



**HAL**  
open science

# Accurate Prediction of Protein NMR Spin Relaxation by Means of Polarizable Force Fields. Application to Strongly Anisotropic Rotational Diffusion

Moreno Marcellini, Minh-Ha Nguyen, Marie Martin, Maggy Hologne, Olivier Walker

► **To cite this version:**

Moreno Marcellini, Minh-Ha Nguyen, Marie Martin, Maggy Hologne, Olivier Walker. Accurate Prediction of Protein NMR Spin Relaxation by Means of Polarizable Force Fields. Application to Strongly Anisotropic Rotational Diffusion. *Journal of Physical Chemistry B*, 2020, 124 (25), pp.5103-5112. 10.1021/acs.jpcc.0c01922 . hal-03968000

**HAL Id: hal-03968000**

**<https://hal.science/hal-03968000v1>**

Submitted on 17 Apr 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accurate Prediction of Protein NMR Spin Relaxation by Means of Polarizable Force Fields. Application to Strongly Anisotropic Rotational Diffusion

Moreno Marcellini, Minh-Ha Nguyen, Marie Martin, Maggy Hologne, and Olivier Walker

*J. Phys. Chem. B*, **Just Accepted Manuscript** • DOI: 10.1021/acs.jpcc.0c01922 • Publication Date (Web): 05 Jun 2020

Downloaded from [pubs.acs.org](https://pubs.acs.org) on June 6, 2020

### Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

# Accurate Prediction of Protein NMR Spin Relaxation by Means of Polarizable Force Fields. Application to Strongly Anisotropic Rotational Diffusion

*Moreno Marcellini, Minh-Ha Nguyen, Marie Martin, Maggy Hologne and Olivier Walker\**

Institut des Sciences Analytiques (ISA), Univ Lyon, CNRS, UMR5280, Université

Claude Bernard Lyon1, Lyon France.

## ABSTRACT

Among the various biophysical methods available to investigate protein dynamics, NMR present the ability to scrutinize protein motions on a broad range of time scales.  $^1\text{H}$ - $^{15}\text{N}$  NMR spin relaxation experiments can reveal the extent of protein motions across the ps-ns dynamics probed by the fundamental parameters  $^{15}\text{N}$ - $R_1$ ,  $^{15}\text{N}$ - $R_2$  and  $^1\text{H}$ - $^{15}\text{N}$  NOE that

1  
2  
3  
4 can be well sampled by molecular dynamics simulations (MD). An accurate prediction of  
5  
6  
7 these parameters is subjected to a proper description of the rotational diffusion and  
8  
9  
10 anisotropy. Indeed, a strong rotational anisotropy has a profound effect on the various  
11  
12  
13 relaxation parameters and could be mistaken for conformational exchange. Although the  
14  
15  
16 principle of NMR spin relaxation predictions from MD is now well established, numerous  
17  
18  
19 NMR/MD comparisons have hitherto focused on proteins that show low to moderate  
20  
21  
22 anisotropy and make use of a scaling factor to remove artifacts arising from water model-  
23  
24  
25 dependence of the rotational diffusion. In the present work, we have used NMR to  
26  
27  
28 characterize the rotational diffusion of the  $\alpha$ -helical STAM2-UIM domain by measuring  
29  
30  
31 the  $^{15}\text{N-R}_1$ ,  $^{15}\text{N-R}_2$  and  $^1\text{H-}^{15}\text{N}$  NOE relaxation parameters. We therefore highlight the use  
32  
33  
34 of the polarizable AMOEBA FF and show that it improves the prediction of the rotational  
35  
36  
37 diffusion in the particular case of strong rotational anisotropy, which in turn enhances the  
38  
39  
40 prediction of the  $^{15}\text{N-R}_1$ ,  $^{15}\text{N-R}_2$  and  $^1\text{H-}^{15}\text{N}$  NOE relaxation parameters without  
41  
42  
43 requirement to a scaling factor. Our findings suggest that the use of polarizable FFs could  
44  
45  
46 potentially enrich our understanding of protein dynamics in situations where charges  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 distribution or protein shape is remodeled over time like in the case of multidomain  
4  
5  
6  
7 proteins or intrinsically disordered proteins.  
8  
9  
10  
11  
12  
13  
14  
15  
16

## 17 INTRODUCTION

18  
19  
20 Biological processes and more particularly cell communication are achieved through  
21  
22  
23 interaction networks where protein dynamics is essential. Among the various biophysical  
24  
25  
26 techniques that allow to investigate protein dynamics, NMR is particularly attractive due  
27  
28  
29 to its capability to accurately explore structure and dynamics at atomic level. From this  
30  
31  
32 perspective, NMR may probe protein dynamics across ps-ms time scale. Faster ps-ns  
33  
34  
35 dynamics are obtained by measuring the most popular  $^{15}\text{N}$  spin relaxation parameters  
36  
37  
38 such as longitudinal relaxation  $R_1$ , transverse relaxation  $R_2$  and  $^1\text{H}$ - $^{15}\text{N}$  steady state  
39  
40  
41 heteronuclear NOE or  $^1\text{H}$ - $^{15}\text{N}$  DD/ $^{15}\text{N}$  CSA longitudinal or transverse cross correlation  
42  
43  
44 experiments while  $\mu\text{s}$ -ms slower motions could be captured by more specific CPMG and  
45  
46  
47  $R_{1\rho}$  relaxation dispersion.<sup>1-2</sup> These parameters characterize heavy atom-proton relaxation  
48  
49  
50 rates, probing both the local and global motion. Their analytical expression is based on a  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 linear combination of spectral densities  $J(\omega)$  operating at different frequencies and are  
4  
5  
6  
7 the Fourier transforms of the heavy atom-proton vector autocorrelation functions  $C(t)$ .<sup>3-4</sup>  
8  
9

10 The most general level of analysis usually requires the characterization of spectral  
11  
12  
13  
14 densities at five different frequencies but necessitates the measurement of extra NMR  
15  
16  
17 cross correlation terms.<sup>5-6</sup> Additionally, the use of the three main relaxation terms cited  
18  
19  
20  
21 above, allows the calculation of reduced spectral densities<sup>7</sup> or quasi spectral density  
22  
23  
24 function<sup>8</sup> that does not necessitate any assumption of a particular motional model. An  
25  
26  
27  
28 accurate description of these motions entails the use of well-established models that  
29  
30  
31 comprise the model-free formalism<sup>9-10</sup>, closely related to the earlier two-step model<sup>11</sup> or  
32  
33  
34 another approach based on the slowly relaxing local structure (SRLS).<sup>12</sup> Due to recent  
35  
36  
37  
38 unprecedented hardware and software improvement<sup>13</sup>, the time scales probed by  
39  
40  
41  
42 relaxation parameters have become accessible through the use of all-atoms molecular  
43  
44  
45 dynamics (MD) in a reasonable computational time. Thus, MD provides a direct method  
46  
47  
48  
49 for cross-validation of force fields (FFs) by spin relaxation<sup>14</sup> or its prediction<sup>15-19</sup> and  
50  
51  
52  
53 necessitates a correct determination of both the local NH bond motion (in case of a NH  
54  
55  
56  
57 probe) and the global molecular tumbling represented by the corresponding  $C_{loc}(t)$  and  
58  
59  
60

1  
2  
3  
4  $C_{\text{global}}(t)$  correlation functions respectively. It requires in turn a correct derivation of the  
5  
6  
7 rotational diffusion  $D_{\text{rot}}$  from the MD trajectory. To determine these parameters, one  
8  
9  
10 generally rely on fitting a sum of exponential functions to  $C_{\text{loc}}(t)$  while the determination of  
11  
12  
13  
14  $D_{\text{rot}}$  is achieved through the use of different mathematical techniques<sup>20-22</sup> in the case of  
15  
16  
17 well-defined structured proteins. This parameter is more questionable in the case of  
18  
19  
20 intrinsically disordered proteins.<sup>23-24</sup> These considerations point to the importance of the  
21  
22  
23 accurate prediction of the overall tumbling ( $D_{\text{rot}}$ ) but also the rotational anisotropy of this  
24  
25  
26 motion ( $D_{\Delta}$ ), since proteins that deviate from a spherical shape will tumble more rapidly  
27  
28  
29 about some directions than others. Additionally, stronger anisotropies can severely affect  
30  
31  
32 spin relaxation values.<sup>25</sup> Since the early work of Smith and Gunsteren about BPTI and  
33  
34  
35 Lysozyme<sup>26</sup>, most of the recent studies that compare NMR spin relaxation and MD,  
36  
37  
38  
39 involve proteins that have a low to moderate anisotropy ( $1.0 < D_{\Delta} < 2.0$ ).<sup>16,21,27</sup> Whatever  
40  
41  
42  
43  
44  
45 the methods used, the different bricks that constitute the relaxation parameters are  
46  
47  
48  
49 constrained by the choice of a given force field (FF). From this perspective, the rotational  
50  
51  
52  
53 diffusion has been proved to be difficult to evaluate and requires corrections to remove  
54  
55  
56 artifacts arising from force fields (FFs) and water model dependence. Most of the  
57  
58  
59  
60

1  
2  
3 corrections consist in using a scaling factor or a correction to the NH bond length to  
4  
5  
6  
7 counterbalance the inaccuracy of water models.<sup>20,28-30</sup> From this point of view, we can  
8  
9  
10 separate the different FFs in two categories. In the most general case, biomolecular  
11  
12  
13 simulations are conducted by using empirical, fixed-charge FF, developed along with their  
14  
15  
16  
17 corresponding water model.<sup>31-32</sup> FFs based on the popular AMBER and CHARMM  
18  
19  
20 families are well suited to model biological phenomena, but suffer from a lack of accuracy  
21  
22  
23 in determining rotational diffusion.<sup>33</sup> Moreover, an improved prediction of NMR spin  
24  
25  
26 relaxation would require the use of a zero-point vibration correction.<sup>15</sup> To account for a  
27  
28  
29 more accurate and realistic description of protein structure and dynamics, polarizable FF  
30  
31  
32 like Drude<sup>34</sup> or AMOEBA (Atomic Multipole Optimized Energetics for Biomolecular  
33  
34  
35 Application)<sup>35</sup> have been developed.<sup>36</sup> Several studies have demonstrated the benefits of  
36  
37  
38 treating biomolecular systems by including polarizability in peptide simulation.<sup>37-38</sup> While  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

FFs are validated against NMR time averaged parameters like the general order parameter  $S^2$ , scalar couplings or residual dipolar couplings, less has been done to validate NMR spin relaxation parameters due to their intrinsic complexity. Thus, one may wonder if it possible to predict NMR spin relaxation parameters in a situation of (i) strong



