	1.6	0.6
Gln17	1	0.88	0.01	-	-	0.88	0.01	-	-	-	-
Glu18	2	0.85	0.01	-	-	0.85	0.01	43.8	196.6	-	-
Trp19	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Leu20	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-
Arg21	1	0.91	0.02	-	-	0.91	0.02	-	-	-	-
Phe22	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-

Val23	3	0.9	0.01	-	-	0.9	0.01	-	-	0.7	0.2
Asp24	3	0.89	0.02	-	-	0.89	0.02	-	-	1.9	0.3
Leu25	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Leu26	-	-	-	-	-	-	-	-	-	-	-
Lys27	3	0.89	0.06	-	-	0.89	0.06	-	-	3.3	1.2
Asn28	3	0.9	0.01	-	-	0.9	0.01	-	-	0.8	0.2
Ala29	1	0.89	0.02	-	-	0.89	0.02	-	-	-	-
Tyr30	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-
Gln31	1	0.9	0.03	-	-	0.9	0.03	-	-	-	-
Asn32	1	0.86	0.01	-	-	0.86	0.01	-	-	-	-
Asp33	1	0.96	0.01	-	-	0.96	0.01	-	-	-	-
Leu34	1	0.89	0.01	-	-	0.89	0.01	-	-	-	-
His35	1	0.94	0.02	-	-	0.94	0.02	-	-	-	-
Leu36	2	0.93	0.01	-	-	0.93	0.01	32	11.3	-	-
Pro37	-	-	-	-	-	-	-	-	-	-	-
Leu38	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Leu39	-	-	-	-	-	-	-	-	-	-	-
Asn40	2	0.89	0.01	-	-	0.89	0.01	22.7	1.5	-	-
Leu41	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Met42	1	0.93	0.03	-	-	0.93	0.03	-	-	-	-
Leu43	1	0.9	0.01	-	-	0.9	0.01	-	-	-	-
Thr44	1	0.86	0.01	-	-	0.86	0.01	-	-	-	-
Pro45	-	-	-	-	-	-	-	-	-	-	-
Asp46	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Glu47	1	0.94	0.02	-	-	0.94	0.02	-	-	-	-
Arg48	-	-	-	-	-	-	-	-	-	-	-
Glu49	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Ala50	1	0.91	0.04	-	-	0.91	0.04	-	-	-	-
Leu51	-	-	-	-	-	-	-	-	-	-	-
Gly52	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Thr53	1	0.87	0.03	-	-	0.87	0.03	-	-	-	-
Arg54	-	-	-	-	-	-	-	-	-	-	-
Val55	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Arg56	1	0.96	0.02	-	-	0.96	0.02	-	-	-	-
Ile57	-	-	-	-	-	-	-	-	-	-	-
Val58	1	0.95	0.02	-	-	0.95	0.02	-	-	-	-
Glu59	1	0.91	0.02	-	-	0.91	0.02	-	-	-	-
Glu60	1	0.98	0.02	-	-	0.98	0.02	-	-	-	-
Leu61	2	0.98	0.04	-	-	0.98	0.04	213.5	695.7	-	-

Leu62	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Arg63	-	-	-	-	-	-	-	-	-	-	-
Gly64	-	-	-	-	-	-	-	-	-	-	-
Glu65	5	0.79	0.02	0.88	0.04	0.89	0.06	916.4	424.5	-	-
Met66	1	0.71	0.01	-	-	0.71	0.01	-	-	-	-
Ser67	1	0.86	0.05	-	-	0.86	0.05	-	-	-	-
Gln68	5	0.76	0.02	0.9	0.02	0.84	0.03	1042.8	370.6	-	-
Arg69	2	0.89	0.01	-	-	0.89	0.01	76.8	5.5	-	-
Glu70	5	0.79	0.01	0.91	0.02	0.87	0.03	948	273	-	-
Leu71	2	0.83	0.01	-	-	0.83	0.01	53.6	8.8	-	-
Lys72	-	-	-	-	-	-	-	-	-	-	-
Asn73	2	0.85	0.01	-	-	0.85	0.01	62	9.3	-	-
Glu74	2	0.79	0.02	-	-	0.79	0.02	1246.1	226.7	-	-
Phe75	4	0.89	0.04	-	-	0.89	0.04	138.5	221.2	1.9	0.7
Gly76	2	0.84	0.01	-	-	0.84	0.01	80.3	19	-	-
Ala77	-	-	-	-	-	-	-	-	-	-	-
Gly78	5	0.68	0.04	0.84	0.03	0.8	0.03	1137.8	445	-	-
Ile79	1	1	0.01	-	-	1	0.01	-	-	-	-
Ala80	1	0.76	0.02	-	-	0.76	0.02	-	-	-	-
Thr81	5	0.89	0.01	0.97	0.01	0.92	0.01	583.5	131.7	-	-
Ile82	3	0.9	0.07	-	-	0.9	0.07	-	-	6.6	1
Thr83	-	-	-	-	-	-	-	-	-	-	-
Arg84	5	0.7	0.02	0.89	0.03	0.78	0.03	1272	196.8	-	-
Gly85	5	0.69	0.01	0.91	0.03	0.76	0.04	980.7	125.3	-	-
Ser86	2	0.86	0.02	-	-	0.86	0.02	296.2	188	-	-
Asn87	-	-	-	-	-	-	-	-	-	-	-
Ser88	4	0.87	0.06	-	-	0.87	0.06	264.4	305.8	3.8	0.9
Leu89	1	0.98	0.02	-	-	0.98	0.02	-	-	-	-
Lys90	2	0.86	0.04	-	-	0.86	0.04	64.9	424.9	-	-
Ala91	5	0.78	0.02	0.9	0.01	0.87	0.02	969	130.7	-	-
Ala92	1	0.85	0.01	-	-	0.85	0.01	-	-	-	-
Pro93	-	-	-	-	-	-	-	-	-	-	-
Val94	1	0.87	0.02	-	-	0.87	0.02	-	-	-	-
Glu95	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Leu96	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Arg97	1	0.88	0.02	-	-	0.88	0.02	-	-	-	-
Gln98	1	0.95	0.01	-	-	0.95	0.01	-	-	-	-
Trp99	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Leu100	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-

Glu101	4	0.88	0.05	-	-	0.88	0.05	41.6	328.8	0.5	0.8
Glu102	2	0.89	0.01	-	-	0.89	0.01	32.8	2.5	-	-
Val103	2	0.88	0.01	-	-	0.88	0.01	14	4.6	-	-
Leu104	2	0.9	0.01	-	-	0.9	0.01	22.9	3.6	-	-
Leu105	1	0.85	0.02	-	-	0.85	0.02	-	-	-	-
Lys106	5	0.37	0.02	0.74	0.06	0.49	0.06	1054.4	85.4	-	-
Ser107	5	0.08	0.01	0.68	0.02	0.12	0.01	841.6	14.8	-	-
Asp108	5	0.04	0.02	0.51	0.04	0.08	0.04	536.7	19.8	-	-

Table 22 – Motional Parameters Computed For Apo-A77V-TrpR Using Model-free Analysis.

Residue	Model	S^2	err	S^2_f	err	S^2_s	err	τ_e (ns)	err (ns)	R_{ex} (s^{-1})	err (s^{-1})
Met1	-	-	-	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	-	-	-	-	-	-	-
Gln3	-	-	-	-	-	-	-	-	-	-	-
Gln4	-	-	-	-	-	-	-	-	-	-	-
Ser5	5	0.09	0.03	0.69	0.1	0.13	0.06	752.3	116.1	-	-
Pro6	-	-	-	-	-	-	-	-	-	-	-
Tyr7	-	-	-	-	-	-	-	-	-	-	-
Ser8	-	-	-	-	-	-	-	-	-	-	-
Ala9	2	0.72	0.04	-	-	0.72	0.04	184.4	23.1	-	-
Ala10	1	0.89	0	-	-	0.89	0	-	-	-	-
Met11	1	0.84	0.01	-	-	0.84	0.01	-	-	-	-
Ala12	5	0.55	0.02	0.86	0.07	0.65	0.07	1035.4	179.3	-	-
Glu13	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Gln14	5	0.63	0.01	0.91	0.02	0.7	0.03	1251.8	154.6	-	-
Arg15	2	0.84	0.03	-	-	0.84	0.03	667.7	191.2	-	-
His16	-	-	-	-	-	-	-	-	-	-	-
Gln17	2	0.61	0.17	-	-	0.61	0.17	536.6	211.2	-	-
Glu18	1	0.89	0.02	-	-	0.89	0.02	-	-	-	-
Trp19	2	0.88	0.02	-	-	0.88	0.02	29.9	11.8	-	-

