Rotational dynamics of proteins from spin relaxation rates and molecular dynamics simulations

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I. INTRODUCTION

Spin relaxation rates measured with NMR techniques give segmental resolution information about rotational dynamics of proteins and macromolecules. Various models have been used to separate internal dynamics and order from overall rotational diffusion of molecules [**?**]. These techniques have been highly useful in characterization of rotational diffusion and internal flexibility of proteins and other biomolecules. On the other hand, segmental level information has been used in validation and improvement of molecular dynamics simulation force fields [**?**]. Segmental order has been also related to conformational entropy of proteins [**?**].

Spin relaxation rates are typically intepreted by using various dynamical models assuming different level of complexity of rotational relaxation processes. The most simplistic models assume single timescale and order parameter for internal motion and isotropic overall rotational diffusion. However, biomolecules often experience anisotropic overall diffusion and several internal timescales. Models for more complicated models have been also introduced, however, there are more fitting parameters in these models and intepretation of experiments becomes extensively difficult.

Classical molecular dynamics simuations contain, in principle, all the complexity of the timescales and could be used to interpret the rotational dynamics measured with spin relaxation experiments. However, this has not been a trivial task due to the force field issues and limited available time scales in the simulations.

In this work we present approach that can be used to analyze spin relaxation experiments by using classical molecular dynamics simulations. The approach can be used for anisotropic molecules and allows a correction of anisotropic overall diffusion. We demonstrate the usage of the approach for two aniotropic protein constructs from engineered from Helicobacteri pyroli and Pseudomonas.

II. METHODS

A. Spin relaxation analysis from MD simulations

Experimentally measurable spin relaxation rates R_1, R_2 and R_{NOE} for N-H bonds are related to the molecular dynamics through equations [**?** **?**]

$$R_1 = \frac{d_{\mathrm{NH}}^2 N_{\mathrm{H}}}{20}\Big[J(\omega_{\mathrm{H}}-\omega_{\mathrm{N}}) + 3J(\omega_{\mathrm{N}}) + 6J(\omega_{\mathrm{N}}+\omega_{\mathrm{H}})\Big] + \frac{(\sigma\omega_{\mathrm{N}})^2}{15} j(\omega_{\mathrm{N}}), \tag{1}$$

$$R_2 = \frac{1}{2}\frac{d_{\mathrm{NH}}^2 N_{\mathrm{H}}}{20}\Big[4J(0) + 3j(\omega_{\mathrm{N}}) + J(\omega_{\mathrm{H}}-\omega_{\mathrm{N}}) + 6J(\omega_{\mathrm{H}}) + 6J(\omega_{\mathrm{N}}+\omega_{\mathrm{H}})\Big] + \frac{(\sigma\omega_{\mathrm{N}})^2}{15*6}[4J(0)+3J(\omega_{\mathrm{N}})], \tag{2}$$

$$R_{\mathrm{NOE}} = 1 + \frac{d_{\mathrm{NH}}^2 N_{\mathrm{H}}}{20}\Big[6J(\omega_{\mathrm{N}}+\omega_{\mathrm{H}}) + J(\omega_{\mathrm{H}}-\omega_{\mathrm{N}}))\Big]\frac{\gamma_{\mathrm{H}}}{\gamma_{\mathrm{N}} R_1}, \tag{3}$$

where ω_{N} and ω_{H} are the Larmor angular frequencies of ^{15}N and ^{1}H respectively, N_{H} is the number of bound protons. The dipolar coupling constant is given by

$$d_{\mathrm{NH}} = -\frac{\mu_0 \hbar \gamma_{\mathrm{H}} \gamma_{\mathrm{N}}}{4\pi \langle r_{\mathrm{CN}}^3 \rangle},$$

where μ_0 is the magnetic constant or vacuum permeability, $\hbar$ is the reduced Planck constant, γ_{N} and γ_{H} are the gyromagnetic constants of ^{13}C and ^{1}H, respectively, and $\langle r_{\mathrm{CN}}^3 \rangle$ is the average cubic length of the C–H chemical bond. Chemical shift anisotropy is approximately $\Delta\sigma = 160 * 10^{-6}$ for N-H bonds in proteins [**?**]. Spectral density $J(\omega)$ is defined as the Fourier transformation of the second order rotational correlation function for N-H bond

