

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 interpreted 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 interpretation of experiments becomes extensively difficult.

Classical molecular dynamics simulations 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 anisotropic protein constructs from engineered from *Helicobacteri pyroli* and *Pseudomonas*.

II. METHODS

A. Spin relaxation and rotational dynamics of molecules

Spin relaxation rates R_1 , R_2 and R_{NOE} measured from NMR experiments for N-H bonds are related to the molecular dynamics through spectral density $J(\omega)$ and equations [?]

[?]

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

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

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

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

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

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 ^{15}N and ^1H , respectively. Average cubic length is $\langle r_{\text{CN}}^3 \rangle \approx$ and the chemical shift anisotropy is $\Delta\sigma \approx 160 * 10^{-6}$ for N-H bonds in proteins [?].

Spectral density $J(\omega)$ is the Fourier transformation of the second order rotational correlation function for N-H bond

$$J(\omega) = 2 \int_0^\infty C(t) \cos(\omega t) dt. \quad (4)$$

The second order rotational correlation is defined as

$$C(t) = \langle (3 \cos^2 \theta_{t'; t'+t} - 1) / 2 \rangle_{t'}, \quad (5)$$

where average is ensemble average and θ is the angle between N-H bonds at times t' and $t' + t$. The rotational correlation function for protein tumbling in solution contains information about overall rotation of the molecule as well as internal relaxation processes. Assuming that these are independent, the rotational correlation function can be written as [?]

$$C(t) = C_I(t) C_O(t), \quad (6)$$

where $C_I(t)$ and $C_O(t)$ are correlation functions for internal and overall rotations, respectively. Correlation function for fully anisotropic overall rotation can be written as a sum of

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five exponentials [?]

$$C_O(t) = \sum_{j=1}^5 A_j e^{-t/\tau_j}, \quad (7)$$

where time constants τ_j are related [?] to the diffusion constants around three principal axes of a molecule D_{xx} , D_{yy} and D_{zz} , defined as

$$\begin{aligned} \langle (\Delta\alpha_{t';t'+t})^2 \rangle_{t'} &= 2D_{xx}t \\ \langle (\Delta\beta_{t';t'+t})^2 \rangle_{t'} &= 2D_{yy}t \\ \langle (\Delta\gamma_{t';t'+t})^2 \rangle_{t'} &= 2D_{zz}t, \end{aligned} \quad (8)$$

where $\langle (\Delta\alpha_{t';t'+t})^2 \rangle_{t'}$, $\langle (\Delta\beta_{t';t'+t})^2 \rangle_{t'}$ and $\langle (\Delta\gamma_{t';t'+t})^2 \rangle_{t'}$ are mean square angle deviations of protein inertia axes. The internal correlation function decays to a plateau, which defines the square of order parameter respect to molecular axes S^2 . The effective internal correlation time can be defined with the help of reduced correlation function $C'_I(t) = (C_I - S^2)/(1 - S^2)$ [?]

$$\tau_{\text{eff}} = \int_0^\infty C'_I(t) dt. \quad (9)$$

Standard analyses of experimental relaxation data usually assume fully or axially isotropic overall rotational motion and single decay constant for internal 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 amount of parameters to be fitted becomes large compared with the typical amount of experimental points.

B. Rotational dynamics from molecular dynamics simulations

Classical molecular dynamics simulations give trajectories of individual atoms as a function of time, which can be used to calculate rotational correlation functions for each bond and explicit separation of internal and overall rotational motions of molecules. The rotational correlation functions can be used to calculate spin relaxation times through Eqs. ??-??, which can be then compared to the experimental data in order to assess simulation model quality [?] and interpret experiments [?]. However, the comparison is often complicated by the short simulation times [?] and incorrect overall rotational diffusion due to water models [?]. These issues have been typically overcome by introducing isotropic rotational diffusion term in the correlation functions [?] or comparing order parameters instead of spin relaxation rates [?]. These approaches are not, however, useful for anisotropic proteins and order parameter comparison is not direct comparison between simulations and experiments in the case of freely rotating molecules.