Leu20	1	0.94	0.02	-	-	0.94	0.02	-	-	-	-
Arg21	1	0.89	0.01	-	-	0.89	0.01	-	-	-	-
Phe22	1	0.94	0.01	-	-	0.94	0.01	-	-	-	-
Val23	1	0.87	0.01	-	-	0.87	0.01	-	-	-	-
Asp24	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-
Leu25	1	0.91	0.02	-	-	0.91	0.02	-	-	-	-
Leu26	1	0.87	0.01	-	-	0.87	0.01	-	-	-	-
Lys27	3	0.84	0.01	-	-	0.84	0.01	-	-	7.2	0.2
Asn28	1	0.91	0	-	-	0.91	0	-	-	-	-
Ala29	3	0.85	0.01	-	-	0.85	0.01	-	-	1.2	0.1
Tyr30	3	0.86	0.04	-	-	0.86	0.04	-	-	2	0.8
Gln31	1	0.89	0.02	-	-	0.89	0.02	-	-	-	-
Asn32	1	0.82	0.01	-	-	0.82	0.01	-	-	-	-
Asp33	3	0.81	0.05	-	-	0.81	0.05	-	-	2.6	0.8
Leu34	2	0.9	0.01	-	-	0.9	0.01	21.4	3.7	-	-
His35	1	0.98	0.01	-	-	0.98	0.01	-	-	-	-
Leu36	1	0.95	0	-	-	0.95	0	-	-	-	-
Pro37	-	-	-	-	-	-	-	-	-	-	-
Leu38	-	-	-	-	-	-	-	-	-	-	-
Leu39	3	0.86	0.03	-	-	0.86	0.03	-	-	1.6	0.5
Asn40	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Leu41	1	0.89	0.05	-	-	0.89	0.05	-	-	-	-
Met42	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Leu43	3	0.83	0.05	-	-	0.83	0.05	-	-	2.1	0.7
Thr44	-	-	-	-	-	-	-	-	-	-	-
Pro45	-	-	-	-	-	-	-	-	-	-	-
Asp46	1	0.86	0.02	-	-	0.86	0.02	-	-	-	-
Glu47	3	0.79	0.01	-	-	0.79	0.01	-	-	2.4	0.6
Arg48	1	0.84	0.06	-	-	0.84	0.06	-	-	-	-
Glu49	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Ala50	1	0.98	0.01	-	-	0.98	0.01	-	-	-	-
Leu51	4	0.8	0.01	-	-	0.8	0.01	15.5	4.6	2.7	0.3
Gly52	3	0.83	0.05	-	-	0.83	0.05	-	-	2	0.7
Thr53	-	-	-	-	-	-	-	-	-	-	-
Arg54	1	0.94	0.04	-	-	0.94	0.04	-	-	-	-
Val55	1	0.28	0	-	-	0.28	0	-	-	-	-
Arg56	-	-	-	-	-	-	-	-	-	-	-
Ile57	1	0.98	0	-	-	0.98	0	-	-	-	-
Val58	1	0.66	0.34	-	-	0.66	0.34	-	-	-	-

Glu59	-	-	-	-	-	-	-	-	-	-	-
Glu60	1	0.93	0	-	-	0.93	0	-	-	-	-
Leu61	1	0.96	0.03	-	-	0.96	0.03	-	-	-	-
Leu62	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Arg63	1	0.91	0.03	-	-	0.91	0.03	-	-	-	-
Gly64	4	0.82	0.01	-	-	0.82	0.01	29	10.1	2.7	0.4
Glu65	1	0.8	0.04	-	-	0.8	0.04	-	-	-	-
Met66	4	0.76	0.03	-	-	0.76	0.03	18.5	3.4	1.2	0.5
Ser67	1	0.82	0.01	-	-	0.82	0.01	-	-	-	-
Gln68	5	0.68	0.04	0.82	0.03	0.83	0.03	1615.8	295.7	-	-
Arg69	5	0.87	0.02	0.93	0.03	0.94	0.03	564.7	304.5	-	-
Glu70	2	0.83	0.03	-	-	0.83	0.03	42.9	362.1	-	-
Leu71	1	0.88	0	-	-	0.88	0	-	-	-	-
Lys72	-	-	-	-	-	-	-	-	-	-	-
Asn73	1	0.88	0	-	-	0.88	0	-	-	-	-
Glu74	1	0.8	0.01	-	-	0.8	0.01	-	-	-	-
Leu75	1	0.92	0.04	-	-	0.92	0.04	-	-	-	-
Gly76	4	0.77	0.02	-	-	0.77	0.02	31.9	8.6	1.7	0.2
Val77	4	0.7	0.05	-	-	0.7	0.05	27.2	7	5.4	1.2
Gly78	4	0.75	0.01	-	-	0.75	0.01	32.3	5.4	2	0.3
Ile79	-	-	-	-	-	-	-	-	-	-	-
Ala80	-	-	-	-	-	-	-	-	-	-	-
Thr81	-	-	-	-	-	-	-	-	-	-	-
Ile82	-	-	-	-	-	-	-	-	-	-	-
Thr83	-	-	-	-	-	-	-	-	-	-	-
Arg84	-	-	-	-	-	-	-	-	-	-	-
Gly85	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Ser86	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Asn87	-	-	-	-	-	-	-	-	-	-	-
Ser88	-	-	-	-	-	-	-	-	-	-	-
Leu89	-	-	-	-	-	-	-	-	-	-	-
Lys90	2	0.89	0.01	-	-	0.89	0.01	31.1	9	-	-
Ala91	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Ala92	3	0.74	0.01	-	-	0.74	0.01	-	-	2	0.1
Pro93	-	-	-	-	-	-	-	-	-	-	-
Val94	1	0.87	0.04	-	-	0.87	0.04	-	-	-	-
Glu95	4	0.8	0.01	-	-	0.8	0.01	13.5	2.8	2	0.2
Leu96	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Arg97	1	1	0.04	-	-	1	0.04	-	-	-	-

Gln98	4	0.78	0.05	-	-	0.78	0.05	13.1	5.7	2.2	0.8
Trp99	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Leu100	3	0.83	0.01	-	-	0.83	0.01	-	-	1.4	0.2
Glu101	4	0.78	0.01	-	-	0.78	0.01	15.9	4.7	1.7	0.2
Glu102	1	0.87	0	-	-	0.87	0	-	-	-	-
Val103	1	0.88	0.03	-	-	0.88	0.03	-	-	-	-
Leu104	1	0.89	0.03	-	-	0.89	0.03	-	-	-	-
Leu105	1	0.86	0.02	-	-	0.86	0.02	-	-	-	-
Lys106	5	0.45	0.06	0.81	0.04	0.55	0.05	1110.5	122.7	-	-
Ser107	5	0.08	0.01	0.66	0.05	0.11	0.02	822.2	22.1	-	-
Asp108	5	0.03	0.01	0.45	0.04	0.07	0.03	530.2	17.9	-	-

Table 23 – Motional Parameters Computed For Holo-WT-TrpR Using Model-free Analysis.

<u>Residue</u>	<u>Model</u>	<u>S²</u>	<u>Err</u>	<u>S²_f</u>	<u>err</u>	<u>S²_s</u>	<u>err</u>	<u>τ_e (ns)</u>	<u>err</u> (ns)	<u>R_{ex}</u> (s ⁻¹)	<u>R_{ex} err</u> (s ⁻¹)
Met1	-	-	-	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	-	-	-	-	-	-	-
Gln3	-	-	-	-	-	-	-	-	-	-	-
Gln4	-	-	-	-	-	-	-	-	-	-	-
Ser5	-	-	-	-	-	-	-	-	-	-	-
Pro6	-	-	-	-	-	-	-	-	-	-	-
Tyr7	5	0.22	0.03	0.83	0.02	0.27	0.03	807.5	56	-	-
Ser8	-	-	-	-	-	-	-	-	-	-	-
Ala9	5	0.45	0	0.8	0.01	0.56	0.01	877.9	41	-	-
Ala10	-	-	-	-	-	-	-	-	-	-	-
Met11	5	0.46	0.02	0.87	0.04	0.53	0.04	1095.6	83.7	-	-
Ala12	5	0.61	0.02	0.85	0.01	0.72	0.01	931.1	38.8	-	-
Glu13	-	-	-	-	-	-	-	-	-	-	-
Gln14	2	0.68	0.03	-	-	0.68	0.03	1322	356.9	-	-
Arg15	-	-	-	-	-	-	-	-	-	-	-
His16	1	0.88	0.03	-	-	0.88	0.03	-	-	-	-

