

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

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## I. SPIN RELAXATION ANALYSIS FROM MD SIMULATIONS

Measurable spin relaxation rates are related to the molecular dynamics through equations

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where  $J(\omega)$  is the spectral density. The spectral density is the fourier transformation of the second order rotational correlation function for the bond under interest

$$C(t) = \langle P_2(\theta) \rangle, \quad (1)$$

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

$$C(t) = C_I(t)C_O(t), \quad (2)$$

where  $C_i(t)$  and  $C_o(t)$  are correlation functions for internal and overall rotations, 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  $C'(t)$

$$C(t) = [C'_I(t)(1 - S^2) + S^2]C_O(t). \quad (3)$$

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

$$\tau = \int_0^\infty g'(\tau) d\tau. \quad (4)$$

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

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

where  $\tau_1 = (4D_{xx} + D_{yy} + D_{zz})^{-1}$ ,  $\tau_2 = (D_{xx} + 4D_{yy} + D_{zz})^{-1}$ ,  $\tau_3 = (D_{xx} + D_{yy} + 4D_{zz})^{-1}$ ,  $\tau_4 = [6(D + (D^2 - L^2)^{-1/2})^{-1}]^{-1}$ ,  $\tau_5 = [6(D - (D^2 - L^2)^{-1/2})^{-1}]^{-1}$ ,  $D = \frac{1}{3}(D_{xx} + D_{yy} + D_{zz})$  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 defined as

$$\begin{aligned} \langle (\Delta\alpha)^2 \rangle &= 2D_{xx}t \\ \langle (\Delta\beta)^2 \rangle &= 2D_{yy}t \\ \langle (\Delta\gamma)^2 \rangle &= 2D_{zz}t. \end{aligned} \quad (6)$$

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 ( $S^2$ ,  $\tau_j$ ,  $A_j$ ) 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 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.

An example of calculated rotational correlation function from molecular dynamics simulation is shown in Fig. ?? . 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 to interpret the experimental data.