1  
2  
3 rotational anisotropy and (ii) without turning to any scaling factor. To answer these  
4  
5  
6  
7 questions, we have used the UIM domain of STAM2, a short 31 amino acids  $\alpha$ -helical  
8  
9  
10 peptide that is characterized by a strong rotational anisotropy. In an effort to predict the  
11  
12  
13  $^{15}\text{N}$   $R_1$ ,  $^{15}\text{N}$   $R_2$  and  $^1\text{H}$ - $^{15}\text{N}$  NOEs NMR relaxation parameters, we have used four different  
14  
15  
16 FF, comprising the polarizable AMOEBA FF and three other non-polarizable FF: the  
17  
18 ff99SB-disp<sup>39</sup>, ff15ipq<sup>40</sup> and C36m.<sup>41</sup> As a first approach, we have replaced the MD  
19  
20  
21 calculated rotational diffusion by its experimentally derived counterpart to circumvent the  
22  
23  
24 problem of the rotational diffusion evaluation and water model. As a second approach,  
25  
26  
27 we have used the rotational diffusion derived from the MD trajectories. Whatever the  
28  
29  
30 approach, we show that the use of a polarizable FF significantly improves the prediction  
31  
32  
33 of the different relaxation parameters without the need of a scaling factor while the ff15ipq  
34  
35  
36 associated to the SPC/E<sub>b</sub> water model gives the best predictions among the non-  
37  
38  
39 polarizable FFs.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51

## 52 EXPERIMENTAL METHODS AND THEORY

53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 *Protein production.* The human STAM2 UIM construct was designed in pETM60  
5  
6  
7 plasmid with NusA and 6-His tag fused to the N-terminus under the regulation of a lac  
8  
9  
10 operon and has been purchased from Genecust. The plasmid was then transformed into  
11  
12  
13 E. coli BL21 GOLD (Millipore). Cells were grown in M9 medium supplemented with 1 mM  
14  
15  
16 MgSO<sub>4</sub>, 1 mM CaCl<sub>2</sub>, 6 mg/L thiamine, 1% (v/v) trace element solution [5 g/L EDTA, 0.5  
17  
18  
19 g/L FeCl<sub>3</sub>·6H<sub>2</sub>O, 5 mg/L ZnO, 1 mg/L CuCl<sub>2</sub>·2H<sub>2</sub>O, 1 mg/L Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, and 1 mg/L  
20  
21  
22 (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O], 50 mg/L kanamycin and 1 g/L <sup>15</sup>NH<sub>4</sub>Cl as sole nitrogen source for  
23  
24  
25 a uniform <sup>15</sup>N labelling and 2.5 g/L of <sup>12</sup>C<sub>6</sub>-D-Glucose. The cells culture was grown at 37  
26  
27  
28 °C to an A600 of 0.6-0.8 and the overexpression is induced by adding 1 mM IPTG. After  
29  
30  
31  
32 5 h of induction at 30 °C, cells were lysed in 50 mM Tris buffer, 250 mM NaCl, 10 mM  
33  
34  
35 imidazole, 0.04 % (v/v) β-mercapto-ethanol, 5 % (v/v) glycerol and 1 tablet of Complete®  
36  
37  
38 protease inhibitors from ROCHE. The clarified cells lysate was loaded on a Ni-NTA Fast  
39  
40  
41  
42 Flow column (GE Healthcare) equilibrated with 50 mM Tris-HCl (pH 7.8), 250 mM NaCl,  
43  
44  
45  
46 10 mM imidazole, 1% (v/v) glycerol and 0.04% (v/v) β-mercaptoethanol. The bound protein  
47  
48  
49  
50  
51  
52 was eluted with a 10-400 mM imidazole gradient. NusA and His6 tag were cleaved by  
53  
54  
55  
56 TEV protease at 4 °C O/N and discarded by a second Ni-NTA column. UIM was then  
57  
58  
59  
60

1  
2  
3 purified by a Superdex 75 gel filtration column (GE Healthcare) equilibrated in 20 mM  
4  
5  
6 sodium phosphate buffer (pH 6.8) and 130 mM NaCl. The elution peak was desalted and  
7  
8  
9 concentrated in a Microcon concentrator tube with 2 kD cut-off.  
10  
11  
12  
13  
14  
15  
16

17 *NMR experiments and processing.* NMR spin relaxation measurements were carried  
18  
19  
20 out at a proton frequency of 600 MHz at 288 K on a Bruker Avance III HD equipped with  
21  
22  
23 a triple HCN probe. Relaxation measurements including  $^{15}\text{N}$  longitudinal ( $R_1$ ), transverse  
24  
25  
26 ( $R_2$ ) relaxation as well as the  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear cross-relaxation rates were performed  
27  
28  
29 using the previously published method<sup>42</sup>. Prior to any experiments, the temperature was  
30  
31  
32 calibrated with a methanol- $\text{d}_4$  sample. NMR spectra were recorded with spectral widths  
33  
34  
35 of 2189 Hz in the  $^{15}\text{N}$  dimension and 11160 Hz in the  $^1\text{H}$  dimension. For the  $R_1$   
36  
37  
38 experiments, we have used ten relaxation delays ranging from 40 to 2400 ms with a  
39  
40  
41 recycling delay of 4 s. The delays are the following: 40, 200, 400 (twice), 600, 800, 1100,  
42  
43  
44 1400 (twice), 1700, 2200 and 2400 ms. In the case of  $R_2$  experiments, we have used 13  
45  
46  
47 relaxation delays ranging from 8 to 448 ms with a recycling delay of 4 s. The delays are  
48  
49  
50 the following: 8, 32, 64, 96 (twice), 160, 192 (twice), 224, 256, 304, 352, 384, 416 and 448 ms.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 For heteronuclear NOE experiments, 2D spectra were recorded with and without  
4  
5  
6  
7 presaturation of amide protons and a recycling delay of 4.5 s. Longitudinal and transverse  
8  
9  
10 relaxation parameters were estimated by fitting the measured peak intensities to a single  
11  
12  
13 exponential decay, using a two-parameter fit. The uncertainty of the relaxation  
14  
15  
16 parameters was estimated by Monte-Carlo simulation of the fitted parameters as  
17  
18  
19 implemented in the Relaxfit program.<sup>42</sup>  
20  
21  
22  
23  
24  
25  
26  
27

28 *NMR spin relaxation simulations as a function of rotational anisotropy.* To model the  
29  
30  
31 effect of an increasing rotational anisotropy on the  $R_1$ ,  $R_2$  and NOE parameters we have  
32  
33  
34 used the standard equations expressed as a linear combination of spectral densities:<sup>4</sup>  
35  
36  
37

$$R_1 = 3(d^2 + c^2)J(\omega_N) + d^2[J(\omega_H - \omega_N) + 6J(\omega_H + \omega_N)] \quad (1)$$

$$R_2 = \frac{1}{2}(d^2 + c^2)[4J(0) + 3J(\omega_N)] + \frac{1}{2}d^2[J(\omega_H - \omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)] + R_{ex} \quad (2)$$

$$NOE = 1 - \left| \frac{\gamma_H}{\gamma_N} \right| d^2 \frac{[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)]}{R_1} \quad (3)$$

Where  $d = -\frac{\mu_0}{4\pi} \times \frac{\gamma_H \gamma_N}{4\pi r_{HN}^3}$  is the strength of the  $^1\text{H}$ - $^{15}\text{N}$  dipolar coupling,  $c = -\frac{\omega_N \times \text{CSA}}{3}$ ,  $\omega_H$  and

$\omega_N$

are the resonance frequencies of  $^1\text{H}$  and  $^{15}\text{N}$  respectively, CSA is the anisotropy of the  $^{15}\text{N}$  chemical shift tensor, assumed axially symmetric,  $\gamma_H$  and  $\gamma_N$  are the gyromagnetic ratios of the nuclei,  $h$  is Plank's constant and  $R_{ex}$  is the conformational exchange contribution (if any) to the measured  $R_2$ . These equations provide the basis for extracting information on protein dynamics from NMR relaxation measurements. To translate into a physical picture of protein dynamics, the spectral densities  $J(\omega)$  are expressed as a combination of local and global reorientation, assuming there is no correlation between the local dynamics and the global molecular tumbling, one can define a global correlation function  $C(t)$ :

$$C(t) = C_{global}(t) \times C_{local}(t) \quad (4)$$

Although extended models exist, we have chosen to use the standard model-free approach<sup>9-10</sup> to represent  $C_{local}(t)$ :

$$C_{local}(t) = S^2 + (1 - S^2) \times \exp\left(-t/\tau_{loc}\right) \quad (5)$$

where  $\tau_{loc}$  represents the correlation time of a bond motion and  $S^2$  stands for the order parameter that represents the dimensionless averaged amplitude of the bond motion. In the case of an axially symmetric diffusion tensor, the global  $J(\omega)$  can be rewritten as<sup>43</sup>:

$$J(\omega) = \frac{2}{5} \sum_{i=1}^3 \frac{A_i^{ax} D_i^{ax}}{(D_i^{ax})^2 + \omega^2} \quad (6)$$

with

$$D_1^{ax} = (5D_{\perp} + D_{\parallel}); \quad D_2^{ax} = (2D_{\perp} + 4D_{\parallel}); \quad D_3^{ax} = 6D_{\perp}$$

$$\text{and } A_1^{ax} = 3z_d^2(1 - z_d^2); \quad A_2^{ax} = \frac{3}{4}(1 - z_d^2)^2; \quad A_3^{ax} = \frac{1}{4}(3z_d^2 - 1)$$

where  $D_{\parallel}$  and  $D_{\perp}$  stand for the principal components of the axially symmetric tensor.  $z_d$  denotes the z coordinates of a NH unit vector in the principal axis frame of the diffusion tensor and is related to the molecular axis frame following the general transformation:

$$\begin{bmatrix} x_d \\ y_d \\ z_d \end{bmatrix} = R(\alpha, \beta, \gamma) \begin{bmatrix} x \\ y \\ z \end{bmatrix} \quad (7)$$

$R$  being the passive rotation matrix defined by its Euler angles according to the  $zyz$  convention in 3D space.<sup>44</sup> For a complete description in the case of a fully anisotropic rotational diffusion tensor, one can refer to previous papers for the analytical expression of the relaxation parameters.<sup>43,45-46</sup>

1  
2  
3  
4  
5  
6  
7 *Rotational diffusion analysis.* The rotational diffusion tensor of the protein was derived  
8  
9  
10 from the orientation dependence of the  $\rho$  factor described previously.<sup>47</sup> The  $\rho$  factor is  
11  
12  
13 expressed as  $\left(\frac{2R_2'}{R_1'} - 1\right)^{-1}$  where  $R_2'$  and  $R_1'$  correspond to the transverse and longitudinal  
14  
15  
16 relaxation rates modified to subtract the contributions from high-frequency motions.<sup>48</sup> The  
17  
18  
19 advantage of using this ratio instead of the individual values of these parameters is that  
20  
21  
22 it is independent, to a first approximation, of the site-specific variations in the strength of  
23  
24  
25  $^1\text{H}$ - $^{15}\text{N}$  dipolar coupling and  $^{15}\text{N}$  chemical-shift anisotropy. Moreover, in the case of protein  
26  
27  
28 core residues, the  $R_2'/R_1'$  ratio primarily depends on the overall tumbling and is practically  
29  
30  
31 insensitive to fast, subnanosecond backbone dynamics.  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