Gln17	5	0.8	0.04	0.93	0.03	0.86	0.02	1378.6	370.9	-	-
Glu18	2	0.9	0.03	-	-	0.9	0.03	29.6	70.2	-	-
Trp19	1	0.89	0.03	-	-	0.89	0.03	-	-	-	-
Leu20	1	0.9	0.01	-	-	0.9	0.01	-	-	-	-
Arg21	1	0.9	0.01	-	-	0.9	0.01	-	-	-	-
Phe22	2	0.92	0.02	-	-	0.92	0.02	1365.1	347.7	-	-
Val23	3	0.88	0.01	-	-	0.88	0.01	-	-	1.7	0.2
Asp24	-	-	-	-	-	-	-	-	-	-	-
Leu25	2	0.96	0.01	-	-	0.96	0.01	1104.7	93.5	-	-
Leu26	-	-	-	-	-	-	-	-	-	-	-
Lys27	3	0.88	0.03	-	-	0.88	0.03	-	-	5.4	1.4
Asn28	3	0.87	0.02	-	-	0.87	0.02	-	-	1.6	0.6
Ala29	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Tyr30	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Gln31	3	0.84	0.03	-	-	0.84	0.03	-	-	1.5	0.5
Asn32	1	0.87	0.02	-	-	0.87	0.02	-	-	-	-
Asp33	2	0.93	0.03	-	-	0.93	0.03	46.9	326.4	-	-
Leu34	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
His35	2	0.94	0.01	-	-	0.94	0.01	42.2	8.2	-	-
Leu36	3	0.85	0	-	-	0.85	0	-	-	2.3	0.9
Pro37	-	-	-	-	-	-	-	-	-	-	-
Leu38	1	0.91	0.03	-	-	0.91	0.03	-	-	-	-
Leu39	1	0.95	0.02	-	-	0.95	0.02	-	-	-	-
Asn40	-	-	-	-	-	-	-	-	-	-	-
Leu41	1	0.93	0.03	-	-	0.93	0.03	-	-	-	-
Met42	1	0.94	0.02	-	-	0.94	0.02	-	-	-	-
Leu43	1	0.95	0.01	-	-	0.95	0.01	-	-	-	-
Thr44	-	-	-	-	-	-	-	-	-	-	-
Pro45	-	-	-	-	-	-	-	-	-	-	-
Asp46	3	0.83	0.02	-	-	0.83	0.02	-	-	2.2	0.3
Glu47	1	0.85	0	-	-	0.85	0	-	-	-	-
Arg48	1	0.9	0.03	-	-	0.9	0.03	-	-	-	-
Glu49	3	0.86	0.04	-	-	0.86	0.04	-	-	2	0.7
Ala50	1	1	0.01	-	-	1	0.01	-	-	-	-
Leu51	1	0.97	0.03	-	-	0.97	0.03	-	-	-	-
Gly52	1	0.92	0.04	-	-	0.92	0.04	-	-	-	-
Thr53	-	-	-	-	-	-	-	-	-	-	-
Arg54	3	0.89	0.01	-	-	0.89	0.01	-	-	1.9	0.2
Val55	1	0.94	0.03	-	-	0.94	0.03	-	-	-	-

Arg56	-	-	-	-	-	-	-	-	-	-	-
Ile57	-	-	-	-	-	-	-	-	-	-	-
Val58	1	0.96	0.03	-	-	0.96	0.03	-	-	-	-
Glu59	-	-	-	-	-	-	-	-	-	-	-
Glu60	1	0.97	0.02	-	-	0.97	0.02	-	-	-	-
Leu61	1	1	0.03	-	-	1	0.03	-	-	-	-
Leu62	4	0.82	0.02	-	-	0.82	0.02	11.2	2.3	2.2	0.9
Arg63	4	0.85	0.01	-	-	0.85	0.01	14	1.6	1.8	0.6
Gly64	2	0.87	0.03	-	-	0.87	0.03	12.4	4.2	-	-
Glu65	2	0.82	0.03	-	-	0.82	0.03	30.3	301	-	-
Met66	-	-	-	-	-	-	-	-	-	-	-
Ser67	2	0.85	0.05	-	-	0.85	0.05	58.9	646.1	-	-
Gln68	2	0.83	0.02	-	-	0.83	0.02	60.2	9.6	-	-
Arg69	-	-	-	-	-	-	-	-	-	-	-
Glu70	2	0.85	0.03	-	-	0.85	0.03	62.8	57.5	-	-
Leu71	2	0.76	0.04	-	-	0.76	0.04	1497.1	509.3	-	-
Lys72	-	-	-	-	-	-	-	-	-	-	-
Asn73	2	0.86	0.02	-	-	0.86	0.02	60.2	25.2	-	-
Glu74	1	0.84	0.01	-	-	0.84	0.01	-	-	-	-
Leu75	-	-	-	-	-	-	-	-	-	-	-
Gly76	-	-	-	-	-	-	-	-	-	-	-
Ala77	-	-	-	-	-	-	-	-	-	-	-
Gly78	1	0.75	0.02	-	-	0.75	0.02	-	-	-	-
Ile79	-	-	-	-	-	-	-	-	-	-	-
Ala80	-	-	-	-	-	-	-	-	-	-	-
Thr81	-	-	-	-	-	-	-	-	-	-	-
Ile82	-	-	-	-	-	-	-	-	-	-	-
Thr83	-	-	-	-	-	-	-	-	-	-	-
Arg84	2	0.95	0.01	-	-	0.95	0.01	1734.1	264.6	-	-
Gly85	1	0.96	0.04	-	-	0.96	0.04	-	-	-	-
Ser86	1	0.98	0.01	-	-	0.98	0.01	-	-	-	-
Asn87	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Ser88	-	-	-	-	-	-	-	-	-	-	-
Leu89	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Lys90	1	0.94	0.02	-	-	0.94	0.02	-	-	-	-
Ala91	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Ala92	3	0.77	0.04	-	-	0.77	0.04	-	-	2.1	0.6
Pro93	-	-	-	-	-	-	-	-	-	-	-
Val94	1	0.88	0.03	-	-	0.88	0.03	-	-	-	-

Glu95	1	0.95	0.03	-	-	0.95	0.03	-	-	-	-
Leu96	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Arg97	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Gln98	1	0.95	0.02	-	-	0.95	0.02	-	-	-	-
Trp99	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Leu100	1	0.96	0.01	-	-	0.96	0.01	-	-	-	-
Glu101	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Glu102	2	0.9	0.03	-	-	0.9	0.03	16.8	180.8	-	-
Val103	2	0.89	0.02	-	-	0.89	0.02	16.3	5	-	-
Leu104	1	0.91	0.03	-	-	0.91	0.03	-	-	-	-
Leu105	2	0.91	0.02	-	-	0.91	0.02	112.3	99.1	-	-
Lys106	5	0.39	0.02	0.84	0.04	0.47	0.04	1152.3	57.4	-	-
Ser107	5	0.09	0.02	0.7	0.02	0.13	0.02	834.6	14.9	-	-
Asp108	-	-	-	-	-	-	-	-	-	-	-

Table 24– Motional Parameters Computed For Holo-L75F-TrpR Using Model-free Analysis.