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

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

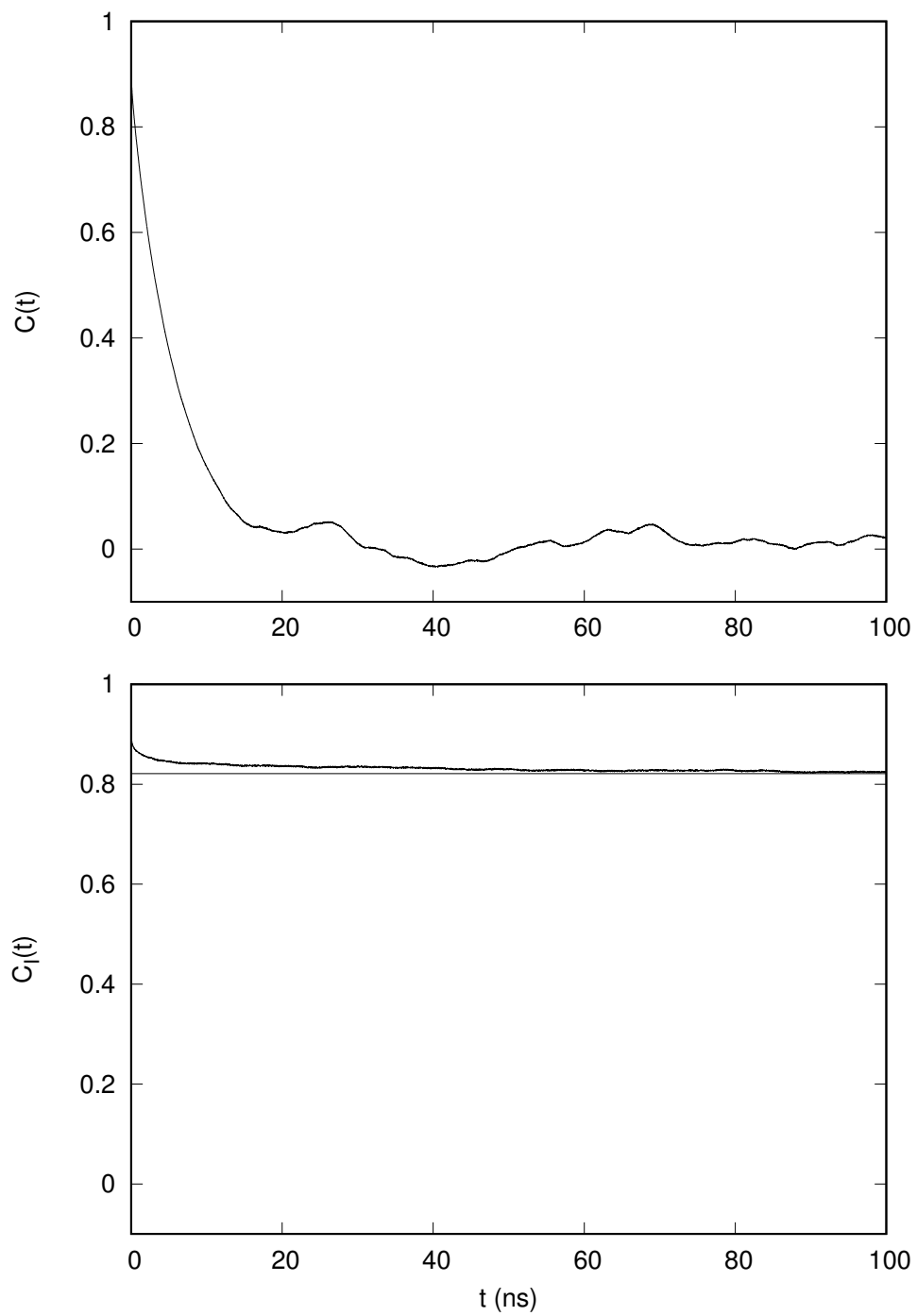


FIG. 1. Example correlation functions from residue 269 of PsTonB simulated with OPC water model. Total correlation function  $g(t)$  on (top). Correlation function for internal motions calculated from trajectory from which overall rotation of protein is removed. The plateau of this gives the order parameter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

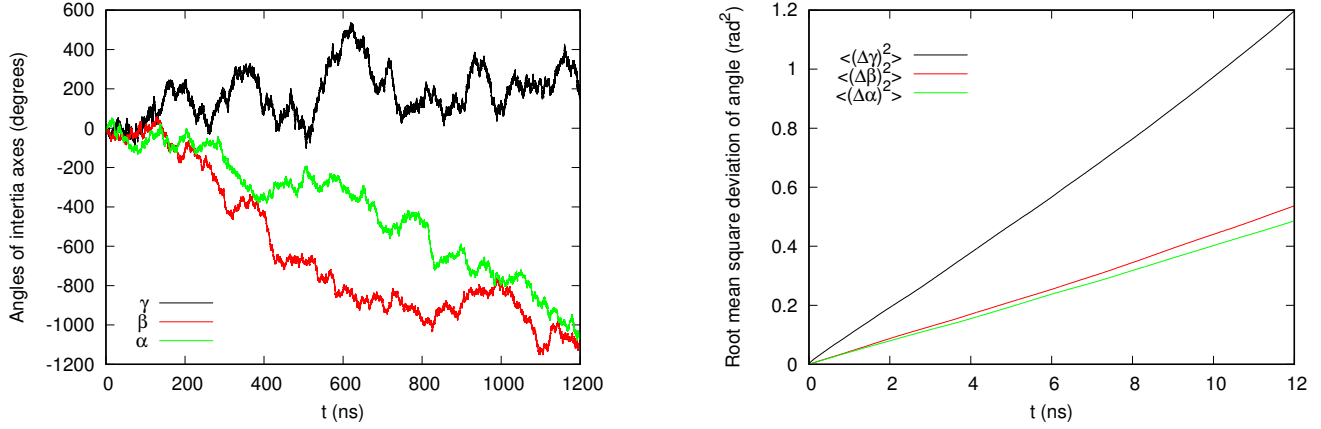


FIG. 2. The inertia tensor angles as a function of time and mean square angular deviations for PsTonB simulation with OPC water model.

Eq. ref??  $C_O(t) = C(t)/C_I(t)$ .

- 3) The protein axes of inertia 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 inertia 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.

## II. RESULTS AND DISCUSSION

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. 3. The rotational diffusion constants for overall

TABLE I. Rotational diffusion coefficients calculated directly from simulations in 303K. OPC RESULTS TO BE CHECKED.