42 *Structure of the UIM domain.* The three-dimensional structure of the UIM domain alone  
43  
44  
45 was obtained by homology modeling. As already demonstrated in earlier studies, the  
46  
47  
48 amino acid sequences of STAM2-UIM and Vps27-UIM1 share 55% identity and 70%  
49  
50  
51 similarity. Moreover, NMR has demonstrated that the STAM2 UIM domain adopts a  $\alpha$ -  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 helical structure in solution<sup>49</sup> from residue E170 to residue E184 (currently renumbered  
4  
5  
6  
7 E10 to E24 for simplicity in the current study, see supporting information). We used the  
8  
9  
10 UIM1 domain<sup>50</sup> of Vps27 (PDB code 1Q0V) as a template to model the structure of the  
11  
12  
13  
14 UIM domain by means of the Modeler program.<sup>51</sup>  
15  
16  
17  
18  
19  
20

21 *MD simulation.* Molecular Dynamics (MD) simulations were carried out on GPUs by  
22  
23  
24 means of GROMACS 5.1.4<sup>52</sup>, software with the CHARMM36m and AMBER ff99SB-disp  
25  
26  
27 force fields, or using ACEMD<sup>53</sup> software with AMBER ff15ipq and finally using Tinker and  
28  
29  
30  
31 OpenMM<sup>54-55</sup> software with AMOEBA force field. For all cases, temperature and pressure  
32  
33  
34 were set to 300 K and 1.013 bar. All simulations include explicit solvent and H-bonds  
35  
36  
37  
38 constrained to the length defined in the force field. MD analysis were performed with  
39  
40  
41  
42 GROMACS software, a forked version of PLUMED (to compute quaternions)<sup>20</sup> and the  
43  
44  
45 SpinRelax<sup>15</sup> program.  
46  
47  
48  
49  
50  
51

52 *GROMACS simulations.* The protein was set in a dodecahedron water box with a  
53  
54  
55  
56 minimum distance between the protein and the side equal to 1.2 nm. Counter ions (Na<sup>+</sup>  
57  
58  
59  
60



1  
2  
3  
4 and Cl<sup>-</sup>) were added to adjust the net charge to zero ( $C_{\text{NaCl}} = 0.15 \text{ M}$ ). Equilibration  
5  
6  
7 involves the removal of isolated intruding water molecules. 2500 steps energy  
8  
9  
10 minimization followed by a gradual relaxation of side-chain and backbone restraints over  
11  
12  
13  
14 2 ns was conducted from 5000 to 0 kJ mol<sup>-1</sup> nm<sup>-2</sup>. Temperature and pressure were  
15  
16  
17 coupled with V-Rescale thermostat<sup>56</sup> and Berendsen's barostat.<sup>57</sup> Electrostatic  
18  
19  
20 interactions were handled by Particle Mesh Ewald (PME) methods<sup>58</sup> and van der Waals  
21  
22  
23 potential was treated by simple cutoff of 1.2 nm. Constraints of all bonds to the length  
24  
25  
26 defined by the FF were processed by LINCS algorithm.<sup>59</sup> The SETTLE algorithm<sup>60</sup> was  
27  
28  
29 used to constrain the rigid water molecule model. The final structure obtained after  
30  
31  
32 equilibration was retained as the starting configuration for 20 quasi-independent sim-  
33  
34  
35 ulations. Each trajectory has been recorded every 5 ps for analysis and the integration  
36  
37  
38 time step was set to 2 fs. The trajectory length was set to 50 ns. For each simulation, the  
39  
40  
41 velocities of the particle were generated at start-up yielding a family of 20 different  
42  
43  
44 trajectories.  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 *ACEMD simulations.* The protein was set in a cubic box by applying 1.2 nm buffer to  
5  
6  
7 the protein coordinates, filled with water molecules and the specific counter-ions as in the  
8  
9  
10 previous case. Energy minimization was run for 1000 steps, followed by NVT equilibration  
11  
12  
13 for 200 ps with time-step of 2 fs and concluded with NPT equilibration in two steps: first  
14  
15  
16 with constraints during 1 ns and secondly without constraints for 1 ns. We used a  
17  
18  
19 Langevin's thermostat with dumping of 1 ps and Berendsen's barostat with pressure  
20  
21  
22 relaxation time of 800 ps. The Coulomb's electrostatic was described by PME (Particle  
23  
24  
25 Mesh Ewald) method with cutoff at 0.9 nm and grid-spacing of 0.1 nm, whereas the van  
26  
27  
28 der Waals forces were described by a switching function with a cutoff at 0.75 nm. The 50  
29  
30  
31 ns runs were carried out in NVT ensemble. For this specific case, we have produced 20  
32  
33  
34 quasi-independent trajectories, by generating new sets of velocity at start-up, giving rise  
35  
36  
37  
38 to a total trajectory of 1 $\mu$ s.  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48

49 *Tinker and OpenMM simulations.* The latest version of TINKER has OpenMP shared-  
50  
51  
52 memory parallelization of AMOEBA simulations, while OpenMM<sup>61</sup> is accelerated for GPU-  
53  
54  
55 based calculations. The generation of protein, water and counter-ions in a cubic box was  
56  
57  
58  
59  
60

1  
2  
3 made by using GROMACS tools, adding a buffer of 1 nm to the protein (due to the com-  
4  
5  
6 putational intensity of the mechanic of the force field, to obtain a reasonable simulation  
7  
8  
9  
10 speed, we needed to reduce the number of water molecules). We used the latest version  
11  
12  
13 of the AMOEBA force field<sup>62</sup>. The energy minimization was run through MINIMIZE. NVT  
14  
15  
16 and NPT equilibrations were run via DYNAMIC. In NVT equilibration (50 ps) the trajectory  
17  
18  
19 was integrated via RESPA integrator with a time step of 2 fs, whereas the NPT (100 ps)  
20  
21  
22 trajectory was integrated via VERLET integrator with a time step of 1 fs. In all cases, the  
23  
24  
25 HEAVY-HYDROGEN keyword was set so that we could use longer time-steps (Tinker  
26  
27  
28 manual suggests time-step below 1 fs with no mass-repartition, up to 3 fs can be used  
29  
30  
31 with RESPA integrator and heavy-hydrogen). Dipole convergence criterion was set  
32  
33  
34 POLAR-EPS = 0.001 D, and the polarization was computed by perturbation theory at the  
35  
36  
37 third order (POLARIZATION = OPT3).<sup>63</sup> Temperature and pressure were coupled with  
38  
39  
40 Bussi thermostat and Montecarlo barostat. During thermalization and density  
41  
42  
43 equilibration, the atomic distances of the heavy atom-hydrogen bonds were restrained by  
44  
45  
46 applying the RATTLE algorithm (water was never restrained because the system  
47  
48  
49 becomes computationally intractable). Production run were integrated via RESPA  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 integrator with a time-step of 2 fs, keeping same condition for barostat and thermostat.  
4  
5

6  
7 Conversion from Tinker .arc file to GROMACS .xtc file format was carried out by awk and  
8

9  
10 Python scripts. In this current case, we performed 10 independent equilibrations. The  
11

12  
13 simulation velocity in a GeForce GTX 980 is approximately ~ 5 ns/day, whereas in  
14

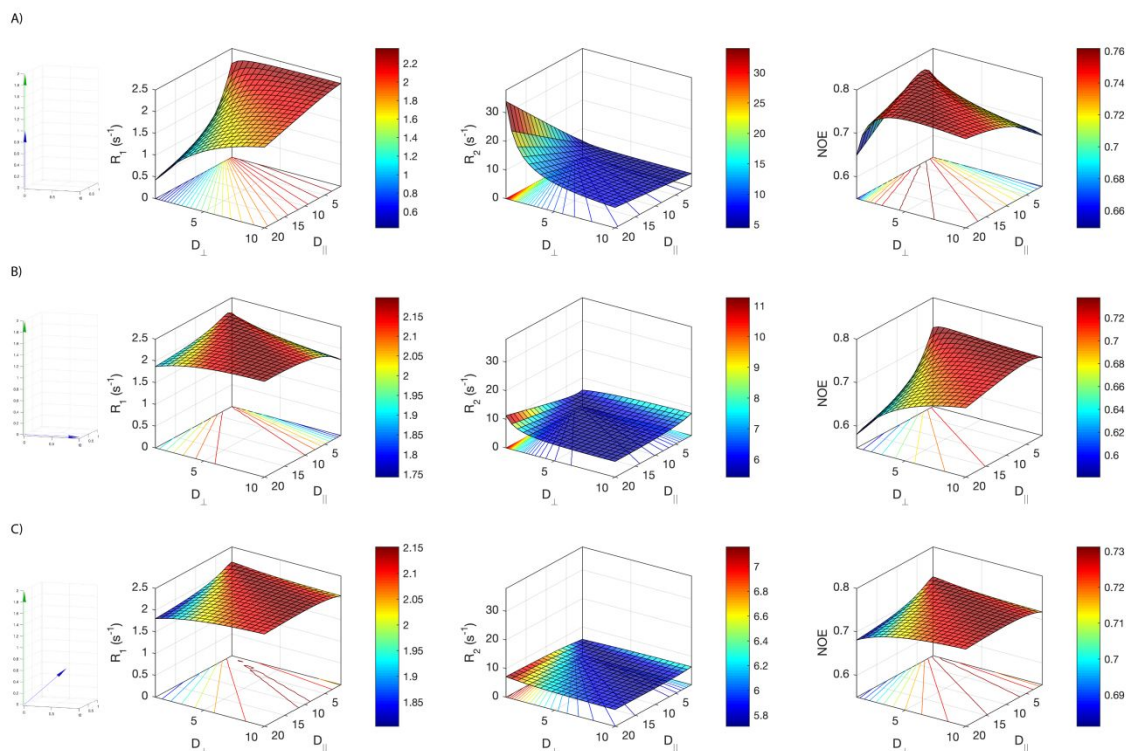
15  
16  
17 GeForce GTX 780 Ti is ~ 3 ns/day, and in a Tesla P100 at CINES is ~ 7 ns/day.  
18  
19  
20  
21  
22  
23