<u>Residue</u>	<u>Model</u>	<u>S²</u>	<u>err</u>	<u>S²_f</u>	<u>err</u>	<u>S²_s</u>	<u>err</u>	<u>τ_e (ns)</u>	<u>err</u> (ns)	<u>R_{ex}</u> (s ⁻¹)	<u>err</u>
Met1	-	-	-	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	-	-	-	-	-	-	-
Gln3	-	-	-	-	-	-	-	-	-	-	-
Gln4	-	-	-	-	-	-	-	-	-	-	-
Ser5	5	0.05	0.05	0.64	0.14	0.07	0.08	614.2	94.7	-	-
Pro6	-	-	-	-	-	-	-	-	-	-	-
Tyr7	5	0.12	0.01	0.77	0.04	0.16	0.02	866.8	22.6	-	-
Ser8	2	0.19	0.01	-	-	0.19	0.01	537.5	87.9	-	-
Ala9	5	0.41	0.05	0.83	0.08	0.49	0.08	974.8	123	-	-
Ala10	-	-	-	-	-	-	-	-	-	-	-
Met11	5	0.42	0.01	0.79	0.03	0.53	0.03	1062.3	58.9	-	-
Ala12	5	0.56	0.11	0.86	0.07	0.65	0.09	1093.6	215.4	-	-
Glu13	5	0.62	0.01	0.83	0.01	0.75	0.01	1041.2	62	-	-

Gln14	5	0.63	0.01	0.84	0.02	0.75	0.02	1123.5	91.9	-	-
Arg15	5	0.75	0.04	0.9	0.02	0.84	0.03	642.9	252.8	-	-
His16	-	-	-	-	-	-	-	-	-	-	-
Gln17	5	0.72	0.03	0.89	0.04	0.81	0.05	1897.6	497.2	-	-
Glu18	2	0.95	0.02	-	-	0.95	0.02	47.7	163.3	-	-
Trp19	1	0.89	0.04	-	-	0.89	0.04	-	-	-	-
Leu20	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Arg21	1	0.92	0	-	-	0.92	0	-	-	-	-
Phe22	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-
Val23	1	0.87	0.03	-	-	0.87	0.03	-	-	-	-
Asp24	-	-	-	-	-	-	-	-	-	-	-
Leu25	1	0.93	0.05	-	-	0.93	0.05	-	-	-	-
Leu26	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Lys27	1	0.89	0.01	-	-	0.89	0.01	-	-	-	-
Asn28	1	0.91	0.05	-	-	0.91	0.05	-	-	-	-
Ala29	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-
Tyr30	1	1	0.02	-	-	1	0.02	-	-	-	-
Gln31	1	0.91	0.03	-	-	0.91	0.03	-	-	-	-
Asn32	1	0.85	0.02	-	-	0.85	0.02	-	-	-	-
Asp33	1	0.9	0.06	-	-	0.9	0.06	-	-	-	-
Leu34	1	0.92	0	-	-	0.92	0	-	-	-	-
His35	1	0.97	0.01	-	-	0.97	0.01	-	-	-	-
Leu36	1	0.87	0.06	-	-	0.87	0.06	-	-	-	-
Pro37	-	-	-	-	-	-	-	-	-	-	-
Leu38	2	0.83	0.06	-	-	0.83	0.06	33.9	504.2	-	-
Leu39	3	0.84	0	-	-	0.84	0	-	-	1.7	0.2
Asn40	1	0.96	0	-	-	0.96	0	-	-	-	-
Leu41	1	0.93	0.06	-	-	0.93	0.06	-	-	-	-
Met42	-	-	-	-	-	-	-	-	-	-	-
Leu43	2	0.97	0	-	-	0.97	0	1062.8	459.4	-	-
Thr44	1	0.93	0.07	-	-	0.93	0.07	-	-	-	-
Pro45	-	-	-	-	-	-	-	-	-	-	-
Asp46	1	0.91	0.04	-	-	0.91	0.04	-	-	-	-
Glu47	1	0.91	0.04	-	-	0.91	0.04	-	-	-	-
Arg48	2	0.86	0.01	-	-	0.86	0.01	76.6	6.6	-	-
Glu49	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Ala50	1	0.9	0.01	-	-	0.9	0.01	-	-	-	-
Leu51	3	0.88	0.01	-	-	0.88	0.01	-	-	2.7	0.2
Gly52	1	0.95	0.05	-	-	0.95	0.05	-	-	-	-

Thr53	-	-	-	-	-	-	-	-	-	-	-
Arg54	1	0.85	0.04	-	-	0.85	0.04	-	-	-	-
Val55	1	0.95	0.03	-	-	0.95	0.03	-	-	-	-
Arg56	1	0.93	0.02	-	-	0.93	0.02	-	-	-	-
Ile57	-	-	-	-	-	-	-	-	-	-	-
Val58	-	-	-	-	-	-	-	-	-	-	-
Glu59	1	0.92	0.03	-	-	0.92	0.03	-	-	-	-
Glu60	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
Leu61	1	0.98	0.04	-	-	0.98	0.04	-	-	-	-
Leu62	1	0.89	0	-	-	0.89	0	-	-	-	-
Arg63	1	0.89	0.03	-	-	0.89	0.03	-	-	-	-
Gly64	2	0.98	0.02	-	-	0.98	0.02	291.4	462.8	-	-
Glu65	1	0.9	0.01	-	-	0.9	0.01	-	-	-	-
Met66	2	0.74	0.02	-	-	0.74	0.02	33.6	8.2	-	-
Ser67	-	-	-	-	-	-	-	-	-	-	-
Gln68	2	0.79	0.08	-	-	0.79	0.08	1319.1	506.9	-	-
Arg69	-	-	-	-	-	-	-	-	-	-	-
Glu70	2	0.77	0.02	-	-	0.77	0.02	38.6	5.4	-	-
Leu71	-	-	-	-	-	-	-	-	-	-	-
Lys72	5	0.77	0.05	0.9	0.03	0.85	0.03	1264.5	244.4	-	-
Asn73	2	0.84	0.02	-	-	0.84	0.02	47.7	13.9	-	-
Glu74	-	-	-	-	-	-	-	-	-	-	-
Phe75	-	-	-	-	-	-	-	-	-	-	-
Gly76	-	-	-	-	-	-	-	-	-	-	-
Ala77	5	0.67	0.07	0.84	0.04	0.8	0.05	1055.4	240.2	-	-
Gly78	5	0.56	0.06	0.77	0.05	0.73	0.07	1280.8	281.4	-	-
Ile79	-	-	-	-	-	-	-	-	-	-	-
Ala80	-	-	-	-	-	-	-	-	-	-	-
Thr81	1	0.83	0.01	-	-	0.83	0.01	-	-	-	-
Ile82	2	0.93	0.04	-	-	0.93	0.04	1124.9	701.3	-	-
Thr83	-	-	-	-	-	-	-	-	-	-	-
Arg84	1	0.86	0.02	-	-	0.86	0.02	-	-	-	-
Gly85	1	0.94	0.05	-	-	0.94	0.05	-	-	-	-
Ser86	1	0.86	0	-	-	0.86	0	-	-	-	-
Asn87	1	0.89	0.04	-	-	0.89	0.04	-	-	-	-
Ser88	-	-	-	-	-	-	-	-	-	-	-
Leu89	1	0.96	0.06	-	-	0.96	0.06	-	-	-	-
Lys90	1	0.87	0.02	-	-	0.87	0.02	-	-	-	-
Ala91	1	0.85	0.12	-	-	0.85	0.12	-	-	-	-

Ala92	1	0.87	0.05	-	-	0.87	0.05	-	-	-	-
Pro93	-	-	-	-	-	-	-	-	-	-	-
Val94	1	0.83	0.01	-	-	0.83	0.01	-	-	-	-
Glu95	1	0.95	0.03	-	-	0.95	0.03	-	-	-	-
Leu96	1	0.93	0	-	-	0.93	0	-	-	-	-
Arg97	3	0.84	0.03	-	-	0.84	0.03	-	-	1.5	0.4
Gln98	1	0.91	0.04	-	-	0.91	0.04	-	-	-	-
Trp99	1	0.81	0.02	-	-	0.81	0.02	-	-	-	-
Leu100	3	0.81	0.04	-	-	0.81	0.04	-	-	2.1	0.5
Glu101	2	0.86	0.01	-	-	0.86	0.01	1500.3	277.1	-	-
Glu102	1	0.88	0.03	-	-	0.88	0.03	-	-	-	-
Val103	4	0.77	0.01	-	-	0.77	0.01	12.8	4.2	2	0.1
Leu104	2	0.87	0.03	-	-	0.87	0.03	48.6	201.6	-	-
Leu105	2	0.8	0.04	-	-	0.8	0.04	1346.4	348.2	-	-
Lys106	5	0.39	0.02	0.82	0.02	0.48	0.02	1093.2	50.2	-	-
Ser107	5	0.08	0.01	0.75	0.05	0.1	0.02	839.5	10.9	-	-
Asp108	5	0.03	0.02	0.51	0.04	0.06	0.04	542.9	22.7	-	-

Table 25 – Motional Parameters Computed For Holo-A77V-TrpR Using Model-free Analysis.