$$C(t) = \langle P_2(\theta) \rangle, \tag{4}$$

where $P_2(\theta) = (3 * \cos^2\theta - 1)/2$ is the second order Legendre polynomial. The rotational correlation function is often separated to overall and internal motions of the molecule. Assuming that these are independent one can write

$$C(t) = C_I(t) C_O(t), \tag{5}$$

where $C_i(t)$ and $C_o(t)$ are correlation functions for internal and overall rotiations, respectively. The internal correlation function decays to a plateau, which is used to defined a order parameter respect to molecular axes S^2. The internal correlation function can be then written by using reduced correlation function $C'(t)$

$$C(t) = [C_I'(t)(1-S^2) + S^2] C_O(t). \tag{6}$$

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The effective correlation time describing relaxation of internal processes is then defined as

$$\tau = \int_0^\infty g'(\tau)\mathrm{d}t. \tag{7}$$

Fully anisotropic overall rotation can be described as a sum of five exponentials

$$C_O(t) = \sum_{j=1}^{5} A_j e^{-t/\tau_j}, \tag{8}$$

where $\tau_1 = (4D_{xx} + D_{yy} + D_{zz})^{-1}$, $\tau_2 = (D_{xx} + 4D_{yy} + D_{zz})^{-1}$, $\tau_3 = (D_{xx} + D_{yy} + 4D_{zz})^{-1}$, $\tau_4 = [6(D + (D^2 - L^2)^{-1/2}]^{-1}$, $\tau_5 = [6(D - (D^2 - L^2)^{-1/2}]^{-1}$, $D = \frac{1}{3}(D_{xx} + D_{yy} + D_{zz})$ and $L^2 = \frac{1}{3}(D_{xx}D_{yy} + D_{xx}D_{zz} + D_{yy}D_{zz})$. The diffusion constants around three principal axes of a molecule D_{xx}, D_{yy} and D_{zz} are are defined as

$$\begin{aligned} \langle(\Delta\alpha)^2\rangle &= 2D_{xx}t \\ \langle(\Delta\beta)^2\rangle &= 2D_{yy}t \\ \langle(\Delta\gamma)^2\rangle &= 2D_{zz}t. \end{aligned} \tag{9}$$

Standard analyses of experimental relaxation data usually assume fully or axially isotropic overall rotational motion and single decay constant for interal motion. Then the free parameters (S^2, τ_j, A_j) are fit against spin relaxation data from experiments. This gives most likely very good results for isotropic molecules for which the assumption of single internal motional timescale is reasonable. However, for molecules with significant shape anisotropy or several timescales in internal motions the amoount parameters to be fitted becomes large compared with the typical amount of experimental points.

On the other hand, trajectories of individual atoms can be calculated from classical molecular dynamics simulations. The rotational correlation functions can be calculated from such trajectories and the internal and overall rotational motions can be explicitly separated. If molecular dynamics simulation model reproduces experimental spin relaxation rates, it can be used to interpret the different dynamical processes present in the system.

The following steps are performed in practise.
1) Total rotational correlation functions $C(t)$ for protein N-H bonds are calculated from MD simulation trajectory. The rotational correlation functions for internal dynamics $C_I(t)$ are calculated from a trajectory from where the overall rotation of protein is removed. The rotation is removed by using fit option in gmx trjconv and rotational correlation functions are calculated with gmx rotacf.
2) The overall and internal motions are assumed independent. Overall rotational correlation function can be then calculated from Eq. ref?? $C_O(t) = C(t)/C_I(t)$.
3) The protein axes of intertia and their root mean square deviations as function of time are calculated from MD simulation trajectory.
4) Rotational diffusion constants D_x, D_y and D_z are calculated by fitting a straight line to root mean square angle deviations of intertia axes.
5) The weighting factors A_j are calculated by fitting Eqs. ref?? in rotational correlation functions of overall rotational motion $C_0(t)$.
6) The rotational diffusion constants are divided by the scaling factor and new overall rotational correlation functions are calculated from Eq. ref by using weights from previous step. New total correlation functions are calculated by multiplying $C_I(t)$ from simulations with new overall correlation functions.