## 24 RESULTS AND DISCUSSION

### 25 *The effect of rotational anisotropy on NMR spin relaxation*

26  
27  
28  
29  
30  
31 To account for the effect of an increasing rotational anisotropy on NMR spin relaxation  
32  
33 data, we have used the analytical expression presented in the theory section above. The  
34  
35 determination of the dynamical properties of a molecule by NMR relies on its shape. While  
36  
37 an ideal sphere tumbles isotropically in solution so that all orientations within a molecule  
38  
39 are equivalent, any deviation of this case will result in an anisotropy of its overall rotational  
40  
41 diffusion in solution, which could be the case for an elongated rod, multidomain proteins  
42  
43 or intrinsically disordered proteins. This, in turn, will lead to an orientational dependence  
44  
45 of the various processes of nuclear spin relaxation that are modulated by molecular  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 motions. Orientational dependence is probed by  $^1\text{H}$ - $^{15}\text{N}$  bond vectors in our case and  
4  
5  
6  
7 appears as a difference in the apparent tumbling rate. If we assume a molecule with its  
8  
9  
10 three principal components of the overall diffusion tensor  $D_x$ ,  $D_y$ ,  $D_z$  with  $D_z > D_y > D_x$ , an  
11  
12  
13 axial rotational tumbling will reduce to two components with  $D_z \equiv D_{||}$  and  $D_y = D_x \equiv D_{\perp}$ . For  
14  
15  
16 a NH vector parallel to the  $||$  axis, its reorientations will be caused by molecular rotations  
17  
18  
19 around the  $\perp$  axis so that the apparent rotational diffusion rate for this vector will be  
20  
21  
22 determined by  $D_{\perp}$ . Conversely, reorientation of a vector perpendicular to the  $||$  axis will be  
23  
24  
25 affected by  $D_{||}$  and thus will proceed faster. These differences in molecular tumbling rates  
26  
27  
28 and internuclear vector orientations will lead to different modulation of the relaxation  
29  
30  
31 parameters and will increase with the rotational anisotropy. Moreover, this effect has to  
32  
33  
34  
35  
36  
37  
38 be accurately treated as it could be mistaken for conformational exchange.<sup>25</sup>  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



**Figure 1.** Dependence of the NMR spin relaxation parameters  $R_1$ ,  $R_2$  and NOE as a function of various degrees of anisotropy ( $D_{\Delta} = D_{\parallel} / D_{\perp}$ ) and NH vector orientation with respect to the principal axis frame (PAF). On the left panel, the  $D_{\parallel}$  axis of the PAF is represented in green along the z axis of the laboratory frame and three NH unit vectors are presented with a 0° (A), 90° (B) and 54° (C) orientation with respect to the  $D_{\parallel}$  axis of the PAF. The synthetic data were obtained by assuming local model-free combined with an axially symmetric molecular reorientation (see analytical expressions in experimental

1  
2  
3 methods). Typical parameters for restricted local backbone dynamics in protein core were  
4  
5  
6 used with  $S^2 = 0.84$ , a local motion  $\tau_{loc} = 20$  ps, a global tumbling  $\tau_c = 4.0$  ns for a  $^1\text{H}$   
7  
8  
9 frequency of 600MHz. Plain lines represent the surface projection for a given anisotropy.  
10  
11  
12  
13

14 For a complete description of the theoretical expressions describing these motions, we  
15  
16 refer the reader to the previous work of Woessner.<sup>64</sup> To account for this theoretical  
17  
18  
19 framework, we have simulated the three relaxation parameters  $R_1$ ,  $R_2$  and NOE in the  
20  
21  
22 case of an axially symmetric model (see experimental methods for a description of the  
23  
24  
25 analytical expression used here) in the case of three distinct orientations of a NH bond  
26  
27  
28  
29 vector. Figure 1 shows the results obtained for three different orientations of a NH vector  
30  
31  
32 with respect to the principal axis frame (PAF). For a given set of dynamical parameters  
33  
34  
35 that corresponds to a protein of ca  $\sim 8\text{kDa}$  ( $\tau_c = 4.0$  ns,  $\tau_{loc} = 20$  ps,  $S^2 = 0.84$ ), there is a  
36  
37  
38 significant difference for the three relaxation parameters as a function of bond vector  
39  
40  
41 orientation and rotational anisotropy. For a rather low anisotropy ( $\sim 1-2$ ), the orientation of  
42  
43  
44 a NH vector has practically no effect on the relaxation parameters. For a bond vector that  
45  
46  
47 would lie parallel to the long axis of the PAF ( $D_{||}$ ) and a significant increase of the  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 anisotropy ( $D_{\Delta} \sim 4$  to 10), one observes a profound modification of each relaxation  
4  
5  
6  
7 parameters. Conversely, for a NH vector orientation that would lie  $90^{\circ}$  or  $54^{\circ}$  away from  
8  
9  
10 the  $D_{\parallel}$ , a change of the anisotropy has a weak or moderate effect on any of the relaxation  
11  
12  
13  
14 parameters. Increasing the rate of the local motion to 200 ps significantly affects the  
15  
16  
17 values of NOE while it has practically no effect on  $R_1$  or  $R_2$  (see figure S1A). Finally, if  
18  
19  
20 one considers the case of a NH vector that undergoes a significant flexibility ( $S^2 = 0.2$  and  
21  
22  
23  $\tau_{loc} = 200$  ps),  $R_1$  and  $R_2$  are less affected by the rotational anisotropy while NOEs  
24  
25  
26  
27 significantly drop and are influenced in the case of a NH vector lying along the  $D_{\parallel}$  axis  
28  
29  
30  
31 (Figure S1B).  
32  
33  
34  
35  
36  
37

### *Rotational diffusion of the UIM domain*

38  
39  
40  
41  
42 Having shown that rotational anisotropy is a crucial parameter in the determination of  
43  
44  
45 the spin relaxation values, we have determined the rotational diffusion parameters from  
46  
47  
48  
49 NMR relaxation data recorded for the UIM domain of the STAM2 protein. The UIM  
50  
51  
52 domain contains 31 amino acids that fold into an  $\alpha$ -helix between E10 and E24 while the  
53  
54  
55 rest of this domain remains flexible (see supporting information).<sup>65</sup> We have used the  $\rho$   
56  
57  
58  
59  
60



1  
2  
3 factor method<sup>66-67</sup> for its ability to be valid for higher anisotropies (see experimental  
4  
5  
6 methods)<sup>47</sup>. Table 1 presents the results obtained for an isotropic and an axially  
7  
8  
9 symmetric model. In the case of an axially symmetric tensor, we have fitted 4 parameters,  
10  
11  
12 the two principal values of the rotational diffusion tensor along with the two angles that  
13  
14  
15 define the position of the PAF with respect to the laboratory frame. Since two of the  
16  
17  
18 eigenvalues are equal in the case of the axially symmetric model, the orientation of the  
19  
20  
21 diffusion tensor can be described by the orientation of the unique eigenvalue  $D_{\parallel}$ .  
22  
23  
24 Therefore, we can express this orientation using only  $\alpha$  and  $\beta$  angles and set  $\gamma = 0$ . It has  
25  
26  
27 to be recalled that the relaxation parameters are not sensitive to the directionality (sign) of the NH-  
28  
29  
30 vector coordinates.<sup>66</sup> Figure S2 shows the orientation of the unique axis of the rotational  
31  
32  
33 diffusion tensor that is roughly aligned along the  $\alpha$ -helical motif of the UIM domain. Each  
34  
35  
36 of the NH bond vector orientations are defined with respect to the PAF. Only residues  
37  
38  
39 that belong to the well-defined  $\alpha$ -helical region of UIM and that do not show any  
40  
41  
42 conformational exchange (A15 and L20 excluded) have been used for the analysis. As  
43  
44  
45 can be seen in Table 1, the axially symmetric model agrees with the experimental data  
46  
47  
48 significantly better than the isotropic model: the F-statistics analysis of the fit results in the  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

probability  $P = 5.3 \times 10^{-10}$  that this could occur by chance. The use of a more complicated, fully anisotropic model, did not improve the fitting procedure and is not presented here.

**Table 1.** Rotational diffusion tensor parameters for UIM derived from  $^{15}\text{N}$  relaxation data using an isotropic or axially symmetric model of the overall tumbling.

Model	$D_x^a (\equiv D_{\perp})$	$D_y^a (\equiv D_{\perp})$	$D_z^a (\equiv D_{\parallel})$	$\alpha^b$	$\beta^b$	$\gamma^b$	$\tau_c^c$	Anisotropy <sup>d</sup>	$\chi^2/\text{df}$ <sup>e</sup>	$P^f$
Isotropic	4.55 (0.07)	4.55 (0.07)	4.55 (0.07)	-	-	-	3.66 (0.06)		8.0	
Axially-symmetric <sup>c</sup>	2.2 (0.3)	2.2 (0.3)	11.7 (0.9)	120 (22)	57 (16)	-	3.10 (0.58)	5.3 (1.1)	1.4	$5.3 \times 10^{-10}$

<sup>a</sup> Principal values (in  $10^7 \text{ s}^{-1}$ ) of the rotational diffusion tensor, ordered so that  $D_x \leq D_y \leq D_z$ . In the case of an axially symmetric model,  $D_z \equiv D_{\parallel}$  and  $D_y = D_x \equiv D_{\perp}$ .

<sup>b</sup> Euler angles  $\{\alpha, \beta, \gamma\}$  (in degrees) describe the orientation of the principal axes frame of the rotational diffusion tensor with respect to protein coordinate frame. Proper Euler angles have been defined with successive rotation around  $z(\alpha)y(\beta)z(\gamma)$ . In the case of an axially symmetric model,  $\gamma = 0$ .

<sup>c</sup> Overall rotational correlation time (in ns) of the molecule,  $\tau_c = \frac{1}{[2 \text{Tr}(\underline{D})]}$

<sup>d</sup> The degree of anisotropy of the diffusion tensor,  $D_{\Delta} = 2D_z / (D_x + D_y)$  or  $D_{\parallel} / D_{\perp}$  in the case of an axially symmetric model.