rad <sup>2</sup> /ns	TIP3P	TIP4P	OPC
$D_{xx}$	0.083	0.038	0.030
$D_{yy}$	0.077	0.033	0.027
$D_{zz}$	0.16	0.059	0.058
$2D_{zz}/(D_{xx}+D_{yy})$	1.99	1.7	2.03
$D_{av}$	0.11	0.043	0.038
tau1	1.76	4.13	4.87
tau2	1.82	4.40	5.14
tau3	1.26	3.25	3.47
tau4	1.05	2.75	2.94
tau5	3.05	6.48	8.43

Results with rotational diffusion coefficient corrected with constant factor are shown in Fig. 4.

TABLE II. Rotational diffusion coefficients scaled with constant factor which gives a good agreement for spin relaxation data, 3.0 for tip3p simulation and by 1.3 for tip4p simulation. OPC RESULTS TO BE CHECKED.

rad <sup>2</sup> /ns	TIP3P	TIP4P
$D_{xx}$	0.028	0.029
$D_{yy}$	0.026	0.025
$D_{zz}$	0.053	0.045
$2D_{zz}/(D_{xx}+D_{yy})$	1.99	1.7
$D_{av}$	0.034	0.033

TABLE III. Rotational diffusion coefficients for different proteins based on MD analysis of NMR relaxation data

rad <sup>2</sup> /ns	HpTonB (short construct)	HpTonB (long construct)
$D_{xx}$	$0.028 \pm 0.001$	0.016
$D_{yy}$	$0.026 \pm 0.001$	0.022
$D_{zz}$	$0.05 \pm 0.01$	0.045
$2D_{zz}/(D_{xx}+D_{yy})$	$1.9 \pm 0.2$	2.3
$D_{av}$	$0.034 \pm 0.01$	0.028
$\tau_c(\text{ns})$	$4.9 \pm 0.2$	5.99