B. Molecular dynamics simulations

Simulations were ran using Gromacs 5 [**?**] and Amber ?? force field for proteins [**?**]. The proteins were solvated to tip3p[**?**], tip4p [**?**] or OPC4 [**?**] water models. Temperature was coupled to desired value with ?? and pressure was isotropically set to 1 bar using ??. The simulated systems are listed in Table **??**

NMR structures from [**?**] and [**?**] are used as initial structure for PaTonB and HpTonB-92, respectively.

III. RESULTS AND DISCUSSION

A. Protein overall rotational diffusion constants and relaxation times

Rotational correlation functions calculated from molecular dynamics simulation are shown in Fig. 1.

The intertia tensor angles as a function of time and mean square angular deviations are shown in Fig. 2

B. Overall rotational diffusion in simulations and experiments

Spin relaxation rates calculated from PaTonB simulations with different water models are shown in Fig. 3. T_1 and T_1/T_2 ratio are systemically underestimated in all simulations, which is an indication of overestimated overall rotational diffusion. Overall rotational diffusion can be slowed down in dynamical model from MD simulations by scaling all diffusion constant by a constant factor and generating new correlation functions representing overall rotational diffusion. Spin relaxation times calculated from corrected rotational diffusion model for PaTonB are shown in Fig. 4.

Spin relaxation rates from dynamical model with 1.2 scaling in overall rotational diffusion are is good agreement with experiments. This suggests that the experimental relaxation data for PaTonB construct can be iterpreted with dynamical model having overall rotational diffusion constants shown in Table II.

The analysis method is demonstrated here for HpTonB short construct. The calculated spin lattice relaxation times from simulations with different water models together with

TABLE I. Simulated systems and rotational diffusion coefficients (rad$^2 \cdot 10^7$/s) calculated from simulations.

Protein	Water model	T (K)	$t_{\rm sim}$ (ns)	$t_{\rm anal}$ (ns)	D_{xx}	D_{yy}	D_{zz}	$D_{||}/D_{+}$	D_{av}	files
PaTonB	tip4p	298	400	390	1.81 ± 0.01	2.06 ± 0.03	4.55 ± 0.03	2.35 ± 0.04	2.80 ± 0.02	[**?**]
PaTonB	tip4p	310	400	390	2.60 ± 0.02	2.22 ± 0.05	5.0 ± 0.1	2.07 ± 0.09	3.26 ± 0.07	[**?**]
PaTonB	OPC4	310	1200	1190	2.01 ± 0.01	2.19 ± 0.01	5.01 ± 0.03	2.39 ± 0.02	3.07 ± 0.01	[**?**]
HpTonB-92	tip3p	310	570	370	8.25 ± 0.05	7.67 ± 0.06	15.9 ± 0.3	1.99 ± 0.06	10.6 ± 0.2	[**?**]
HpTonB-92	tip3p	303	400	390	[**?**]					
HpTonB-92	tip4p	310	470	370	3.6 ± 0.1	3.24 ± 0.01	6.3 ± 0.3	1.8 ± 0.1	4.4 ± 0.2	[**?**]
HpTonB-92	tip4p	303			2.7 ± 0.1	2.71 ± 0.02	5.6 ± 0.5	2.1 ± 0.2	3.7 ± 0.2	[**?**]
HpTonB-92	OPC4	310			[**?**]					