Method presented here can be applied also to anisotropic

proteins simulated with timescales routinely accessible with state of the art computational infrastructure. The key idea of the method is to calculate the rotational diffusion constants and then use Eq. 7 to determine the correlation function related to the overall rotational diffusion.

Here we present method to analyse rotational dynamics. The analysis of rotational dynamics performed in this work can be divided in essentially six steps listed here.

- 1) Total rotational correlation functions $C(t)$ for protein N-H bonds are calculated from MD simulation trajectory by applying Eq. 5.
- 2) Rotational correlation functions for internal dynamics $C_I(t)$ are calculated from a trajectory from where the overall rotation of protein is removed.
- 3) The overall and internal motions are assumed to be independent and overall rotational correlation function is calculated as $C_O(t) = C(t)/C_I(t)$ according to Eq. 6.
- 4) The protein axes of inertia and their mean square deviations as function of time are calculated from MD simulation trajectory.
- 5) Rotational diffusion constants D_x , D_y and D_z are calculated by fitting a straight line to mean square angle deviations of inertia axes according to Eq. 8.
- 6) Timescales in Eq. 7 are calculated from diffusion constants and weighting factors A_j are determined by fitting the equation to rotational correlation functions of overall rotational motion $C_O(t)$ determined in step 3).
- 7) New total rotational correlation functions $C_N(t)$ are determined by multiplying internal correlation function $C_I(t)$ from step 2) by Eq. ?? with parameters from step 6). Rotational diffusion constants (and τ_i values in Eq. 7) can be also divided by a scaling factor at this point to tune overall rotational diffusion in rotational dynamics model close to experimental value.

C. Simulation and analysis details

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.

The rotation is removed by using fit option in gmxf trjconv and rotational correlation functions are calculated with gmxf rotacf. Inertia axes of protein are calculated as ??.

III. RESULTS AND DISCUSSION

A. Protein overall rotational diffusion constants and relaxation times

The presented rotational dynamics analysis from MD simulations is exemplified in this section for residue ?? located in

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

Protein	Water model	T (K)	t_{sim} (ns)	t_{anal} (ns)	D_{xx}	D_{yy}	D_{zz}	$D_{ }/D_{\perp}$	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	800	790	6.24 ± 0.02	7.04 ± 0.03	11.9 ± 0.2	1.80 ± 0.03	8.40 ± 0.07	[?]
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	800	790	2.85 ± 0.01	2.70 ± 0.01	5.56 ± 0.01	2.00 ± 0.01	3.70 ± 0.01	[?]

alpha helix of PaTonB protein. The analysis of all other bonds are essentially similar.

The total correlation functions calculated from original MD trajectory are shown in Fig. 1 A). As expected, they decay to fluctuate approximately around zero after $\sim 20\text{ns}$. Fluctuations from ideal relaxation behaviour are seen already with shorter times, which is also expected because good statistic for single molecule correlation functions is expected only for timescales less than hundredth of total simulation time [?], i.e. approximately 4-12ns for the studied systems. Internal correlation functions calculated from trajectory with overall protein rotation removed are shown in Fig. 1 B). As expected, they decay very fast to a plateau value, which is used to determine S^2 . Overall rotational correlation functions calculated as $C_O(t) = C(t)/C_I(t)$ are shown in Fig. 1 C). MAYBE WE SHOULD SHOW THE FITS HERE?

Overall rotational correlation functions are used to determine prefactors A_j in Eq. 7 by fitting the equation to the calculated correlation functions. For this purpose timescales τ_j in Eq. 7 are determined by calculating the rotational diffusion coefficients of protein inertia axes. Mean square angle deviations of protein inertia axes from different simulations are shown in Fig. 2. By taking in to account only hundredth of the total simulation length, mean square angle deviations can be considered linear and diffusion constants can be calculated from linear fit. The results for diffusion constants are shown in Table II.