<u>Residue</u>	<u>Model</u>	<u>S²</u>	<u>Err</u>	<u>S²_f</u>	<u>err</u>	<u>S²_s</u>	<u>err</u>	<u>τ_e(ns)</u>	<u>err</u> (ns)	<u>R_{ex}</u> (s ⁻¹)	<u>err</u>
Met1	-	-	-	-	-	-	-	-	-	-	-
Ala2	-	-	-	-	-	-	-	-	-	-	-
Gln3	-	-	-	-	-	-	-	-	-	-	-
Gln4	-	-	-	-	-	-	-	-	-	-	-
Ser5	-	-	-	-	-	-	-	-	-	-	-
Pro6	-	-	-	-	-	-	-	-	-	-	-
Tyr7	5	0.21	0.01	0.79	0.01	0.26	0.01	837.8	5.6	-	-
Ser8	5	0.24	0.06	0.75	0.04	0.32	0.07	1000.3	77.4	-	-
Ala9	5	0.33	0.08	0.61	0.06	0.54	0.11	999.6	179.5	-	-
Ala10	5	0.44	0.01	0.8	0.01	0.55	0.01	986.2	43.5	-	-
Met11	5	0.44	0.02	0.83	0.03	0.54	0.03	1057.9	65	-	-
Ala12	5	0.55	0.01	0.81	0.02	0.69	0.02	1062.3	60.5	-	-

Glu13	5	0.59	0.03	0.84	0.04	0.7	0.05	1115.3	136.2	-	-
Gln14	5	0.7	0.04	0.91	0.02	0.77	0.02	1028.4	97	-	-
Arg15	1	0.85	0.02	-	-	0.85	0.02	-	-	-	-
His16	4	0.78	0	-	-	0.78	0	11.5	1.8	1.7	0.1
Gln17	-	-	-	-	-	-	-	-	-	-	-
Glu18	2	0.88	0.02	-	-	0.88	0.02	32.3	269.8	-	-
Trp19	2	0.87	0.01	-	-	0.87	0.01	41.2	7.8	-	-
Leu20	-	-	-	-	-	-	-	-	-	-	-
Arg21	1	0.92	0.01	-	-	0.92	0.01	-	-	-	-
Phe22	3	0.89	0.01	-	-	0.89	0.01	-	-	1.3	0.4
Val23	-	-	-	-	-	-	-	-	-	-	-
Asp24	1	0.98	0.02	-	-	0.98	0.02	-	-	-	-
Leu25	1	0.89	0	-	-	0.89	0	-	-	-	-
Leu26	3	0.83	0.03	-	-	0.83	0.03	-	-	1.8	0.5
Lys27	4	0.82	0.01	-	-	0.82	0.01	19.8	5.6	3.7	0.4
Asn28	4	0.85	0	-	-	0.85	0	59.5	3.2	0.9	0.2
Ala29	2	0.94	0.02	-	-	0.94	0.02	48.1	368.9	-	-
Tyr30	3	0.81	0.02	-	-	0.81	0.02	-	-	2.7	0.7
Gln31	2	0.87	0	-	-	0.87	0	21.9	0.9	-	-
Asn32	2	0.81	0.01	-	-	0.81	0.01	8.6	2.9	-	-
Asp33	3	0.82	0.03	-	-	0.82	0.03	-	-	2	0.5
Leu34	2	0.92	0.01	-	-	0.92	0.01	39.8	4.6	-	-
His35	1	0.91	0	-	-	-	-	-	-	-	-
Leu36	1	0.91	0.02	-	-	0.91	0.02	-	-	-	-
Pro37	-	-	-	-	-	-	-	-	-	-	-
Leu38	3	0.85	0	-	-	0.85	0	-	-	1.5	0.3
Leu39	2	0.86	0	-	-	0.86	0	16.9	3.4	-	-
Asn40	1	0.9	0.01	-	-	0.9	0.01	-	-	-	-
Leu41	1	0.91	0.01	-	-	0.91	0.01	-	-	-	-
Met42	1	0.92	0.04	-	-	0.92	0.04	-	-	-	-
Leu43	1	0.83	0.01	-	-	0.83	0.01	-	-	-	-
Thr44	-	-	-	-	-	-	-	-	-	-	-
Pro45	-	-	-	-	-	-	-	-	-	-	-
Asp46	1	0.89	0.01	-	-	0.89	0.01	-	-	-	-
Glu47	1	0.92	0.03	-	-	0.92	0.03	-	-	-	-
Arg48	4	0.86	0.01	-	-	0.86	0.01	25.2	2.4	0.9	0.3
Glu49	1	0.88	0.03	-	-	0.88	0.03	-	-	-	-
Ala50	1	0.87	0.01	-	-	0.87	0.01	-	-	-	-
Leu51	3	0.8	0.02	-	-	0.8	0.02	-	-	2.1	0.4

[illegible]

Ala91	1	0.83	0.01	-	-	0.83	0.01	-	-	-	-
Ala92	1	0.84	0.03	-	-	0.84	0.03	-	-	-	-
Pro93	-	-	-	-	-	-	-	-	-	-	-
Val94	1	0.86	0.01	-	-	0.86	0.01	-	-	-	-
Glu95	-	-	-	-	-	-	-	-	-	-	-
Leu96	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Arg97	2	0.85	0.02	-	-	0.85	0.02	56.8	121.2	-	-
Gln98	4	0.82	0.03	-	-	0.82	0.03	12.2	4.1	2.4	0.6
Trp99	1	0.92	0.02	-	-	0.92	0.02	-	-	-	-
Leu100	4	0.84	0.03	-	-	0.84	0.03	14.3	4.5	1.6	0.4
Glu101	1	0.93	0	-	-	0.93	0	-	-	-	-
Glu102	1	0.89	0	-	-	0.89	0	-	-	-	-
Val103	1	0.91	0.02	-	-	0.91	0.02	-	-	-	-
Leu104	1	0.9	0.02	-	-	0.9	0.02	-	-	-	-
Leu105	2	0.83	0.01	-	-	0.83	0.01	32.2	6	-	-
Lys106	5	0.4	0.02	0.75	0.05	0.54	0.05	1075.8	83.8	-	-
Ser107	-	-	-	-	-	-	-	-	-	-	-
Asp108	5	0.02	0.01	0.53	0.03	0.04	0.01	546.2	5.9	-	-

Table 26 – ^{15}N -Relaxation Parameters Measured For E73 At pH 5.0 And 312 K.

<u>Residue</u> <u>Type</u>	<u>Residue</u> <u>number</u>	$\frac{^{15}\text{N}-\{^1\text{H}\}}{\text{nOe (I/Io)}}$	<u>err (I/Io)</u>	$\frac{^{15}\text{N}-\text{T}_1}{(\text{ms})}$	$\frac{^{15}\text{N}-\text{T}_1}{\text{error (ms)}}$	$\frac{^{15}\text{N}-\text{T}_2}{(\text{ms})}$	$\frac{^{15}\text{N}-\text{T}_2}{\text{err (ms)}}$
MET	1	-	-	-	-	-	-
VAL	2	-0.29	0.01	772.9	7.4	245.7	14.9
GLU	3	-0.28	0.01	758.1	52.5	311.1	3.7
SER	4	-	-	-	-	-	-
LYS	5	-	-	-	-	-	-
LYS	6	-	-	-	-	-	-
ILE	7	0.12	0.01	725.8	6.2	156.4	1.2
ALA	8	0.34	0.01	684.7	4.0	113.7	1.8
LYS	9	0.57	0.03	758.1	33.0	89.9	2.2
LYS	10	0.70	0.01	734.8	0.2	84.9	3.5
LYS	11	-	-	-	-	-	-