1  
2  
3  
4     <sup>e</sup> Residuals of the fit divided by the number of degrees of freedom

5  
6     <sup>f</sup> Probability that the reduction in  $\chi^2$  (compared to the isotropic model) could occur by  
7 chance. The axially symmetric model gives a statistically much better fit than the isotropic  
8 model.  
9  
10

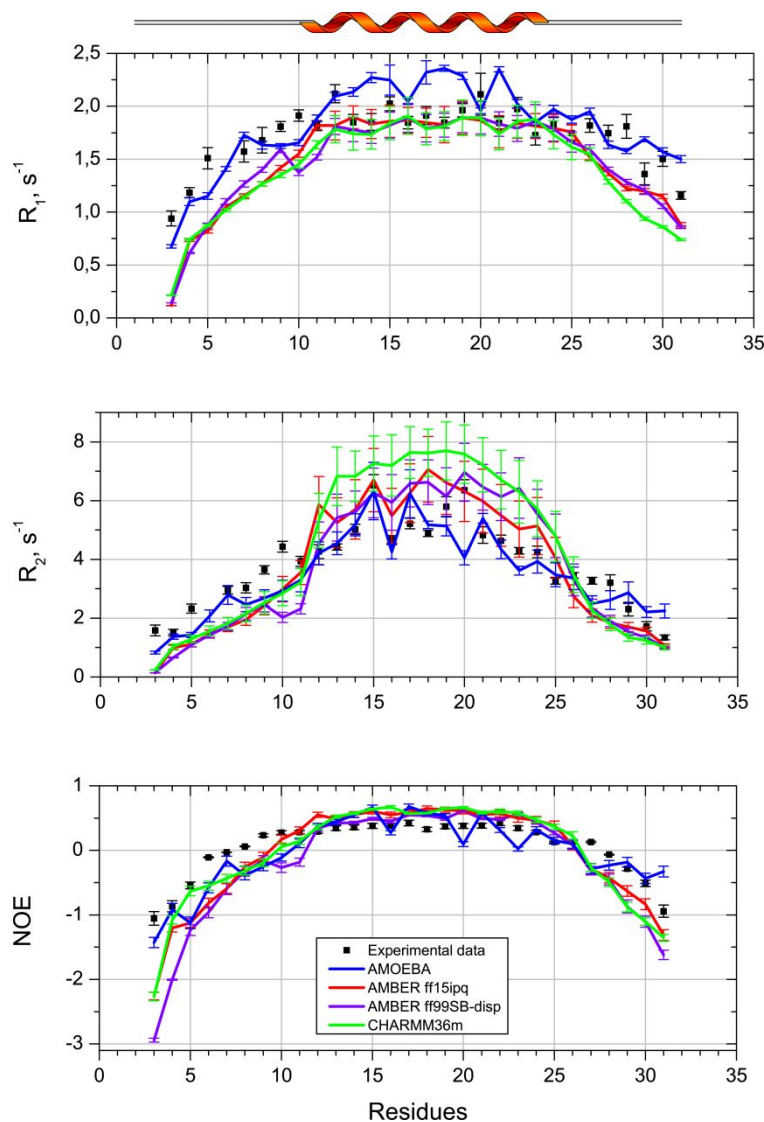
11  
12  
13  
14  
15  
16     *Using the experimental rotational diffusion to predict relaxation parameters*

17  
18  
19     That UIM is affected by a significant anisotropy has encouraged us to compare different  
20 FFs for their ability to predict rotational diffusion and relaxation parameters. While  
21 computing  $D_{\text{rot}}$  by means of non-polarizable FFs could give rise to overestimated values,  
22 we were seeking to understand if the additional physics found in the AMOEBA polarizable  
23 FF allows a more accurate prediction of the standard spin relaxation parameters, i.e.  $^{15}\text{N}$ -  
24  $R_1$ ,  $R_2$  and  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear NOEs. To address this question, we have performed MD  
25 simulations of the short UIM helical domain. As a sake of comparison, we have used  
26 different FFs of the AMBER (ff99SB-disp<sup>39</sup> and ff15ipq<sup>40</sup>) and CHARMM family (C36m).<sup>41</sup>  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

All simulations were run in explicit water (see experimental methods). While FFs were developed and improved with their respective water-models, we have used the SPC/ $E_b$  and AMOEBA water models associated with the ff15ipq and AMOEBA FFs respectively.

1  
2  
3  
4 The SPC/E<sub>b</sub> water model has been developed from the original SPC/E model with a slight  
5  
6  
7 increase in the O-H bond. It has been successfully tested and validated on four globular  
8  
9  
10 proteins (ubiquitin, protein G, barstar and BPTI) that exhibit a weak rotational  
11  
12  
13 anisotropy.<sup>29</sup> We have used the TIP3P-Charmm and a99SB-disp water models along with  
14  
15  
16 the respective Charmm36m and ff99SB-disp FFs. The specific TIP3P-Charmm water  
17  
18  
19 model is a TIP3P modified in the dispersion coefficient of the LJ interaction<sup>41</sup> while the  
20  
21  
22 a99SB-disp is based on the original TIP4P-D water model and has introduced small  
23  
24  
25 changes in the water vdW interaction terms.<sup>39</sup> All simulations have been run with a total  
26  
27  
28 trajectory duration of 1 $\mu$ s divided into 20 replicas of 50 ns. Indeed, it has been  
29  
30  
31 demonstrated that the simulations issued from several replicas are more reliable  
32  
33  
34 compared to a single, long simulation.<sup>68</sup> This duration is in agreement with 1-2 orders of  
35  
36  
37 magnitude above the expected tumbling time recommended previously<sup>69</sup> and ensures a  
38  
39  
40 sufficient conformational sampling for the estimation of the different relaxation  
41  
42  
43 parameters. Furthermore, the UIM domain keeps its  $\alpha$ -helical fold for the majority of the  
44  
45  
46 simulations and FFs (Figure S3). The calculation of the relaxation parameters has been  
47  
48  
49 carried out by analyzing the MD trajectories following our well established protocol.<sup>15,20</sup>  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Here we have compared two different methods to predict the relaxation parameters. First  
4  
5  
6  
7 and foremost, we have chosen to introduce the values of  $D_{\text{iso}}$  and  $D_{\Delta}$  that were derived  
8  
9  
10 from the NMR analysis. In this way, we avoid the problem of both the water-model<sup>33</sup> and  
11  
12  
13 the finite-size effects of the simulation box.<sup>70-71</sup> Secondly, we have used the MD-predicted  
14  
15  
16  
17  $D_{\text{iso}}$  and  $D_{\Delta}$ . For the first method,  $D_{\text{rot}}$  has been extracted from NMR spin relaxation  
18  
19  
20  
21 experiments (see Table 1).  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



**Figure 2.** Experimental (black symbols) and computed NMR spin relaxation parameters (solid lines)  $^{15}\text{N}$   $R_1$ ,  $^{15}\text{N}$   $R_2$  and  $^1\text{H}$ - $^{15}\text{N}$  NOE for the different FFs used in this study. The global rotational diffusion  $D_{\text{iso}}$  and rotational anisotropy  $D_{\Delta}$  are fixed by the experiment while the zero order correction parameter  $\zeta$  has been fixed to 0.89.<sup>72</sup> The UIM secondary structure is represented at the top of the relaxation parameters.

1  
2  
3  
4 The use of an axially symmetric model is also confirmed by MD and the values of the  
5  
6  
7 average radii of gyration obtained by the four FFs (see Table S1). The axial components  
8  
9  
10 of the diffusion tensor  $D_{\parallel}$  and  $D_{\perp}$  (Table 1) were experimentally derived by considering  
11  
12  
13 the residues of the well folded helical region and that do not show any conformational  
14  
15  
16 exchange (A15 and L20 excluded). As a matter of consistency, the computation of the  
17  
18  
19 internal correlation function from MD simulations was conducted by selecting similar  
20  
21  
22 residues and using the following expression:  $D_{\Delta} = D_{\parallel} / D_{\perp}$  and  $D_{\text{iso}} = 1/3 \cdot (D_{\parallel} / D_{\Delta}) \cdot (2 + D_{\Delta})$ .<sup>73</sup>  
23  
24  
25  
26  
27  
28 No further optimization of parameters such as  $D_{\text{iso}}$ ,  $D_{\Delta}$ , CSA and  $\xi$  has been introduced  
29  
30  
31 in the analysis,  $\xi$  being the correction constant that describes the zero-point vibration of  
32  
33  
34 the N-H bonds.<sup>72</sup>  
35  
36  
37

38 To account for the goodness of the predicted relaxation parameters compared to the  
39  
40  
41 experimental ones, we defined the  $\chi^2$  parameter:  
42  
43  
44

$$\chi^2 = \sum_k \sum_{j=1}^n \left( \frac{O_j - P_j}{\sigma_k} \right)^2 \quad (8)$$

45  
46  
47  
48 Where  $O_j$  is the experimentally observed value for the residue  $j$  and  $P_j$  is the  
49  
50  
51 corresponding predicted value derived from the analysis of the MD simulation,  $k \in (R_1,$   
52  
53  
54  $R_2, \text{NOE})$  and  $\sigma_k$  is the standard deviation of the corresponding relaxation parameter.  
55  
56  
57  
58  
59  
60

**Table 2.** Values of  $\chi^2$  indicating the goodness of the predicted  $R_1$ ,  $R_2$  and NOE for the different FFs and water-models used in this study.

Force Field (water model)	$\chi^2 (D_{\text{iso}}, D_{\Delta})^{\text{exp}}$	$\chi^2 (D_{\text{iso}}, D_{\Delta})^{\text{MD}}$
ff99SB-disp (TIP4P-disp)	60.9	105.3
ff15ipq (SPC/E <sub>b</sub> )	41.2	71.7
C36m (TIP3P-charmm)	71.1	131.7
AMOEBA (AMOEBA)	23.3	22.6

$(D_{\text{iso}}, D_{\Delta})^{\text{exp}}$ : spin relaxation parameters are predicted according to the experimental rotational diffusion and anisotropy.  $(D_{\text{iso}}, D_{\Delta})^{\text{MD}}$ : spin relaxation parameters are predicted according to the calculated rotational diffusion and anisotropy derived from MD simulations.

As can be seen in Figure 2 and Table 2, the agreement between experimental and predicted relaxation data depends on the considered FF. While polarized and empirical FFs show roughly the same agreement with respect to the NOE data, they exhibit a profound difference in the prediction of longitudinal and transverse relaxation rates. Non-polarizable FFs underestimate  $R_1$  while the AMOEBA FF displays a good match with the



1  
2  
3 experimental  $R_1$  all along the UIM sequence. In the case of  $R_2$  that shows a significant  
4  
5  
6  
7 increase in the helical part of UIM, non-polarizable FFs overestimate  $R_2$  in this region  
8  
9  
10 while they underestimate  $R_2$  in the flexible N- and C-terminus part of UIM. Moreover, the  
11  
12  
13 gap between the maximum and the minimum values predicted for  $R_2$  is larger when a  
14  
15  
16  
17 non-polarizable FF is used.  
18  
19  
20

21 Our data clearly evidence that the use of the polarizable AMOEBA FF improves the  
22  
23  
24 prediction of NMR spin relaxation parameters compared to empirical FFs in the particular  
25  
26  
27 case of an elongated rod. To understand this discrepancy, we have to pinpoint the most  
28  
29  
30 important parameters responsible for these differences. As illustrated by Figure S4, we  
31  
32  
33  
34 can notice a clear contrast in the decay of the correlation functions associated with some  
35  
36  
37 selected residues K7, I14, Q25 or Q28 located in different regions of UIM, which in turn  
38  
39  
40 induces a significant difference in the internal correlation function  $C_i(t)$  as seen in Figure  
41  
42  
43  
44  
45 S5. Consequently, our observations suggest that the AMOEBA polarizable FF allows to  
46  
47  
48 grasp a more intricate motional network.  
49  
50  
51  
52  
53  
54  
55