After determining the prefactors by fitting Eq. 7, new correlation functions were calculated as $C_N(t) = C_I(t) \sum_{j=1}^5 A_j e^{-t/\tau_j}$. These functions for the example case are shown in Fig. 1 D).

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

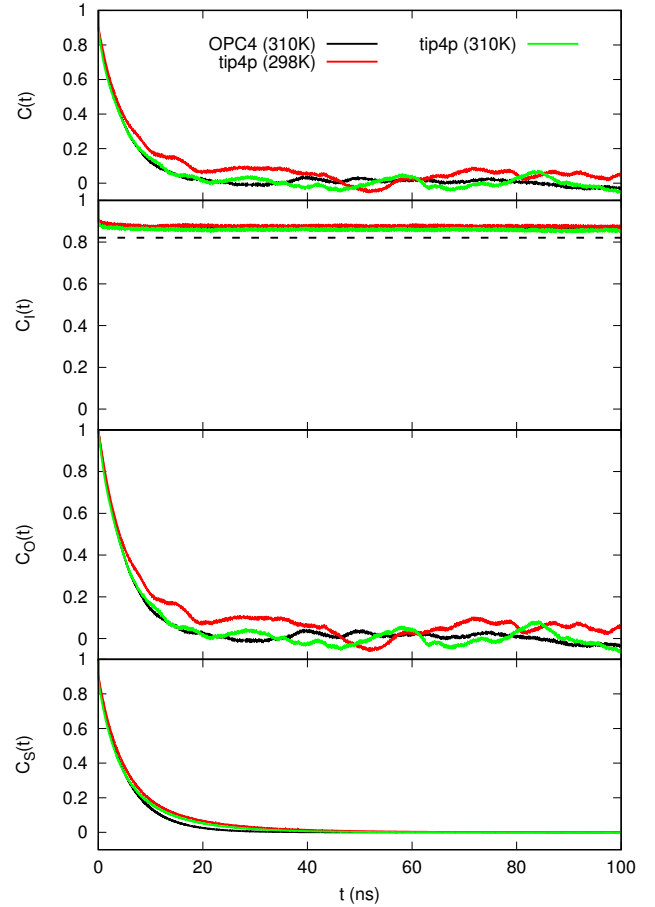


FIG. 1. Example correlation functions for residue ?? of PsTonB calculated from MD simulations with different water models. A) total correlation function $C(t)$, B) correlation function for internal motions and S^2 values determined from the plateau, C) correlation function for overall motions determined from Eq. 6 ($C_O(t) = C(t)/C_I(t)$) and D) new correlation functions determined from Eqs. 6 and 7 by using rotational diffusion constants and fitted prefactors (see section II B)

PaTonB are shown in Fig. 4.

Spin relaxation rates from dynamical model with 1.2 scaling in overall rotational diffusion are in good agreement with experiments. This suggests that the experimental relaxation

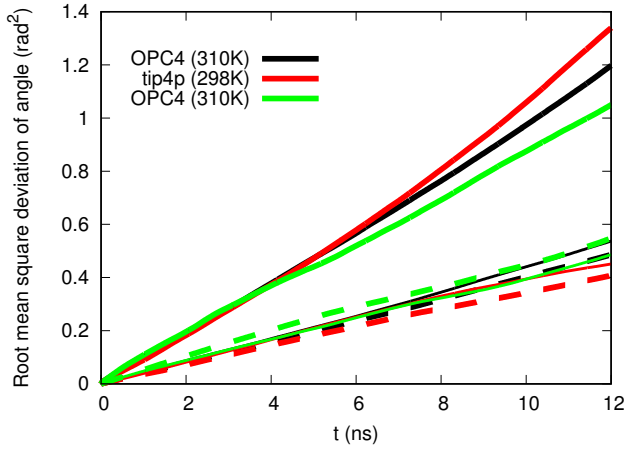


FIG. 2. The inertia tensor angles as a function of time and mean square angular deviations for PsTonB simulation with OPC water model.