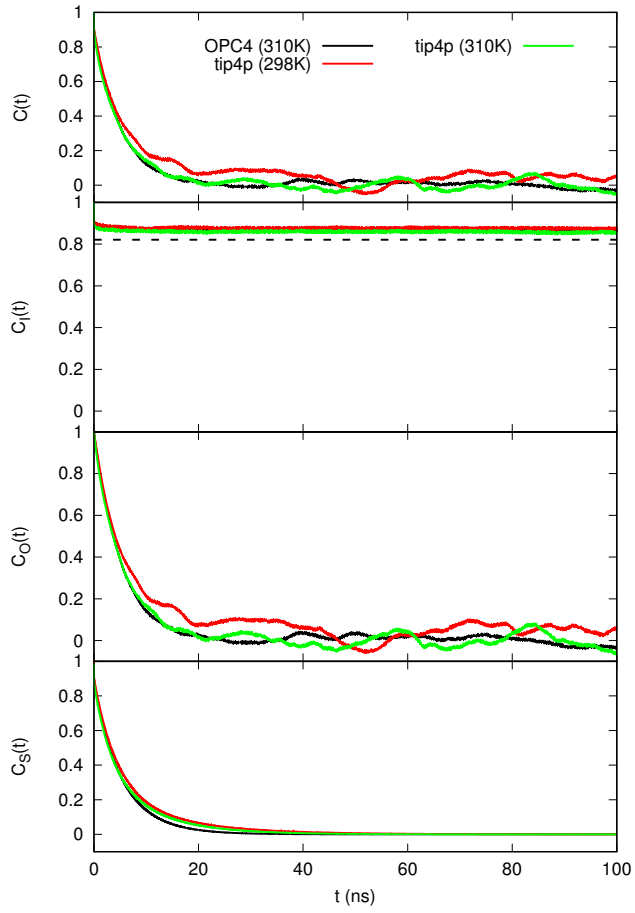

FIG. 1. Example correlation functions from residue 269 of PsTonB simulated with OPC water model. Total correlation function $g(t)$ on (top). Correlation function for internal motions calculated from trajectory from which overall rotation of protein is removed. The plateau of this gives the order paramater square S^2.

experimental data [**?**] are shown in Fig. 5. The rotational diffusion constants for overall

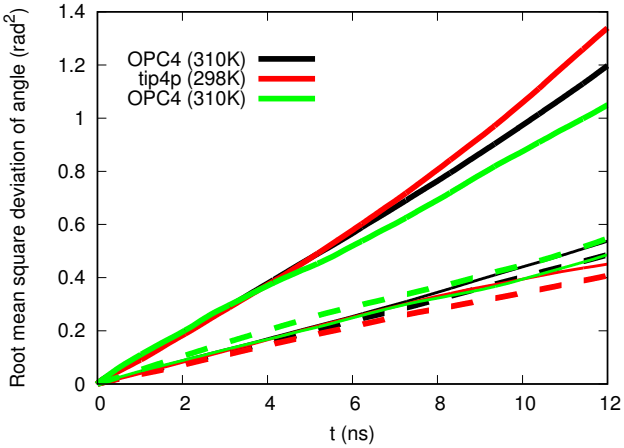

FIG. 2. The intertia tensor angles as a function of time and mean square angular deviations for PsTonB simulation with OPC water model.

C. Protein internal relaxation

Fourier transform of the total correlation function $g(t)$ gives spectral density $J(\omega)$, which can be then embedded in Eqs. ?? to calculate the experimentally measurable spin relaxation rates. If MD results agree with experiments, the simulation can be used intepret the experimental data.