56 *The AMOEBA FF accurately predicts  $D_{rot}$*   
57  
58  
59  
60

1  
2  
3  
4 Thusly, as a second method, we have investigated the ability of the different FFs to  
5  
6  
7 predict the rotational diffusion of UIM. The isotropic value of the rotational diffusion  $D_{\text{iso}}$  is  
8  
9  
10 an essential parameter that remains a staple in the determination of relaxation rates. It  
11  
12  
13 contributes to the global tumbling of the correlation function through its axial components  
14  
15  
16  
17  $D_{\parallel}$  and  $D_{\perp}$ .<sup>15</sup> The calculated values of  $D_{\parallel}$ , that represents the axis of fast overall rotation,  
18  
19  
20 span a large range from  $8.79 \times 10^7$  to  $36.60 \times 10^7 \text{ s}^{-1}$  with respect to the used FFs (see  
21  
22  
23  
24 Table 3).  
25  
26  
27  
28  
29  
30

31 **Table 3.** Characterization of the rotational diffusion tensor of the UIM domain for the  
32  
33  
34 different FFs used in this study  
35  
36  
37  
38

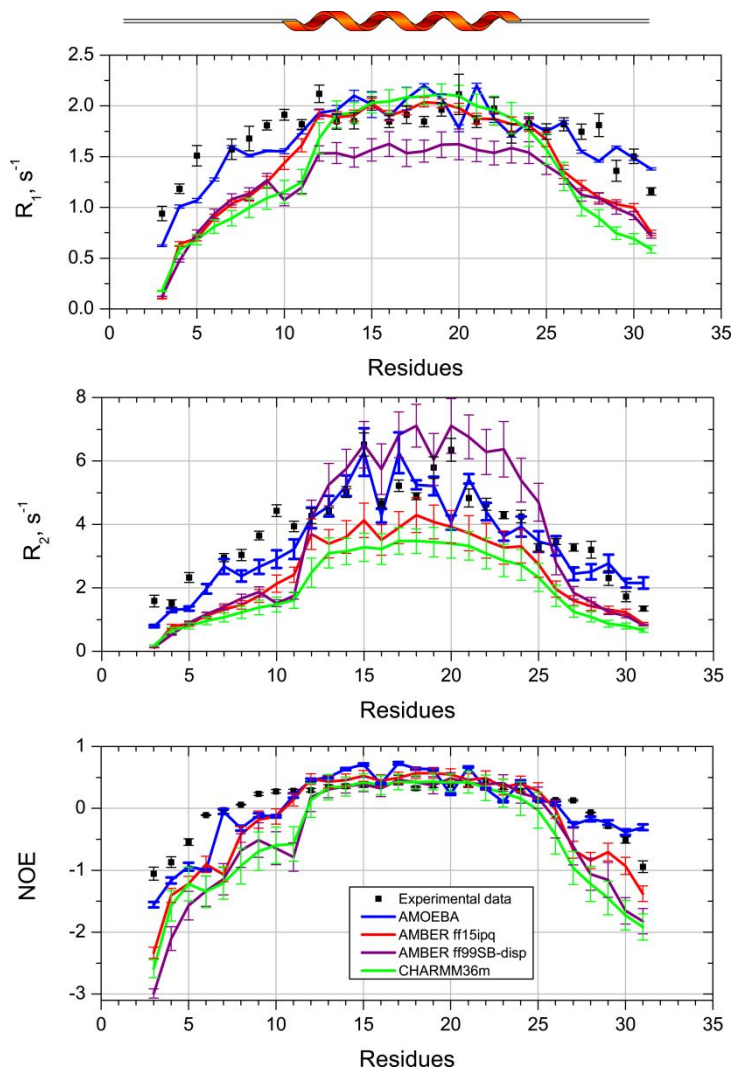
	Exp	ff99SB-disp	ff15ipq	C36m	AMOEBA
$D_{\parallel}^a$	11.70 (0.90)	31.70 (3.50)	17.40 (2.30)	36.60 (5.10)	8.79 (0.60)
$D_{\perp}^a$	2.20 (0.30)	1.53 (0.20)	4.13 (0.50)	5.83 (0.80)	2.28 (0.20)
$D_{\Delta}$	5.30 (1.10)	20.80 (2.30)	4.21 (0.50)	6.29 (0.90)	3.86 (0.30)
$D_{\text{iso}}$	5.40 (0.50)	11.60 (1.30)	8.55 (1.10)	16.10 (2.30)	4.45 (0.30)

53 a  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4<sup>a</sup>Values are given in  $10^7 \text{ s}^{-1}$ . Errors are specified into parentheses.  
5  
6  
7  
8

9  
10 This is not surprising as  $D_{\text{iso}}$  from MD simulations is known to be overestimated due to  
11  
12 the low viscosity of water models (TIP3P and SPC/E) associated with FFs.<sup>69,74</sup> From the  
13  
14 non-polarizable FFs, the ff15ipq FF coupled with the SPC/E<sub>b</sub> water model shows the best  
15  
16 prediction of the relaxation parameters. This is in agreement with a recent study<sup>75</sup> and  
17  
18 highlights the fact that it has been explicitly optimized to mimic the rotational diffusion of  
19  
20 proteins.<sup>29</sup> As can be seen on Figure 3 and Table 3, the prediction of relaxation terms is  
21  
22 significantly different from what is predicted with the use of the experimental  $D_{\text{rot}}$ , except  
23  
24 for the AMOEBA FF and to a lesser extent for ff99SB-disp. For these FFs, the shape of  
25  
26 the relaxation parameters values plotted along the UIM sequence experiences a slight  
27  
28 shift compared to the relaxation parameters values when the experimental  $D_{\text{iso}}$  is included  
29  
30 in the analysis. The most striking difference occurs for the ff15ipq and C36m FFs where  
31  
32 residues located in the  $\alpha$ -helix region have a marked decrease in  $R_2$  and increase in  $R_1$   
33  
34 compared to their values predicted with the experimental  $D_{\text{iso}}$  and  $D_{\Delta}$ . This observation  
35  
36 directly recalls that the rotational anisotropy is a major component of the relaxation  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 parameters and also reflects the orientation of NH bond vectors with respect to the  
4  
5  
6 rotational diffusion tensor. This orientation will align the NH vectors in the  $\alpha$ -helix along  
7  
8  
9 the axis of fast overall rotation (see Figure S2). As a result, they will experience slower  
10  
11 rates of overall tumbling (hence higher  $R_2$ s) compared to the rest of the protein, as it has  
12  
13  
14 been demonstrated in Figure 1. In the case of ff15ipq or C36m, the situation is contrasted  
15  
16  
17 with a significant contribution of  $D_{\perp}$  that represent the axis of slower overall rotation (see  
18  
19  
20 Table 3). For these FFs, NH vectors will be affected more drastically by  $D_{\perp}$  and will  
21  
22  
23  
24  
25  
26  
27  
28 experience faster rates of overall tumbling (hence a decrease of their  $R_2$ s).  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



**Figure 3.** Experimental (black symbols) and computed NMR spin relaxation parameters (lines)  $R_1$ ,  $R_2$  and NOE for the different FFs used in this study. The global rotational diffusion  $D_{\text{iso}}$  is determined by MD while the zero order correction parameter  $\zeta$  has been fixed to 0.89.<sup>72</sup> The UIM secondary structure is sketched at the top of the relaxation parameters.

1  
2  
3  
4 On the other hand, the ff99SB-disp and AMOEBA FFs have a comparable contribution  
5  
6  
7 to  $D_{\perp}$  compared to the experimental one. As a consequence, the values of the relaxation  
8  
9  
10 parameters for the residues in the  $\alpha$ -helix region are less affected when the experimental  
11  
12  
13  $D_{\text{iso}}$  is used (Figure 3). For the residues in the flexible regions, practically no difference  
14  
15  
16 could be noticed. This is likely due to the orientation of NH vectors which coordinates  
17  
18  
19 combine components on  $D_{\parallel}$  and  $D_{\perp}$  and differ from the orientation of the NH vectors  
20  
21  
22 located in the  $\alpha$ -helix. Thus, they are less sensitive to any change of the components of  
23  
24  
25 the diffusion tensor, as can be seen on the simulated relaxation parameters (see Figure  
26  
27  
28 S1B lower row). This observation has been made also in the past and revealed that a  
29  
30  
31 marked anisotropy combined with different orientation of the NH vectors may lead to  
32  
33  
34 important deviation of the transverse relaxation rates.<sup>47</sup>  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44

## 45 CONCLUSION

46  
47  
48 Our results clearly indicate that the introduction of polarizable effects improve not only  
49  
50  
51 the modeling of fast local motion but also the global molecular reorientation delineated by  
52  
53  
54 its rotational diffusion tensor  $D_{\text{rot}}$  in case of a significant rotational anisotropy. Moreover,  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 an excellent prediction of the relaxation parameters has been reached without any  
5  
6  
7 requirement to a scaling factor used to compensate the prediction of the rotational  
8  
9  
10 diffusion. Overall, several conclusions may be drawn from our calculations: i) While  
11  
12  
13 polarization is highly anisotropic in water, the polarizable AMOEBA FF quantitatively  
14  
15  
16 predicts  $D_{\text{iso}}$  and  $D_{\Delta}$  or their components  $D_{\parallel}$  and  $D_{\perp}$ . ii) The AMOEBA FF allows the  
17  
18  
19 accurate prediction of the  $^{15}\text{N}$   $R_1$ ,  $^{15}\text{N}$   $R_2$  and heteronuclear  $^1\text{H}$ - $^{15}\text{N}$  NOE relaxation  
20  
21  
22 parameters not only in well-structured but also in more flexible regions. This being said,  
23  
24  
25 we have shown that the use of the polarizable AMOEBA FF presents a convincing  
26  
27  
28 alternative to non-polarizable FFs when dealing with a mix of well-structured and flexible  
29  
30  
31 parts. Although its high computational cost with respect to conventional FFs, we are  
32  
33  
34 convinced that the next generation of hardware development will furnish sufficient  
35  
36  
37 computational power to alleviate the problem of computational time. We think that the use  
38  
39  
40 of polarizable FF will improve our understanding of protein dynamics especially in the  
41  
42  
43 case of IDPs, multidomain proteins or in crowded environment where charge distributions  
44  
45  
46 or protein shape are constantly remodeled over time.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 ASSOCIATED CONTENT  
5  
6  
7

8       **Supporting Information.** Details regarding structure of UIM domain, rotational diffusion  
9  
10  
11 analysis, radius of gyration, correlation function analysis and figures S1, S2, S3, S4 and  
12  
13  
14  
15 S5 (PDF).  
16  
17  
18

19 AUTHOR INFORMATION  
20  
21  
22

23 **Corresponding Author**  
24  
25

26  
27 \*olivier.walker@univ-lyon1.fr  
28  
29  
30

31 **Author Contributions**  
32  
33  
34

35 The manuscript was written through contributions of all authors. All authors have given  
36  
37  
38 approval to the final version of the manuscript.  
39  
40  
41  
42