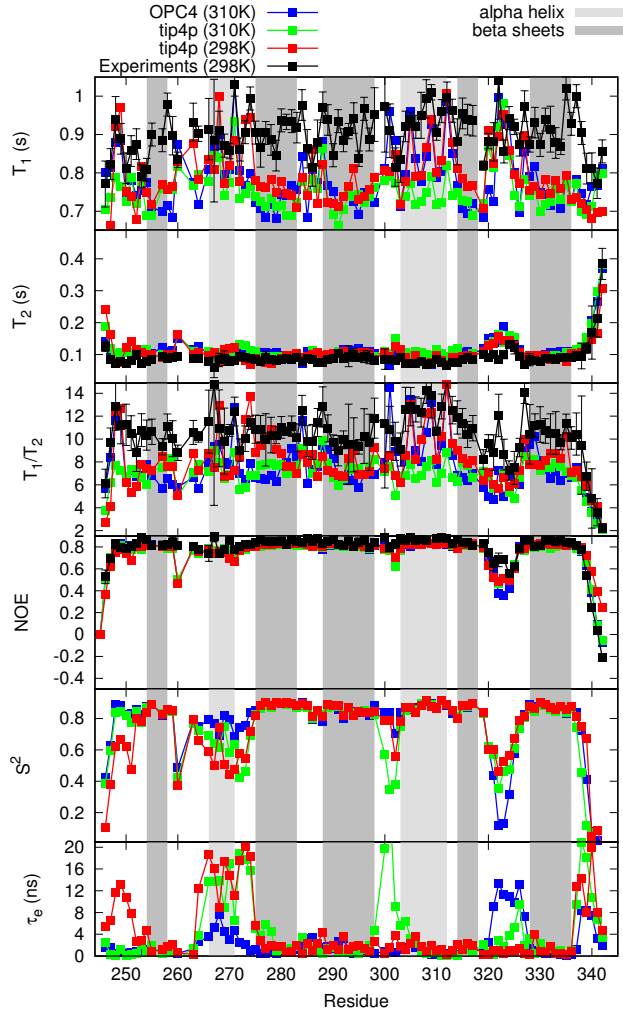


FIG. 3. Relaxation parameters for PsTonB from experiments and simulations with Amber-ildn and different water models