IV. CONCLUSIONS

ACKNOWLEDGMENTS

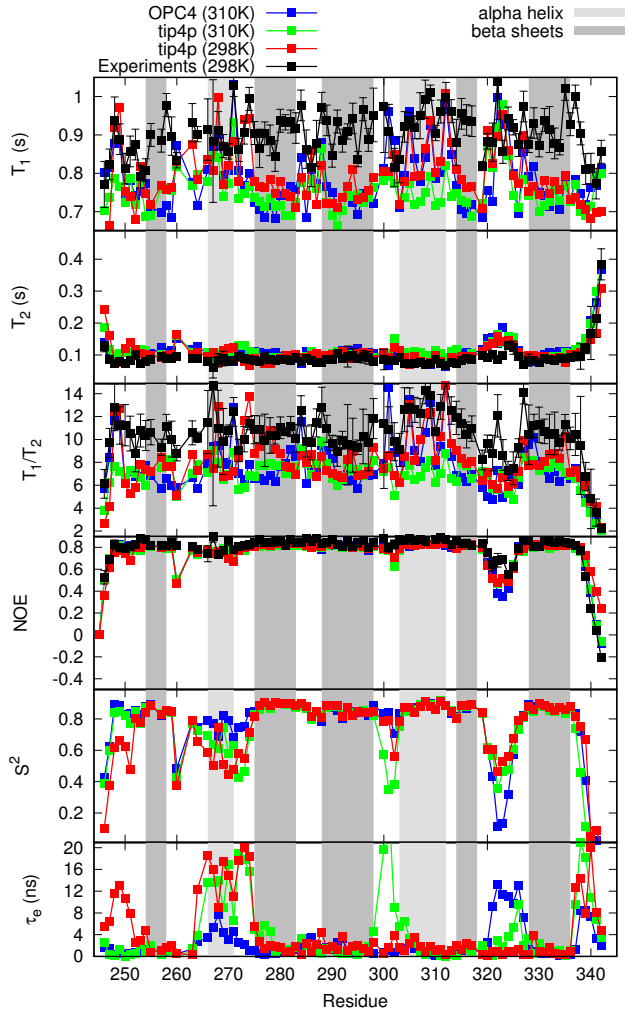

FIG. 3. Relaxation parameters for PsTonB from experiments and simulations with Amber-ildn and different water models

TABLE II. Rotational diffusion coefficients scaled with constant factor which gives a good agreement for spin relaxation data, ?? for tip3p simulation of HpTonB and by 1.2 for tip4p simulation of PsTonB.

	HpTonB-92	PsTonB
D_{xx}	0.027 ± 0.001	1.51 ± 0.01
D_{yy}	0.027 ± 0.001	1.72 ± 0.03
D_{zz}	0.055 ± 0.005	3.79 ± 0.03
D_{av}	0.036 ± 0.003	2.3 ± 0.02
τ_c(ns)	4.6 ± 0.4	7.2 ± 0.1

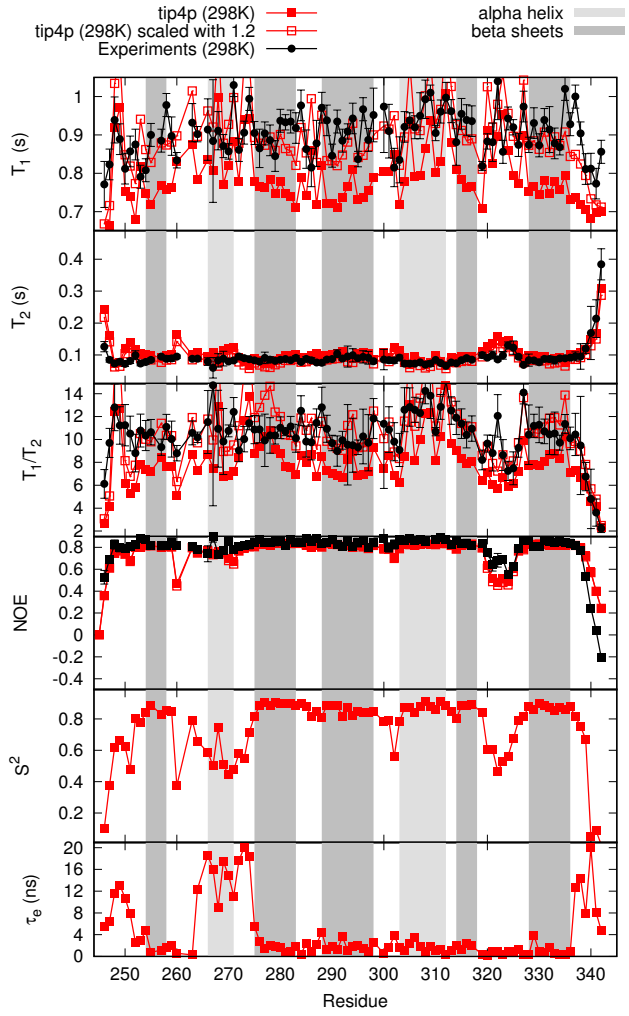

FIG. 4. Relaxation parameters for PsTonB from experiments and simulations with Amber-ildn and different water models. Overall rotational diffusion corrected with factor 1.2.