### III. RESULTS FOR PSTONB

TABLE IV.

rad <sup>2</sup> /ns	TIP4P	OPC
$D_{xx}$	0.026	0.020
$D_{yy}$	0.022	0.022
$D_{zz}$	0.049	0.048
$D_{  }/D_{+}$	2.04	2.31
$D_{av}$	0.03	0.030
tau1	5.67	6.70
tau2	6.05	6.47
tau3	4.06	4.29
tau4	3.45	3.57
tau5	9.83	12.87

### IV. RESULTS FOR CALMODULIN

#### ACKNOWLEDGMENTS

TABLE V. Overall rotational diffusion coefficients of Calmodulin from simulations by Pauline and hydrodynamical models with experimental data used by Barbato et al. [? ].

rad <sup>2</sup> /ns	AMBER	CHARMM	Hydrodynamics
$D_{xx}$	0.038	0.033	$0.015 \pm 0.5$
$D_{yy}$	0.033	0.034	$0.015 \pm 0.5$
$D_{zz}$	0.086	0.065	$0.04 \pm 1$
$D_{  }/D_{+}$	2.396	1.96	$2.5 \pm 0.3$
$D_{av}$	0.053	0.044	
tau1	3.66	4.36	
tau2	3.88	4.30	
tau3	2.40	3.06	
tau4	2.05	2.54	
tau5	6.91	7.61	

### SUPPLEMENTARY INFORMATION

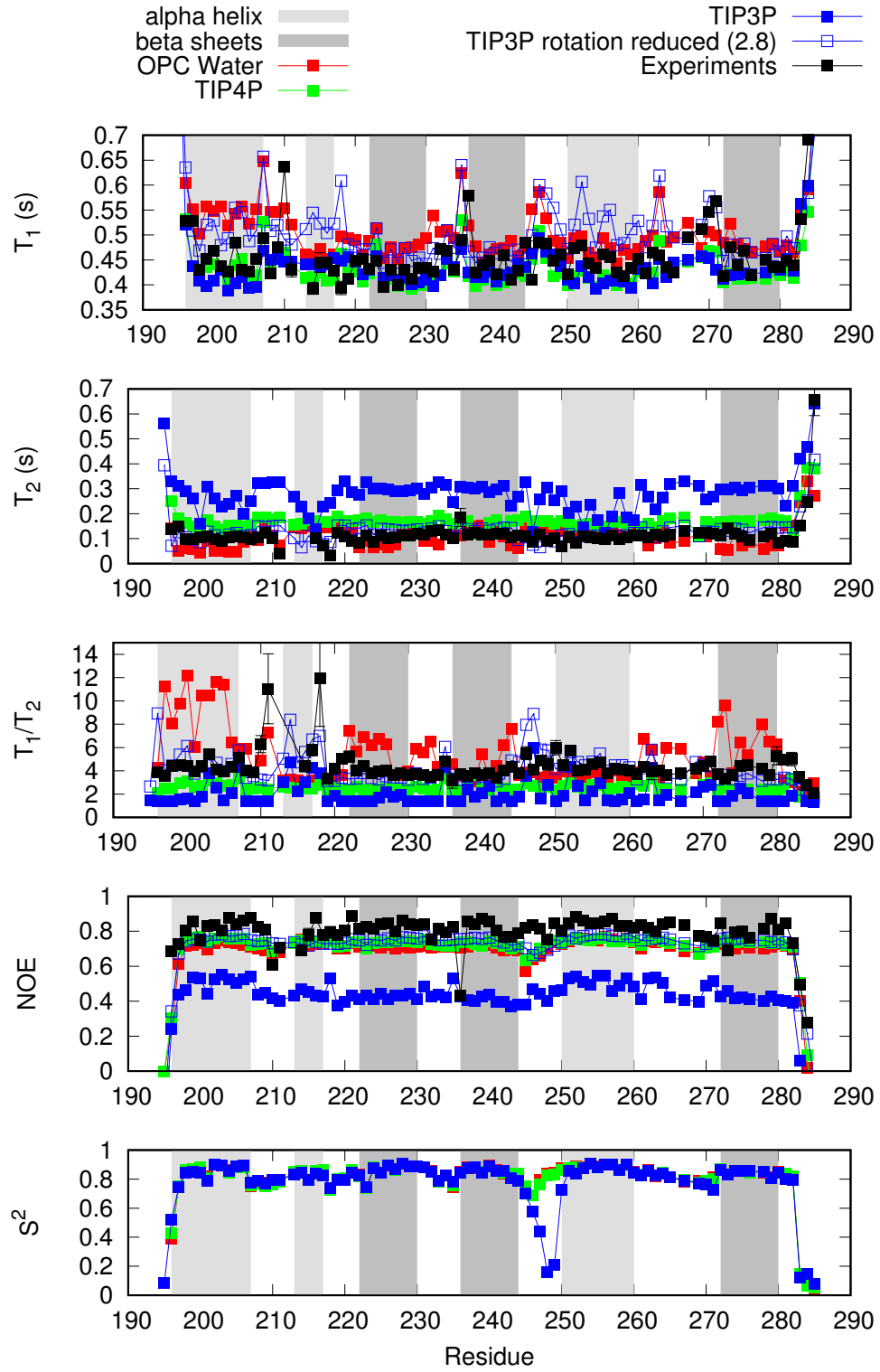


FIG. 3. Relaxation parameters for HpTonB short construct from experiments and simulations with Amber-ildn and different water models