data for PaTonB construct can be iterpreted with dynamical

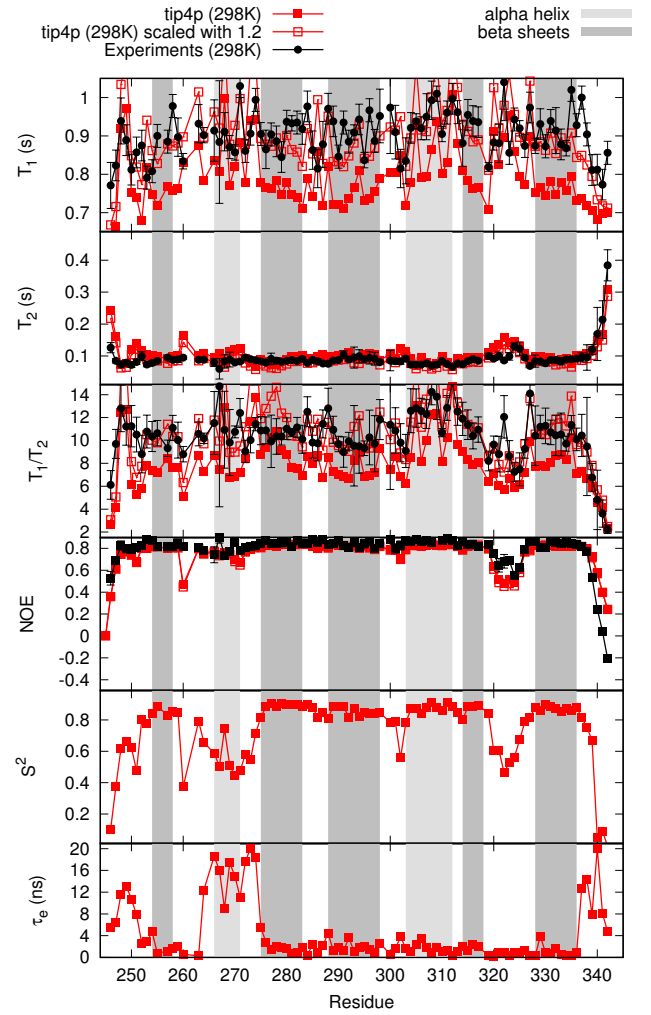


FIG. 4. Relaxation parameters for PsTonB from experiments and simulations with Amber-ildn and different water models. Overall rotational diffusion corrected with factor 1.2.

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

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 interpret the experimental data.

IV. CONCLUSIONS

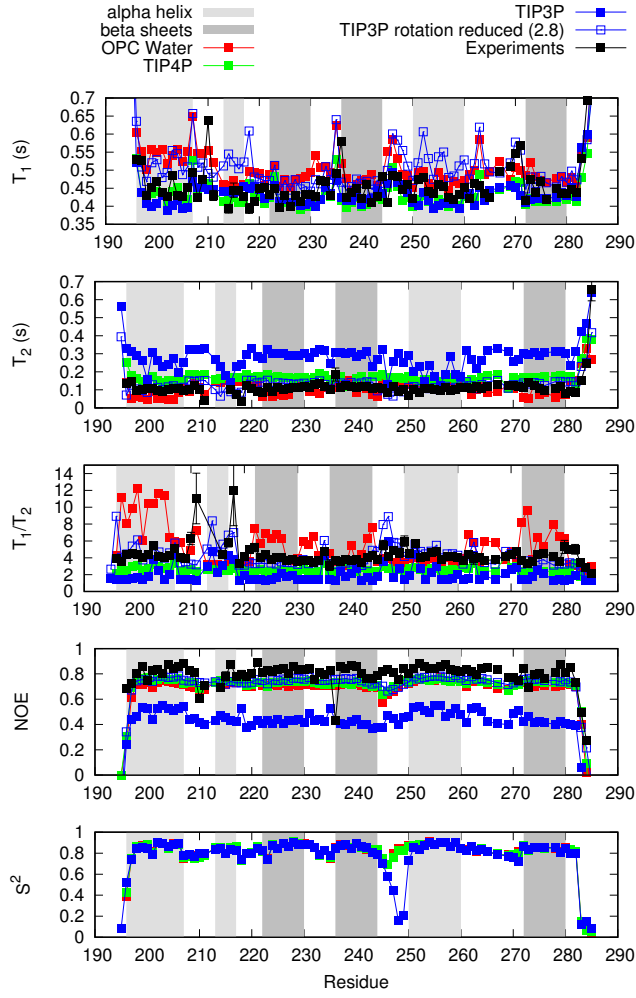


FIG. 5. Relaxation parameters for HpTonB short construct from experiments and simulations with Amber-ildn and different water models

ACKNOWLEDGMENTS

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
$\tau_c(\text{ns})$	4.6 ± 0.4	7.2 ± 0.1