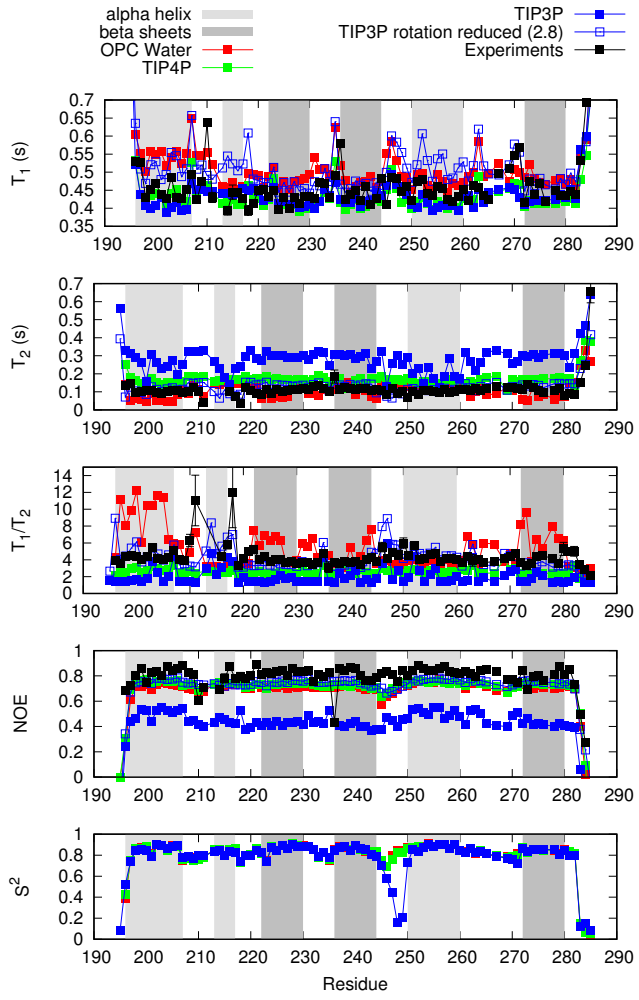

FIG. 5. Relaxation parameters for HpTonB short construct from experiments and simulations with Amber-ildn and different water models