43 **Notes**  
44  
45  
46

47 The authors declare no competing financial interests.  
48  
49  
50

51 ACKNOWLEDGMENT  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3  
4 The current study was supported by a grant overseen by the French National Research  
5  
6  
7 Agency (ANR) as part of the “OH risque” programme (Metadyn, ANR-14-OHRI-0006-01).  
8  
9

10 This work was performed using HPC resources from GENCI-CINES (Grant 2019-  
11  
12  
13 A0060707607). We thank the Rovaltain Foundation and ICL for providing experimental  
14  
15  
16  
17 NMR time. We are grateful to Dr. Paul Robustelli for providing us the Amber ff99SB-disp  
18  
19  
20  
21 formatted for use in Gromacs, Prof. Odile Eisenstein for useful discussion regarding  
22  
23  
24 polarizable force fields and Dr. Po-Chia Chen for fruitful discussion regarding SpinRelax.  
25  
26  
27

## 28 ABBREVIATIONS

29  
30  
31 STAM2 (Signal Transducing Adaptator Molecule 2), UIM (Ubiquitin Interacting Motif),  
32  
33  
34 AMOEBA (Atomic Multipole Optimized Energetics for Biomolecular Application)  
35  
36  
37  
38  
39  
40  
41

## 42 REFERENCES

43  
44  
45  
46 1. Davis, D. G.; Perlman, M. E.; London, R. E., Direct Measurements of the Dissociation-  
47  
48  
49 Rate Constant for Inhibitor-Enzyme Complexes Via the T1 $\rho$  and T2 (CPMG) Methods. *J*  
50  
51  
52  
53 *Magn Reson B* **1994**, *104* (3), 266-275.  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 2. Peng, J. W., Exposing the Moving Parts of Proteins with NMR Spectroscopy. *J Phys*  
5  
6  
7 *Chem Lett* **2012**, *3* (8), 1039-1051.

8  
9  
10  
11 3. Palmer, A. G., NMR Characterization of the Dynamics of Biomacromolecules. *Chem*  
12  
13  
14 *Rev* **2004**, *104* (8), 3623-3640.

15  
16  
17  
18  
19 4. Abragam, P. A.; Abragam, A., *The Principles of Nuclear Magnetism*. Clarendon  
20  
21  
22 Press: 1961.

23  
24  
25  
26  
27 5. Peng, J. W.; Wagner, G., Mapping of the Spectral Densities of N-H Bond Motions in  
28  
29  
30 Eglin C Using Heteronuclear Relaxation Experiments. *Biochemistry* **1992**, *31* (36), 8571-  
31  
32  
33 8586.

34  
35  
36  
37  
38 6. Peng, J. W.; Wagner, G., Mapping of Spectral Density Functions Using Heteronuclear  
39  
40  
41 NMR Relaxation Measurements. *J Magn Reson* **1992**, *98* (2), 308-332.

42  
43  
44  
45  
46 7. Farrow, N. A.; Zhang, O.; Szabo, A.; Torchia, D. A.; Kay, L. E., Spectral Density  
47  
48  
49 Function Mapping Using <sup>15</sup>N Relaxation Data Exclusively. *J Biomol NMR* **1995**, *6* (2),  
50  
51  
52 153-162.

- 1  
2  
3  
4 8. Ishima, R.; Nagayama, K., Protein Backbone Dynamics Revealed by Quasi Spectral  
5  
6  
7 Density Function Analysis of Amide N-15 Nuclei. *Biochemistry* **1995**, *34* (10), 3162-3171.  
8  
9  
10  
11 9. Lipari, G.; Szabo, A., Model-Free Approach to the Interpretation of Nuclear Magnetic  
12  
13  
14 Resonance Relaxation in Macromolecules. 1. Theory and Range of Validity. *J Am Chem*  
15  
16  
17  
18 *Soc* **1982**, *104* (17), 4546-4559.  
19  
20  
21  
22 10. Lipari, G.; Szabo, A., Model-Free Approach to the Interpretation of Nuclear Magnetic  
23  
24  
25 Resonance Relaxation in Macromolecules. 2. Analysis of Experimental Results. *J Am*  
26  
27  
28  
29 *Chem Soc* **1982**, *104* (17), 4559-4570.  
30  
31  
32  
33  
34 11. Halle, B.; Wennerström, H., Interpretation of Magnetic Resonance Data from Water  
35  
36  
37 Nuclei in Heterogeneous Systems. *J Chem Phys* **1981**, *75* (4), 1928-1943.  
38  
39  
40  
41 12. Tchaicheyan, O.; Mendelman, N.; Zerbetto, M.; Meirovitch, E., Local Ordering at  
42  
43  
44 Mobile Sites in Proteins: Combining Perspectives from NMR Relaxation and Molecular  
45  
46  
47 Dynamics. *J Phys Chem B* **2019**, *123* (13), 2745-2755.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 13. Giupponi, G.; Harvey, M. J.; De Fabritiis, G., The Impact of Accelerator Processors  
5  
6  
7 for High-Throughput Molecular Modeling and Simulation. *Drug Discov Today* **2008**, *13*  
8  
9  
10 (23–24), 1052-1058.

11  
12  
13  
14  
15 14. Showalter, S. A.; Brüschweiler, R., Validation of Molecular Dynamics Simulations of  
16  
17  
18 Biomolecules Using NMR Spin Relaxation as Benchmarks: Application to the Amber99sb  
19  
20  
21 Force Field. *J. Chem. Theory Comput* **2007**, *3*(3), 961-975.

22  
23  
24  
25  
26 15. Chen, P. C.; Hologne, M.; Walker, O.; Hennig, J., Ab Initio Prediction of NMR Spin  
27  
28  
29 Relaxation Parameters from Molecular Dynamics Simulations. *J. Chem. Theory Comput*  
30  
31  
32 **2018**, *14*(2), 1009-1019.

33  
34  
35  
36  
37 16. Anderson, J. S.; Hernández, G.; LeMaster, D. M., Prediction of Bond Vector  
38  
39  
40 Autocorrelation Functions from Larmor Frequency-Selective Order Parameter Analysis of  
41  
42  
43 NMR Relaxation Data. *J. Chem. Theory Comput* **2017**, *13*(7), 3276-3289.

44  
45  
46  
47  
48 17. Prompers, J. J.; Brüschweiler, R., General Framework for Studying the Dynamics  
49  
50  
51 of Folded and Nonfolded Proteins by NMR Relaxation Spectroscopy and MD Simulation.  
52  
53  
54 *J Am Chem Soc* **2002**, *124*(16), 4522-4534.

- 1  
2  
3  
4 18. Hoffmann, F.; Xue, M.; Schäfer, L. V.; Mulder, F. A. A., Narrowing the Gap between  
5  
6  
7 Experimental and Computational Determination of Methyl Group Dynamics in Proteins.  
8  
9  
10 *Phys Chem Chem Phys* **2018**, *20* (38), 24577-24590.  
11  
12  
13  
14  
15 19. Hoffmann, F.; Mulder, F. A. A.; Schäfer, L. V., Accurate Methyl Group Dynamics in  
16  
17  
18 Protein Simulations with Amber Force Fields. *J Phys Chem B* **2018**, *122* (19), 5038-5048.  
19  
20  
21  
22  
23 20. Chen, P. C.; Hologne, M.; Walker, O., Computing the Rotational Diffusion of  
24  
25  
26 Biomolecules Via Molecular Dynamics Simulation and Quaternion Orientations. *J Phys*  
27  
28  
29 *Chem B* **2017**, *121* (8), 1812-1823.  
30  
31  
32  
33  
34 21. Ollila, O. H. S.; Heikkinen, H. A.; Iwai, H., Rotational Dynamics of Proteins from Spin  
35  
36  
37 Relaxation Times and Molecular Dynamics Simulations. *J Phys Chem B* **2018**, *122* (25),  
38  
39  
40 6559-6569.  
41  
42  
43  
44  
45 22. Polimeno, A.; Zerbetto, M., Evaluating Rotation Diffusion Properties of Molecules  
46  
47  
48 from Short Trajectories. *Phys Chem Chem Phys* **2019**, *21* (7), 3662-3668.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 23. Kämpf, K.; Izmailov, S. A.; Rabdano, S. O.; Groves, A. T.; Podkorytov, I. S.;  
5  
6  
7 Skrynnikov, N. R., What Drives <sup>15</sup>N Spin Relaxation in Disordered Proteins? Combined  
8  
9  
10 NMR/MD Study of the H4 Histone Tail. *Biophys J* **2018**, *115* (12), 2348-2367.  
11  
12

13  
14  
15 24. Salvi, N.; Abyzov, A.; Blackledge, M., Solvent-Dependent Segmental Dynamics in  
16  
17  
18 Intrinsically Disordered Proteins. *Sci Adv* **2019**, *5* (6), eaax2348.  
19  
20

21  
22  
23 25. Hall, J. B.; Fushman, D., Characterization of the Overall and Local Dynamics of a  
24  
25  
26 Protein with Intermediate Rotational Anisotropy: Differentiating between Conformational  
27  
28  
29 Exchange and Anisotropic Diffusion in the B3 Domain of Protein G. *J Biomol NMR* **2003**,  
30  
31  
32  
33 *27* (3), 261-75.  
34  
35

36  
37  
38 26. Smith, P. E.; van Gunsteren, W. F., Translational and Rotational Diffusion of  
39  
40  
41 Proteins. *J Mol Biol* **1994**, *236* (2), 629-636.  
42  
43

44  
45  
46 27. Wong, K.-B.; Daggett, V., Barstar Has a Highly Dynamic Hydrophobic Core:  
47  
48  
49 Evidence from Molecular Dynamics Simulations and Nuclear Magnetic Resonance  
50  
51  
52 Relaxation Data. *Biochemistry* **1998**, *37* (32), 11182-11192.  
53  
54

1  
2  
3  
4 28. Anderson, J. S.; LeMaster, D. M., Rotational Velocity Rescaling of Molecular  
5  
6  
7 Dynamics Trajectories for Direct Prediction of Protein NMR Relaxation. *Biophys Chem*  
8  
9  
10 **2012**, *168-169*, 28-39.

11  
12  
13  
14  
15 29. Takemura, K.; Kitao, A., Water Model Tuning for Improved Reproduction of  
16  
17  
18 Rotational Diffusion and NMR Spectral Density. *J Phys Chem B* **2012**, *116* (22), 6279-  
19  
20  
21 6287.

22  
23  
24  
25  
26 30. Robustelli, P.; Trbovic, N.; Friesner, R. A.; Palmer, A. G., Conformational Dynamics  
27  
28  
29 of the Partially Disordered Yeast Transcription Factor Gcn4. *J. Chem. Theory Comput*  
30  
31  
32 **2013**, *9*(11), 5190-5200.