THR	12	0.72	0.02	760.8	8.8	74.0	0.7
THR	13	-	-	-	-	-	-
LEU	14	0.70	0.02	725.7	5.7	81.1	0.2
ALA	15	0.67	0.03	721.7	41.5	89.7	1.9
PHE	16	-	-	-	-	-	-
ASP	17	0.78	0.01	783.2	9.4	79.6	5.3
GLU	18	0.76	0.01	705.1	14.5	67.1	0.1
ASP	19	0.74	0.01	735.6	20.6	64.2	1.7
VAL	20	0.74	0.04	759.3	9.9	68.0	1.8
TYR	21	0.79	0.01	767.4	82.4	61.1	2.0
HIS	22	0.80	0.01	675.7	7.6	70.4	1.0
THR	23	0.74	0.02	769.4	91.9	63.6	0.5
LEU	24	-	-	-	-	-	-
LYS	25	0.80	0.01	797.6	6.8	62.2	0.3
LEU	26	0.78	0.01	795.1	2.1	64.3	0.1
VAL	27	0.70	0.03	739.3	10.3	81.8	1.6
SER	28	0.80	0.03	743.3	1.6	62.8	0.1
VAL	29	0.81	0.02	777.6	29.1	62.8	3.1
TYR	30	0.78	0.01	749.3	15.1	67.3	0.1
LEU	31	0.73	0.02	718.9	25.3	66.9	0.1
ASN	32	0.75	0.01	919.2	8.3	64.7	0.3
ARG	33	0.78	0.01	864.2	19.9	61.5	1.4
ASP	34	0.68	0.01	847.1	10.8	74.2	0.8
MET	35	0.71	0.03	728.5	17.0	68.6	3.2
THR	36	0.75	0.01	675.5	11.9	73.6	0.2
GLU	37	0.75	0.06	706.3	13.2	73.8	0.9
ILE	38	-	-	-	-	-	-
ILE	39	0.83	0.01	690.7	13.9	70.5	2.5
GLU	40	0.80	0.02	683.4	7.8	69.7	0.7
GLU	41	0.81	0.01	702.3	26.8	70.3	1.7
ALA	42	0.79	0.01	683.1	1.8	68.5	0.7
VAL	43	0.75	0.01	760.1	8.4	71.2	0.6
VAL	44	0.77	0.02	716.0	5.2	62.6	0.2
MET	45	0.75	0.02	753.0	15.2	59.6	0.5
TRP	46	0.78	0.01	690.2	89.7	71.2	0.8
LEU	47	0.75	0.03	727.3	1.2	68.5	2.4
ILE	48	0.76	0.03	701.7	20.2	70.0	1.1
GLN	49	0.77	0.01	734.5	15.0	67.2	0.4
ASN	50	0.78	0.01	791.5	11.0	73.9	1.7

LYS	51	0.71	0.02	758.7	6.9	70.5	0.4
GLU	52	0.74	0.01	676.0	8.8	73.8	0.8
LYS	53	0.69	0.01	725.6	7.7	73.8	0.3
LEU	54	0.68	0.01	767.7	8.9	76.6	0.6
PRO	55	-	-	-	-	-	-
ASN	56	0.75	0.03	727.3	1.2	68.5	2.4
GLU	57	-	-	-	-	-	-
LEU	58	0.73	0.01	734.8	7.8	68.9	0.3
LYS	59	0.79	0.01	708.6	17.6	69.9	0.1
PRO	60	-	-	-	-	-	-
LYS	61	0.69	0.02	767.5	0.4	70.3	1.7
ILE	62	0.74	0.01	737.6	93.3	69.9	0.9
ASP	63	-	-	-	-	-	-
GLU	64	0.75	0.01	772.2	13.1	68.9	0.5
ILE	65	0.68	0.05	753.3	7.6	67.1	0.2
SER	66	0.79	0.01	705.9	3.1	68.6	0.4
LYS	67	0.74	0.00	692.2	9.6	70.6	0.9
ARG	68	0.75	0.01	731.4	4.0	69.1	0.6
PHE	69	0.69	0.01	733.8	10.2	72.9	2.1
PHE	70	0.62	0.01	749.9	59.0	87.1	0.4
PRO	71	-	-	-	-	-	-
ALA	72	0.16	0.01	779.6	50.7	177.1	5.0
LYS	73	-0.64	0.01	1003.2	9.7	471.1	1.6

Table 27. Spectral Density Values For E73 Measured At 14.1 T.

<u>Res type</u>	<u>Res #</u>	<u>$J_{\text{eff}}(0)$, ns/rad</u>			<u>$J(w_N)$, ns/rad</u>			<u>$J(0.87w_H)$, ps/rad</u>		
MET	1	-			-			-		
VAL	2	1.28	±	0.10	0.27	±	0.01	25.96	±	0.30
GLU	3	0.94	±	0.02	0.28	±	0.02	26.35	±	1.78
SER	4	-			-			-		
LYS	5	-			-			-		
LYS	6	-			-			-		

ILE	7	2.16	±	0.02	0.31	±	0.01	19.16	±	0.26
ALA	8	3.06	±	0.05	0.34	±	0.01	15.03	±	0.46
LYS	9	3.98	±	0.10	0.32	±	0.01	8.44	±	1.09
LYS	10	4.23	±	0.18	0.33	±	0.01	6.36	±	0.01
LYS	11	-			-			-		
THR	12	4.90	±	0.05	0.32	±	0.01	5.93	±	0.86
THR	13	-			-			-		
LEU	14	4.43	±	0.01	0.34	±	0.01	6.89	±	0.89
ALA	15	3.98	±	0.10	0.34	±	0.02	7.59	±	1.40
PHE	16	-			-			-		
ASP	17	4.55	±	0.32	0.32	±	0.01	4.59	±	0.58
GLU	18	5.41	±	0.01	0.35	±	0.01	5.32	±	0.44
ASP	19	5.67	±	0.16	0.33	±	0.01	5.52	±	0.17
VAL	20	5.35	±	0.15	0.33	±	0.01	4.74	±	1.51
TYR	21	5.99	±	0.19	0.32	±	0.03	4.26	±	0.46
HIS	22	5.13	±	0.08	0.37	±	0.01	4.62	±	0.43
THR	23	5.74	±	0.05	0.32	±	0.04	4.87	±	1.09
LEU	24	-			-			-		
LYS	25	5.90	±	0.03	0.31	±	0.01	3.90	±	0.19
LEU	26	5.69	±	0.01	0.31	±	0.01	4.32	±	0.40
VAL	27	4.40	±	0.09	0.33	±	0.01	5.90	±	1.22
SER	28	5.81	±	0.00	0.33	±	0.01	4.63	±	1.30
VAL	29	5.83	±	0.31	0.32	±	0.01	4.02	±	0.76
TYR	30	5.41	±	0.01	0.33	±	0.01	4.56	±	0.10
LEU	31	5.43	±	0.01	0.34	±	0.01	5.42	±	0.91
ASN	32	5.68	±	0.02	0.27	±	0.01	4.08	±	0.17
ARG	33	5.98	±	0.14	0.29	±	0.01	3.98	±	0.20
ASP	34	4.91	±	0.06	0.29	±	0.01	5.89	±	0.56
MET	35	5.30	±	0.25	0.34	±	0.01	5.77	±	1.28
THR	36	4.90	±	0.02	0.36	±	0.01	6.00	±	0.12
GLU	37	4.90	±	0.06	0.35	±	0.01	4.43	±	2.63
ILE	38	-			-			-		
ILE	39	5.14	±	0.19	0.36	±	0.01	4.07	±	0.42
GLU	40	5.19	±	0.06	0.36	±	0.01	5.01	±	1.11
GLU	41	5.15	±	0.14	0.35	±	0.01	3.99	±	0.46
ALA	42	5.29	±	0.06	0.36	±	0.01	5.01	±	0.45
VAL	43	5.10	±	0.05	0.33	±	0.01	4.94	±	0.40
VAL	44	5.82	±	0.02	0.35	±	0.01	4.80	±	0.71
MET	45	6.14	±	0.06	0.33	±	0.01	4.98	±	0.83

TRP	46	5.08	± 0.07	0.36	± 0.05	4.98	± 0.64
LEU	47	5.31	± 0.20	0.34	± 0.01	4.95	± 1.48
ILE	48	5.17	± 0.08	0.35	± 0.01	4.91	± 1.34
GLN	49	5.42	± 0.04	0.34	± 0.01	4.67	± 0.45
ASN	50	4.92	± 0.12	0.31	± 0.01	4.13	± 0.40
LYS	51	5.16	± 0.03	0.32	± 0.01	5.77	± 0.63
GLU	52	4.88	± 0.06	0.36	± 0.01	6.00	± 0.26
LYS	53	4.90	± 0.02	0.34	± 0.01	6.89	± 0.05
LEU	54	4.73	± 0.04	0.32	± 0.01	6.29	± 0.43
PRO	55	-		-		-	
ASN	56	5.31	± 0.19	0.34	± 0.01	4.95	± 1.52
GLU	57	-		-		-	
LEU	58	5.27	± 0.02	0.33	± 0.01	5.73	± 0.23
LYS	59	5.18	± 0.01	0.35	± 0.01	4.84	± 0.67
PRO	60	-		-		-	
LYS	61	5.17	± 0.14	0.32	± 0.01	5.88	± 0.97
ILE	62	5.19	± 0.07	0.33	± 0.04	5.52	± 0.70
ASP	63	-		-		-	
GLU	64	5.28	± 0.04	0.32	± 0.01	4.87	± 0.39
ILE	65	5.42	± 0.02	0.33	± 0.01	6.02	± 1.85
SER	66	5.29	± 0.04	0.35	± 0.01	4.65	± 0.43
LYS	67	5.12	± 0.07	0.35	± 0.01	5.84	± 0.25
ARG	68	5.25	± 0.05	0.34	± 0.01	5.13	± 0.42
PHE	69	4.97	± 0.15	0.33	± 0.01	6.58	± 0.23
PHE	70	4.12	± 0.03	0.32	± 0.02	7.68	± 0.69
PRO	71	-		-		-	
ALA	72	1.89	± 0.06	0.29	± 0.02	16.77	± 1.10
LYS	73	0.59	± 0.01	0.20	± 0.01	25.58	± 0.30

Table 28 – Motional Parameters Computed For E73 Using Model-free Analysis.