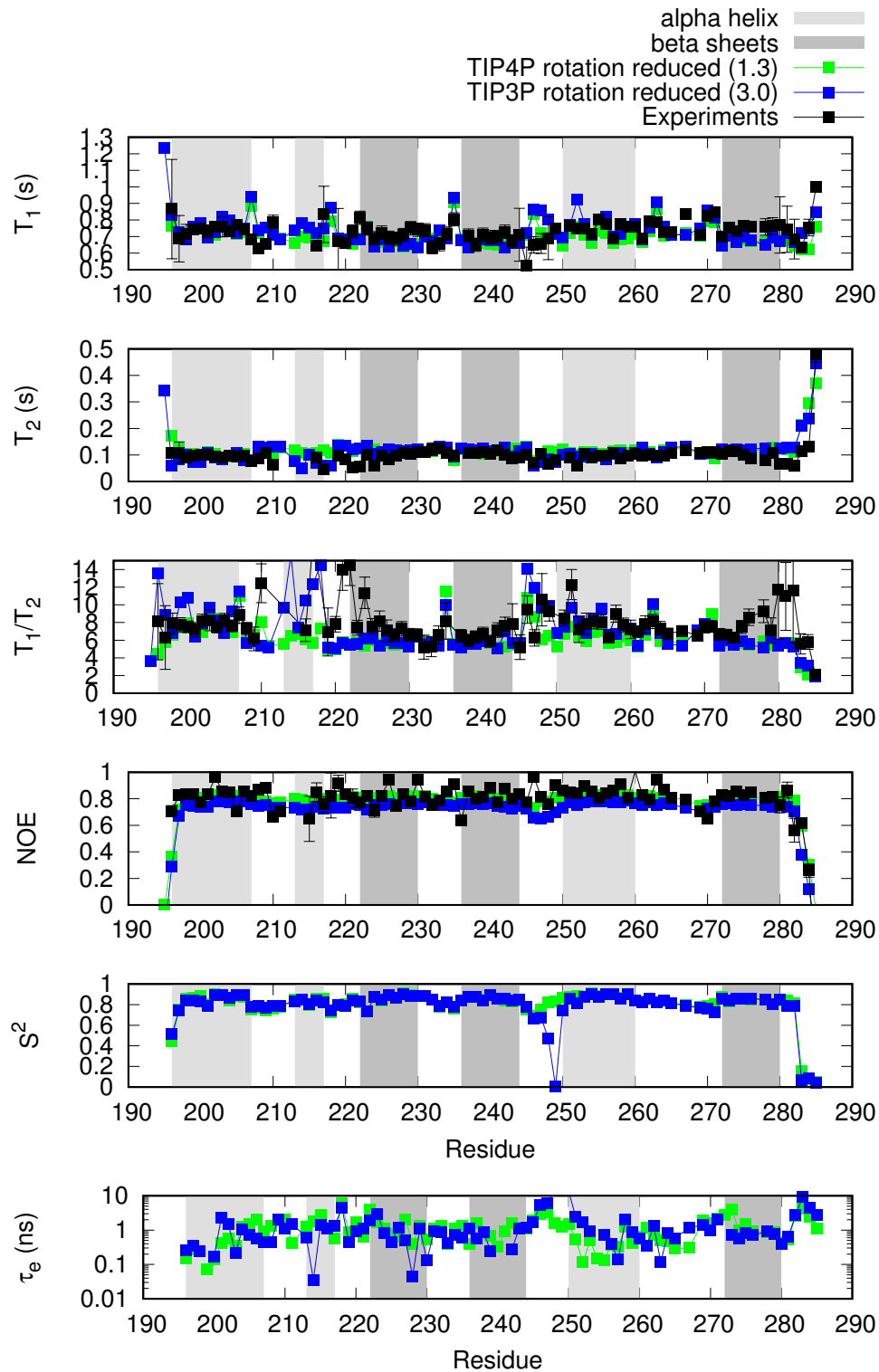


FIG. 4. Relaxation parameters for HpTonB short construct from experiments and simulations with Amber-ildn and different water models. The rotational diffusion coefficients are divided by 3.0 for tip3p simulation and by 1.3 for tip4p simulation. Experiments are done in 303K and simulations in 310K, simulations in 303K are running.