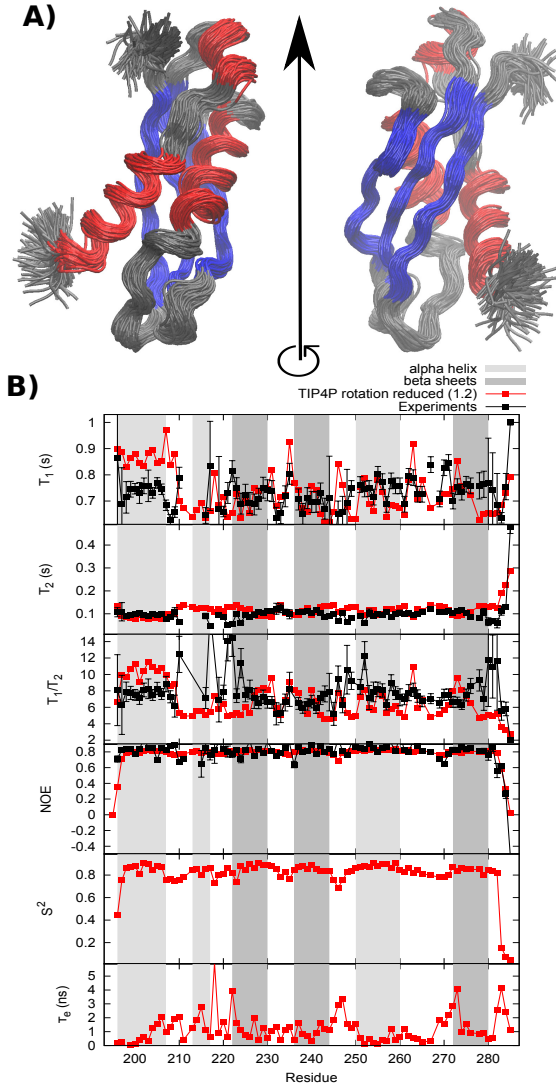


FIG. 6. A) Structures sampled by HpTonB-92 from MD simulations (100 structures from 300ns long trajectory). B) Spin relaxation rates, order parameters and effective correlation times for internal protein dynamics from experiments and simulations.

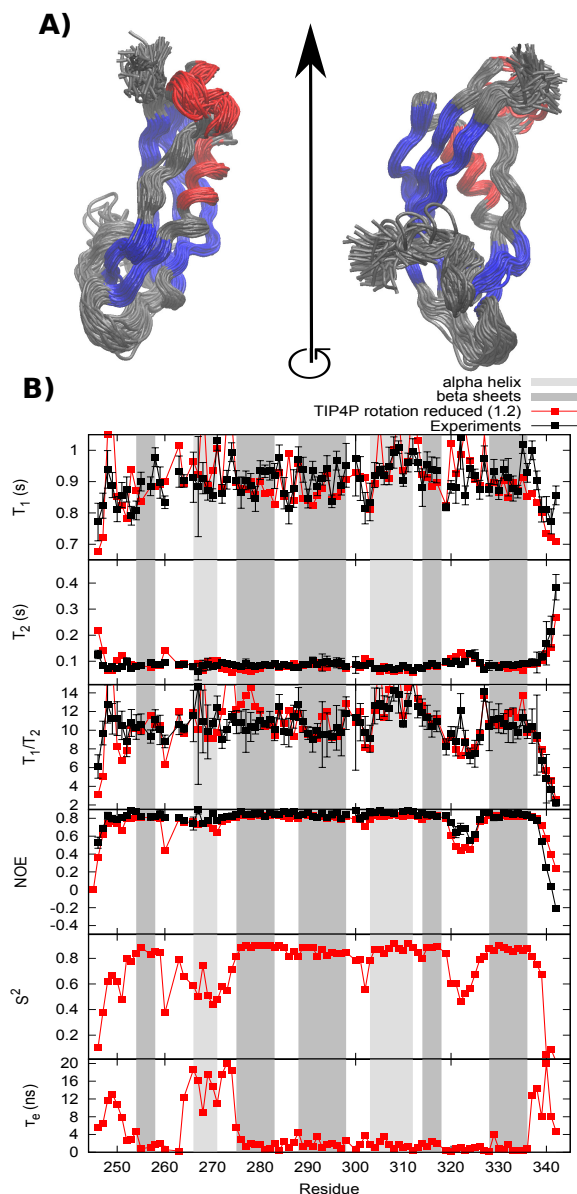


FIG. 7. A) Structures sampled by PsTonB from MD simulations (100 structures from 400ns long trajectory). B) Spin relaxation rates, order parameters and effective internal correlation times from experiments and simulations. Spin relaxation rates indicate increased flexibility only for the last two residues 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.