Res	Res #	Model	S ²	err	S ² _f	err	S ² _s	err	τ_e (ns)	$\tau_{e(ns)}$	R _{ex} (s ⁻¹)	err (s ⁻¹)
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MET	1	-	-	-	-	-	-	-	-	-	-	-
VAL	2	5	0.22	0.02	0.79	0.01	0.28	0.03	772	20	-	-
GLU	3	5	0.14	0.01	0.76	0.04	0.18	0.02	847	16	-	-
SER	4	-	-	-	-	-	-	-	-	-	-	-
LYS	5	-	-	-	-	-	-	-	-	-	-	-
LYS	6	-	-	-	-	-	-	-	-	-	-	-
ILE	7	5	0.39	0.01	0.84	0.01	0.46	0.01	914	10	-	-
ALA	8	5	0.56	0.01	0.91	0.01	0.61	0.01	1009	33	-	-
LYS	9	5	0.77	0.02	0.91	0.02	0.84	0.03	919	233	-	-
LYS	10	5	0.82	0.04	0.92	0.02	0.89	0.02	1267	225	-	-
LYS	11	-	-	-	-	-	-	-	-	-	-	-
THR	12	1	0.96	0.01	-	-	0.96	0.01	-	-	-	-
THR	13	-	-	-	-	-	-	-	-	-	-	-
LEU	14	5	0.88	0.01	0.96	0.01	0.91	0.01	846	231	-	-
ALA	15	5	0.75	0.02	0.91	0.01	0.83	0.04	1333	872	-	-
PHE	16	-	-	-	-	-	-	-	-	-	-	-
ASP	17	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
GLU	18	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
ASP	19	4	0.97	0.02	-	-	0.97	0.02	222	387	1.9	0.5
VAL	20	3	0.93	0.01	-	-	0.93	0.01	-	-	1.9	0.4
TYR	21	4	0.89	0.06	-	-	0.89	0.06	15	532	4.6	0.9
HIS	22	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
THR	23	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
LEU	24	-	-	-	-	-	-	-	-	-	-	-
LYS	25	-	-	-	-	-	-	-	-	-	-	-
LEU	26	4	0.89	0.01	-	-	0.89	0.01	19	7	3.4	0.1
VAL	27	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
SER	28	3	0.92	0.01	-	-	0.92	0.01	-	-	3.8	0.1
VAL	29	3	0.89	0.03	-	-	0.89	0.03	-	-	4.0	0.9
TYR	30	4	0.93	0.02	-	-	0.93	0.02	34	53	2.1	0.3
LEU	31	4	0.95	0.03	-	-	0.95	0.03	87	401	2.2	0.4
ASN	32	4	0.76	0.01	-	-	0.76	0.01	12	2	5.0	0.1
ARG	33	4	0.81	0.02	-	-	0.81	0.02	11	2	5.1	0.4
ASP	34	4	0.79	0.02	-	-	0.79	0.02	33	7	2.8	0.2
MET	35	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
THR	36	4	0.90	0.02	-	-	0.90	0.02	1659	195	0.5	0.2
GLU	37	2	0.94	0.01	-	-	0.94	0.01	1409	481	-	-
ILE	38	-	-	-	-	-	-	-	-	-	-	-
ILE	39	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-

GLU	40	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
GLU	41	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
ALA	42	1	1.00	0.01	-	-	1.00	0.00	-	-	-	-
VAL	43	1	0.98	0.01	-	-	0.98	0.01	-	-	-	-
VAL	44	-	-	-	-	-	-	-	-	-	-	-
MET	45	3	0.98	0.02	-	-	0.98	0.02	-	-	2.8	0.3
TRP	46	2	0.98	0.01	-	-	0.98	0.01	500	419	-	-
LEU	47	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
ILE	48	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
GLN	49	1	1.00	0.01	-	-	1.00	0.00	-	-	-	-
ASN	50	1	0.93	0.01	-	-	0.93	0.01	-	-	-	-
LYS	51	4	0.94	0.01	-	-	0.94	0.01	113	30	0.7	0.2
GLU	52	4	0.89	0.01	-	-	0.89	0.01	1669	158	0.5	0.2
LYS	53	4	0.94	0.01	-	-	0.94	0.01	473	118	0.2	0.1
LEU	54	4	0.90	0.01	-	-	0.90	0.01	70	14	0.7	0.2
PRO	55	-	-	-	-	-	-	-	-	-	-	-
ASN	56	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
GLU	57	-	-	-	-	-	-	-	-	-	-	-
LEU	58	4	0.93	0.01	-	-	0.93	0.01	73	11	2.0	0.1
LYS	59	1	1.00	0.01	-	-	-	-	-	-	-	-
PRO	60	-	-	-	-	-	-	-	-	-	-	-
LYS	61	4	0.90	0.01	-	-	0.90	0.01	64	24	1.7	0.4
ILE	62	4	0.96	0.06	-	-	0.96	0.06	546	812	0.6	0.9
ASP	63	-	-	-	-	-	-	-	-	-	-	-
GLU	64	4	0.93	0.02	-	-	0.93	0.02	48	21	1.4	0.2
ILE	65	3	0.98	0.01	-	-	0.98	0.01	-	-	1.0	0.1
SER	66	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
LYS	67	4	0.92	0.01	-	-	0.92	0.01	1298	194	0.9	0.2
ARG	68	1	1.00	0.01	-	-	1.00	0.01	-	-	-	-
PHE	69	2	0.95	0.01	-	-	0.95	0.01	324	196	-	-
PHE	70	2	0.82	0.01	-	-	0.82	0.01	56	7	-	-
PRO	71	-	-	-	-	-	-	-	-	-	-	-
ALA	72	5	0.33	0.02	0.76	0.03	0.44	0.03	1002	41	-	-
LYS	73	5	0.08	0.01	0.62	0.01	0.14	0.01	696	4	-	-

Table 29. Regulon of TrpR Transcription Factor In *Escherichia coli* Strain K-12.

<u>Operon</u>	<u>Position</u>	<u>Sequence</u>
trpE-trpD-trpC-trpB-trpA	-183	CGAACTAGTTAACTAGTACG
trpR	-66	CGTACTCTTTAGCGAGTACA
mtr	-72	TGTACTCGTGTACTGGTACA
aroL-yaiA-aroM	-79	TGTACTAGTTTGATGGTATG
aroH	-164	TGTACTAGAGAACTAGTGCA

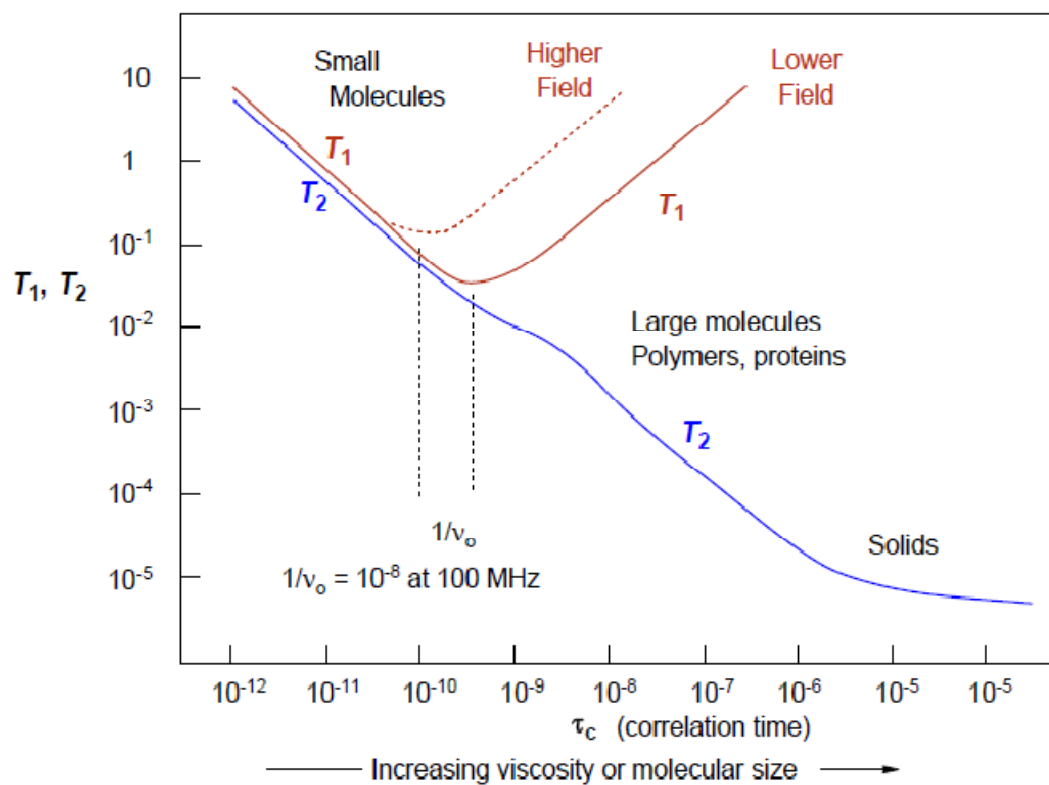


Figure 39. Variation of Relaxation Constants T_1 and T_2 With Correlation Time (Or Tumbling Time), τ_c .