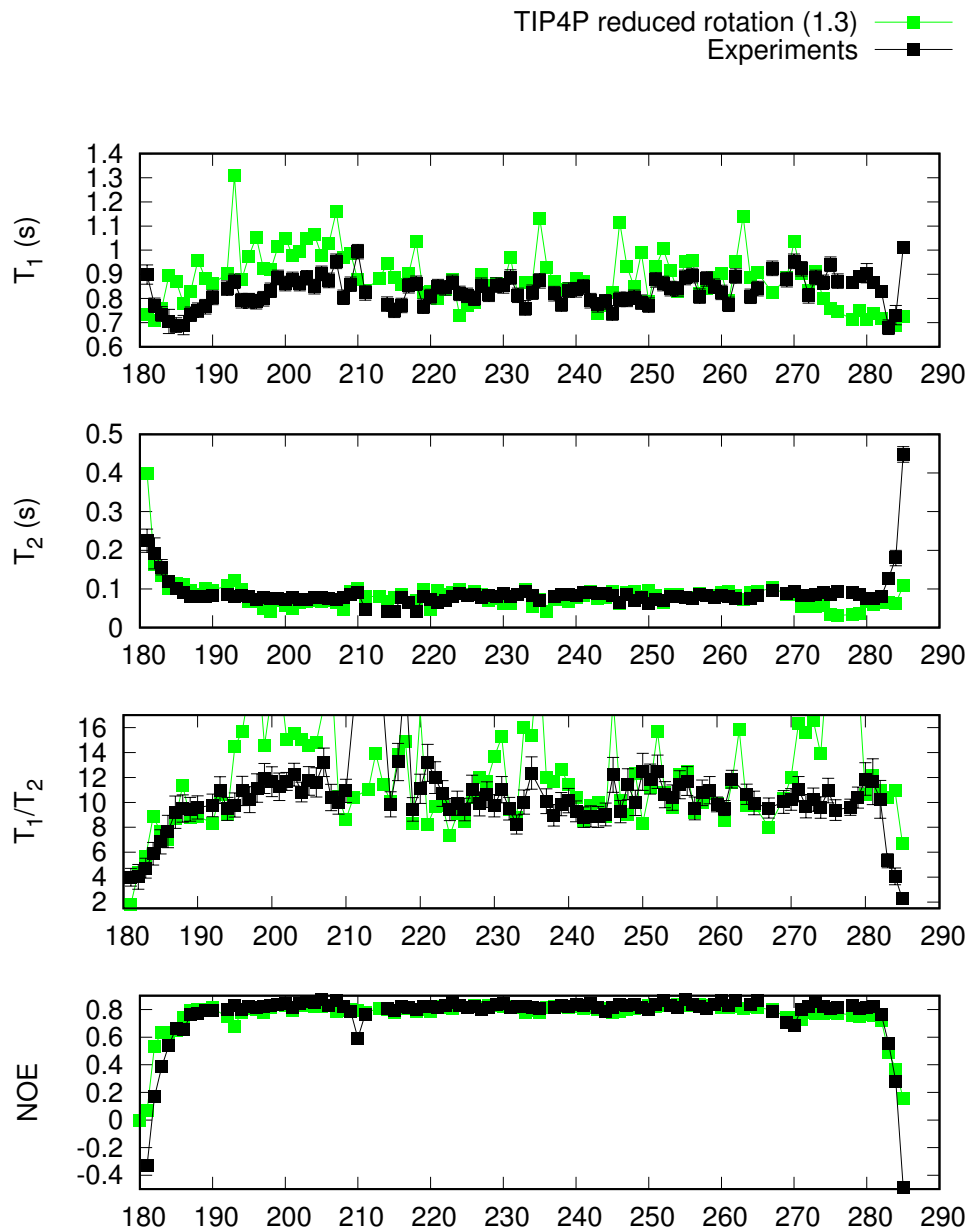


FIG. 5. PRELIMINARY RESULTS for relaxation parameters for HpTonB longer construct (107) from experiments and simulations with Amber-ildn and tip4p water models. The rotational diffusion coefficients are divided by 1.3. Experiments and simulations are done in 303K.