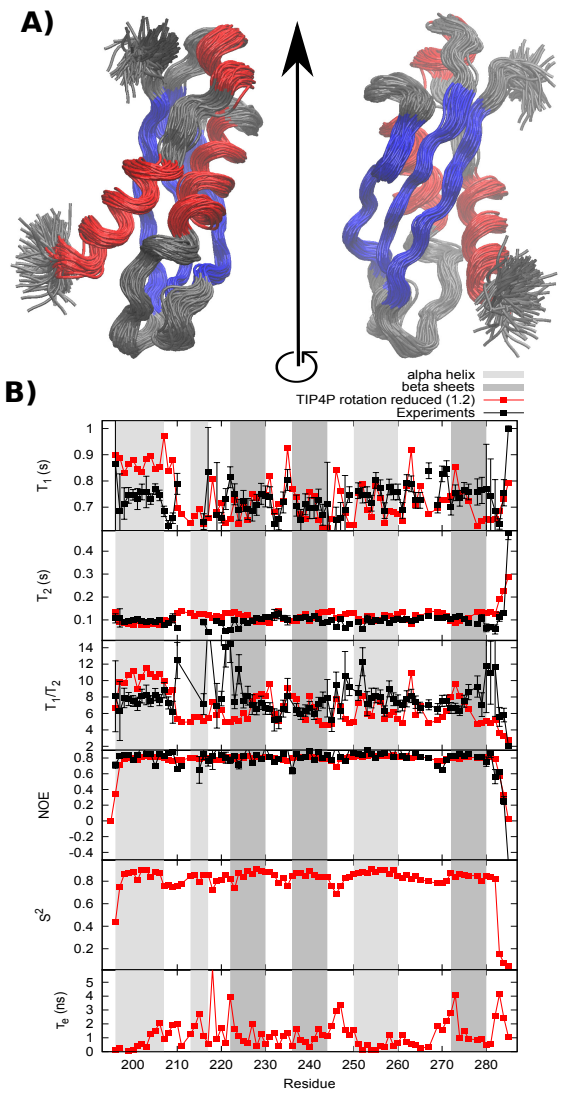

FIG. 6. A) Structures sampled by HpTonB-92 from MD simulations (100 structures from 300ns long trajectory). B) Spin relaxation rates, order parameters and effective correlation times for internal protein dynamics from experiments and simulations.

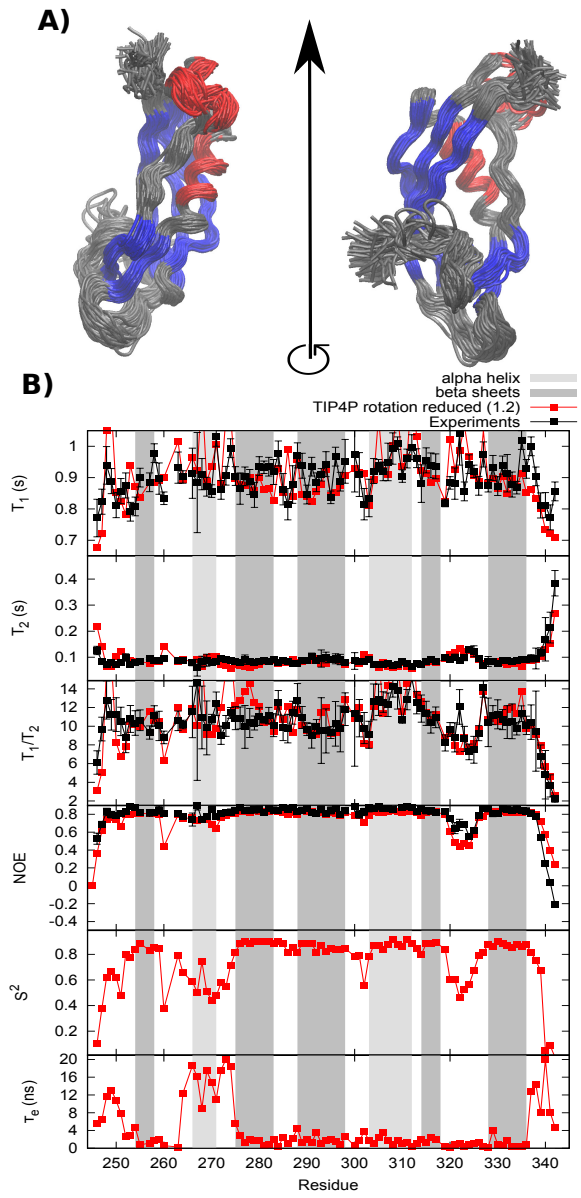

FIG. 7. A) Structures sampled by PsTonB from MD simulations (100 structures from 400ns long trajectory). B) Spin relaxation rates, order parameters and effective internal correlation times from experiments and simulations. Spin relaxation rates indicate increased flexibility only for the last two residues in N terminus. However, MD simulations suggest somewhat smaller order parameters and longer τ_e for residues 246-253. By looking at the sampled structures, it seems that lower order parameters and longer correlation times arise from slower and less extensive conformational sampling than in HpTonB-107. Lower order parameters and larger correlation times are also observed in PsTonB simulations between residues 265-275. This can be explained by the changes in orientation of alpha helix close to this region, as seen in the sampled conformations. Such effect was not seen in the HpTonB simulations, however, NMR data for this region showed some unclarities which may arise from such conformational exchange. Enhanced sampling is also observed between residues 320-326. This was not observed in HpTonB samples, which may be related to the formation of beta sheet between residues 315-318 in PsTonB.