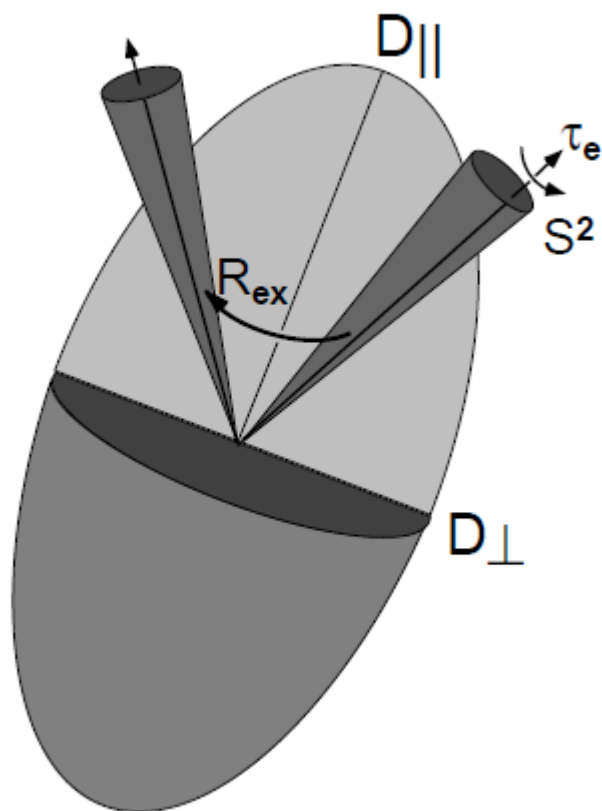


Figure 40. Figurative Representation Of Molecular Reorientation And Internal Motions Parameters Calculated From Model-free Approach In A Prolate Ellipsoid Molecule.

FAST Modelfree ProtocolPreparation of Input Files

Prior to beginning an analysis, several files must be created. Separate files for T1, T2 and NOE data files for each static field (only one in our case because only 600 MHz used) should be made. The form of the file should be one line per residue, with residue number, relaxation rate and error specified. The fields may be separated by any combination of spaces and tabs. An example is illustrated below (the first line should not be included in the data file):

Res #	Rate	Err
2	152	34
5	164	42
6	183	20
9	167	43

If an axially symmetric diffusion tensor is desired, a coordinate PDB file must also be properly prepared. Preparation of the PDB file requires several steps. First, all H atoms must be added to the structure file if using a xtal structure pdb. This can be done by a number of programs, including the freely available program MolMol (<http://www.mol.biol.ethz.ch/wutrich/software/molmol>). If MolMol is used, the necessary H atoms may be added via the following MolMol commands:

```
ReadPdb input.pdb
```

```
CalcAtom "H*"
```

```
SelectAtom 'atom.name "*"Q*"'
```

```
RemoveAtom 'selected'
```

```
WritePdb output.pdb
```

Next, the coordinate system of the PDB file should be moved to the center of mass of the molecule. Finally, an estimate of the diffusion tensor should be made and the axis of the PDB coordinate system should be rotated so that they are aligned with the principal axis of the diffusion tensor.

These final two transformations may be accomplished with the programs "pdbinertia" and "r2r1_diffusion" which are available from the website:

<http://www.palmer.hs.columbia.edu/software/diffusion.html>

Details for the operation of these programs can be found on the Palmer laboratory website (the link given above). (Optional) The diffusion tensor values from r2r1_diffusion can be confirmed by comparing with those obtained from NORMAdyn program.

The user should be aware that the program ModelFree will not tolerate anything other than ATOM statements. All other statements, headers, etc. should be removed before running ModelFree.

Parameter Input

The graphical user interface may be invoked with the command "setupFMF". The user may then specify the various parameters as desired. These parameters include:

FASTModelfree Parameters

Name For Output Files: This is the name that will be given to some of the output files created by FASTModelfree.

SSECutoff: This is the α -critical value for the synthetic distribution of Γ_i . The Γ_i for a given spin is compared to the Γ_i of the α -critical value as part of the protocol to determine if a particular model is appropriate for the given spin. The most commonly used value is 0.95. (SSE = Sum of Squared Errors)

FTest Cutoff: This parameter is similar to the SSECutoff, but refers to the critical value used during the statistical F-test needed to assign two parameter models. The value most commonly used is 0.80.

Model 1 Only: In some cases the authors have noticed a significant improvement in speed when initially only those spins which fit model 1 are used to estimate the diffusion tensor.

In cases where there are a large number of spins or multiple static magnetic fields it can be beneficial to perform analysis using only model 1, then after reaching a tensor which

is self consistent with the set of spins assigned to model 1 begin the calculation again with this new tensor and allow all models to be fitted.

S2 Cutoff: Spins whose calculated S^2 value is below this cutoff will be excluded during diffusion tensor optimizations.

Random Number Seed: Seed number given to the random number generator. Never changed.

Number Of MC Simulations: Number of Monte-Carlo simulations used for statistical error analysis.

MFPAR Parameters

^{15}N Magnetogyric Ratio: Magnetogyric ratio of ^{15}N . Typically = -2.71.

N-H Bond Distance: N-H Bond distance, in Angstroms. Typical value used = 1.02.

^{15}N CSA: ^{15}N chemical shift anisotropy, in ppm. Typical value used = -160

Data Files

The necessary input data files and magnetic field strength may be selected here. Clicking on the box next to the text entry will open a file selection window. A PDB file is not required for use with an isotropic diffusion tensor.

Tensor Optimization Parameters

Diffusion Tensor: Axially symmetric or isotropic diffusion tensor may be selected.

Optimize Tensor?: If this option is set to "No", then only a single round of model selection will be performed and subsequently the diffusion tensor will not be optimized. In general, this should be set to "yes". The remaining dialogue boxes allow specification of parameters related to the diffusion tensor.

For each parameter, initial estimates, gridsearch ranges and number of gridsearch steps may be specified. The convergence limit indicates the maximum amount of change allowed in the tensor compared to the previous iteration to determine whether the system has converged to a diffusion tensor which is self consistent with the set of assigned models.

Running FASTModelfree

Once all parameters have been entered as desired, the user should select the SAVE CONFIG or

SAVE CONFIG and EXIT button. This will write a file in the current directory named FMF.config which contains all of the desired parameters. This file is intended to be

human readable, although all parameters may be specified within the graphical user interface.

The user may now invoke the second part of FASTModelfree by running the module "fastMF".

This module reads the FMF.config file and begins the actual data analysis. A great deal of diagnostic output is created by this module, which details the decisions made in assigning models and optimizing the tensor.

FASTModelfree Output

The output from FASTModelfree is grouped into several files. First, the module "fastMF" will directly output a great deal of information on the particulars of the model assignment. In addition, several other files will be created to further detail both the process and results. These files are typically named according to the name for output files specified during setup. In this example, the name for output files specified in the graphical user interface is assumed to be TrpR.

TrpR.log: This will detail the model assignments and tensor data for each individual iteration, as well as whether the system has converged within the user defined limits.

TrpR.x.par: The model assignments and spin parameters for each iteration are stored in separate tab delimited files. These files contain the final assigned models and relevant motional parameters.

TrpR.x.pdb: These files are created only if an axially symmetric diffusion tensor is used. They contain the original PDB file with the coordinate system rotated to the principal axis of the diffusion tensor used during the current iteration.