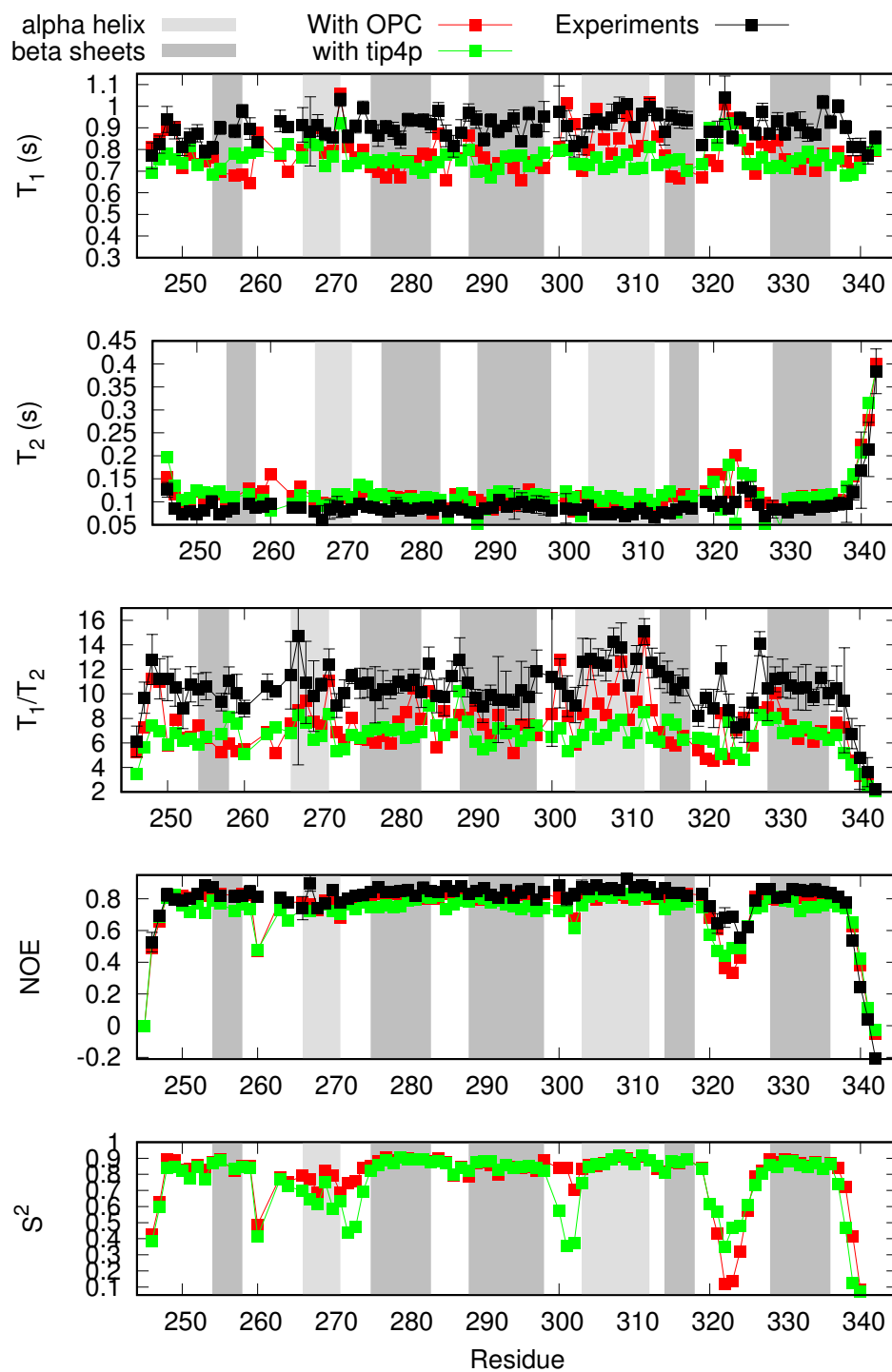
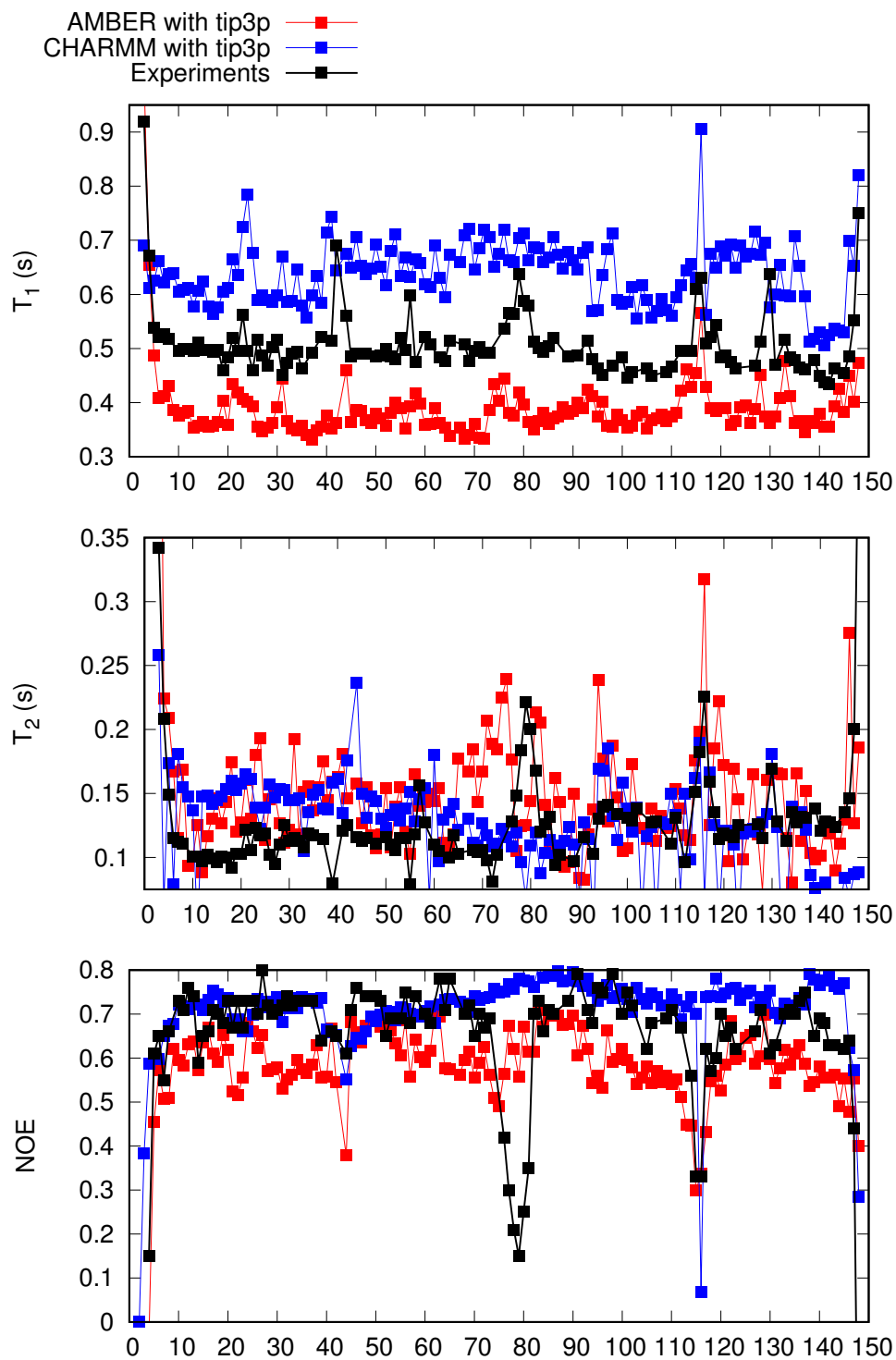


FIG. 6. Relaxation parameters for PsTonB short construct from experiments and simulations with Amber-ildn and different water models



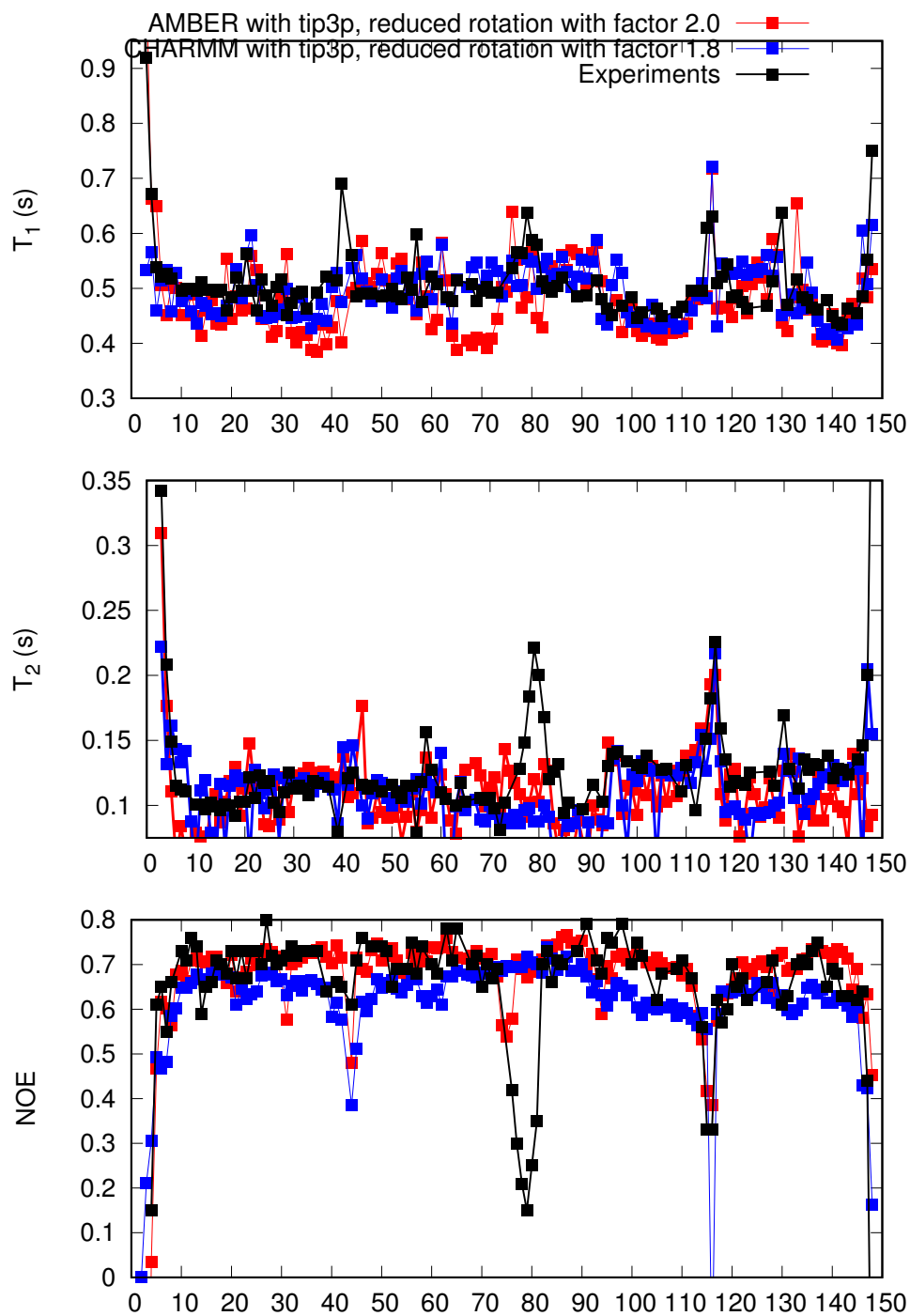


FIG. 8. Relaxation parameters for Calmodulin from experiments and simulations with Amber and CHARMM in tip3p. The overall rotation diffusion coefficients are reduced to get better agreement with experimental spin relaxation times.

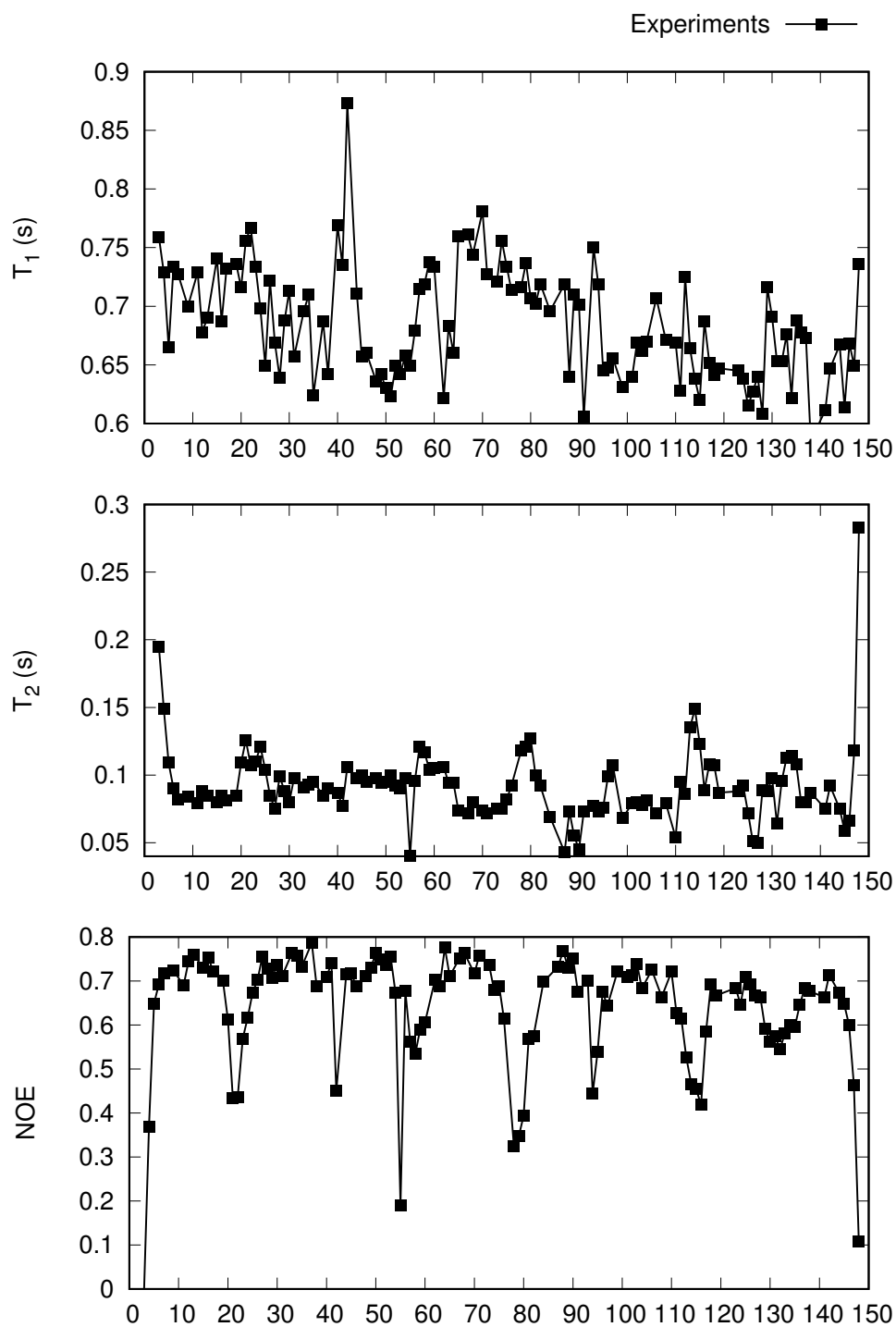


FIG. 9. Relaxation parameters for  $\text{Ca}^{2+}$  free Calmodulin from experiments [? ]