33  
34  
35  
36  
37 31. Dauber-Osguthorpe, P.; Hagler, A. T., Biomolecular Force Fields: Where Have We  
38  
39  
40 Been, Where Are We Now, Where Do We Need to Go and How Do We Get There? *J*  
41  
42  
43 *Comput Aided Mol Des* **2019**, *33* (2), 133-203.

44  
45  
46  
47  
48 32. Nerenberg, P. S.; Head-Gordon, T., New Developments in Force Fields for  
49  
50  
51 Biomolecular Simulations. *Curr Opin Struct Biol* **2018**, *49*, 129-138.

1  
2  
3  
4 33. Zhang, H.; Yin, C.; Jiang, Y.; van der Spoel, D., Force Field Benchmark of Amino  
5  
6  
7 Acids: I. Hydration and Diffusion in Different Water Models. *J Chem Inf Model* **2018**, *58*  
8  
9  
10 (5), 1037-1052.

11  
12  
13  
14  
15 34. Lemkul, J. A.; Huang, J.; Roux, B.; MacKerell, A. D., An Empirical Polarizable Force  
16  
17  
18 Field Based on the Classical Drude Oscillator Model: Development History and Recent  
19  
20  
21 Applications. *Chem Rev* **2016**, *116* (9), 4983-5013.

22  
23  
24  
25  
26 35. Shi, Y.; Xia, Z.; Zhang, J.; Best, R.; Wu, C.; Ponder, J. W.; Ren, P., Polarizable  
27  
28  
29 Atomic Multipole-Based Amoeba Force Field for Proteins. *J. Chem. Theory Comput* **2013**,  
30  
31  
32  
33 *9* (9), 4046-4063.

34  
35  
36  
37 36. Jing, Z.; Liu, C.; Cheng, S. Y.; Qi, R.; Walker, B. D.; Piquemal, J.-P.; Ren, P.,  
38  
39  
40 Polarizable Force Fields for Biomolecular Simulations: Recent Advances and  
41  
42  
43 Applications. *Annu Rev Biophys* **2019**, *48* (1), 371-394.

44  
45  
46  
47  
48 37. Ouyang, J. F.; Bettens, R. P. A., When Are Many-Body Effects Significant? *J. Chem.*  
49  
50  
51  
52 *Theory Comput* **2016**, *12* (12), 5860-5867.



- 1  
2  
3  
4 38. Lin, Z.; van Gunsteren, W. F., Effects of Polarizable Solvent Models Upon the  
5  
6  
7 Relative Stability of an  $\alpha$ -Helical and a  $\beta$ -Hairpin Structure of an Alanine Decapeptide. *J.*  
8  
9  
10 *Chem. Theory Comput* **2015**, *11* (5), 1983-1986.  
11  
12  
13  
14  
15 39. Robustelli, P.; Piana, S.; Shaw, D. E., Developing a Molecular Dynamics Force Field  
16  
17  
18 for Both Folded and Disordered Protein States. *Proc Natl Acad Sci U S A* **2018**, *115* (21),  
19  
20  
21 E4758-E4766.  
22  
23  
24  
25  
26 40. Debiec, K. T.; Cerutti, D. S.; Baker, L. R.; Gronenborn, A. M.; Case, D. A.; Chong,  
27  
28  
29 L. T., Further Along the Road Less Traveled: Amber Ff15ipq, an Original Protein Force  
30  
31  
32 Field Built on a Self-Consistent Physical Model. *J. Chem. Theory Comput* **2016**, *12* (8),  
33  
34  
35  
36 3926-3947.  
37  
38  
39  
40  
41 41. Huang, J.; Rauscher, S.; Nawrocki, G.; Ran, T.; Feig, M.; de Groot, B. L.;  
42  
43  
44 Grubmuller, H.; MacKerell, A. D., Jr., Charmm36m: An Improved Force Field for Folded  
45  
46  
47 and Intrinsically Disordered Proteins. *Nat Methods* **2017**, *14* (1), 71-73.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 42. Fushman, D.; Cahill, S.; Cowburn, D., The Main-Chain Dynamics of the Dynamain  
5  
6  
7 Pleckstrin Homology (Ph) Domain in Solution: Analysis of  $^{15}\text{N}$  Relaxation with  
8  
9  
10 Monomer/Dimer Equilibration. *J Mol Biol* **1997**, *266* (1), 173-94.

11  
12  
13  
14  
15 43. Ghose, R.; Fushman, D.; Cowburn, D., Determination of the Rotational Diffusion  
16  
17  
18 Tensor of Macromolecules in Solution from NMR Relaxation Data with a Combination of  
19  
20  
21 Exact and Approximate Methods--Application to the Determination of Interdomain  
22  
23  
24 Orientation in Multidomain Proteins. *J Magn Reson* **2001**, *149* (2), 204-217.

25  
26  
27  
28  
29 44. Mueller, L. J., Tensors and Rotations in NMR. *Concept Magn Reson A* **2011**, *38A*  
30  
31  
32 (5), 221-235.

33  
34  
35  
36  
37 45. Tjandra, N.; Feller, S. E.; Pastor, R. W.; Bax, A., Rotational Diffusion Anisotropy of  
38  
39  
40 Human Ubiquitin from  $^{15}\text{N}$  NMR Relaxation. *J Am Chem Soc* **1995**, *117* (50), 12562-  
41  
42  
43 12566.

44  
45  
46  
47  
48 46. Lee, L. K.; Rance, M.; Chazin, W. J.; Palmer, A. G., Rotational Diffusion Anisotropy  
49  
50  
51 of Proteins from Simultaneous Analysis of  $^{15}\text{N}$  and  $^{13}\text{C}\alpha$  Nuclear Spin Relaxation. *J*  
52  
53  
54  
55 *Biomol NMR* **1997**, *9* (3), 287-298.

1  
2  
3  
4 47. Fushman, D.; Cowburn, D., Characterization of Inter-Domain Orientations in  
5  
6  
7 Solution Using the NMR Relaxation Approach. In *Protein NMR for the Millennium*,  
8  
9  
10 Krishna, N. R.; Berliner, L. J., Eds. Springer: 2002; pp 53-77.

11  
12  
13  
14  
15 48. Fushman, D.; Xu, R.; Cowburn, D., Direct Determination of Changes of Interdomain  
16  
17  
18 Orientation on Ligation: Use of the Orientational Dependence of  $^{15}\text{N}$  NMR Relaxation in  
19  
20  
21 Abl Sh(32). *Biochemistry* **1999**, *38* (32), 10225-30.

22  
23  
24  
25  
26 49. Lange, A.; Castañeda, C.; Hoeller, D.; Lancelin, J.-M.; Fushman, D.; Walker, O.,  
27  
28  
29 Evidence for Cooperative and Domain-Specific Binding of the Signal Transducing  
30  
31  
32 Adaptor Molecule 2 (Stam2) to Lys63-Linked Diubiquitin. *J Biol Chem* **2012**, *287* (22),  
33  
34  
35  
36 18687-18699.

37  
38  
39  
40  
41 50. Swanson, K. A.; Kang, R. S.; Stamenova, S. D.; Hicke, L.; Radhakrishnan, I.,  
42  
43  
44 Solution Structure of Vps27 Uim-Ubiquitin Complex Important for Endosomal Sorting and  
45  
46  
47 Receptor Downregulation. *EMBO J* **2003**, *22* (18), 4597-4606.

48  
49  
50  
51  
52 51. Sali, A.; Blundell, T. L., Comparative Protein Modelling by Satisfaction of Spatial  
53  
54  
55  
56 Restraints. *J Mol Biol* **1993**, *234* (3), 779-815.

1  
2  
3  
4 52. Berendsen, H. J. C.; van der Spoel, D.; van Drunen, R., Gromacs: A Message-  
5  
6  
7 Passing Parallel Molecular Dynamics Implementation. *Comput Phys Commun* **1995**, *91*  
8  
9  
10 (1), 43-56.

11  
12  
13  
14  
15 53. Harvey, M. J.; Giupponi, G.; Fabritiis, G. D., Acemd: Accelerating Biomolecular  
16  
17  
18 Dynamics in the Microsecond Time Scale. *J. Chem. Theory Comput* **2009**, *5* (6), 1632-  
19  
20  
21 1639.

22  
23  
24  
25  
26 54. Eastman, P.; Swails, J.; Chodera, J. D.; McGibbon, R. T.; Zhao, Y.; Beauchamp, K.  
27  
28  
29 A.; Wang, L.-P.; Simmonett, A. C.; Harrigan, M. P.; Stern, C. D.; et al., Openmm 7: Rapid  
30  
31  
32 Development of High Performance Algorithms for Molecular Dynamics. *PLoS Comput*  
33  
34  
35 *Biol* **2017**, *13* (7), e1005659.

36  
37  
38  
39  
40  
41 55. Harger, M.; Li, D.; Wang, Z.; Dalby, K.; Lagardère, L.; Piquemal, J.-P.; Ponder, J.;  
42  
43  
44 Ren, P., Tinker-Openmm: Absolute and Relative Alchemical Free Energies Using  
45  
46  
47 Amoeba on Gpus. *J Comput Chem* **2017**, *38* (23), 2047-2055.

48  
49  
50  
51  
52 56. Bussi, G.; Donadio, D.; Parrinello, M., Canonical Sampling through Velocity  
53  
54  
55 Rescaling. *J Chem Phys* **2007**, *126* (1), 014101-014101.

1  
2  
3  
4 57. Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J.  
5  
6  
7 R., Molecular Dynamics with Coupling to an External Bath. *J Chem Phys* **1984**, *81* (8),  
8  
9  
10 3684-3690.

11  
12  
13  
14  
15 58. Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G., A  
16  
17  
18 Smooth Particle Mesh Ewald Method. *J Chem Phys* **1995**, *103* (19), 8577-8593.  
19  
20

21  
22 59. Hess, B., P-Lincs: A Parallel Linear Constraint Solver for Molecular Simulation. *J.*  
23  
24  
25  
26 *Chem. Theory Comput* **2008**, *4* (1), 116-122.  
27  
28

29  
30 60. Miyamoto, S.; Kollman, P. A., Settle: An Analytical Version of the Shake and Rattle  
31  
32  
33  
34 Algorithm for Rigid Water Models. *J Comput Chem* **1992**, *13* (8), 952-962.  
35  
36

37  
38 61. Friedrichs, M. S.; Eastman, P.; Vaidyanathan, V.; Houston, M.; Legrand, S.; Beberg,  
39  
40  
41 A. L.; Ensign, D. L.; Bruns, C. M.; Pande, V. S., Accelerating Molecular Dynamic  
42  
43  
44  
45 Simulation on Graphics Processing Units. *J Comput Chem* **2009**, *30* (6), 864-872.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 62. Zhang, C.; Lu, C.; Jing, Z.; Wu, C.; Piquemal, J.-P.; Ponder, J. W.; Ren, P., Amoeba  
5  
6  
7 Polarizable Atomic Multipole Force Field for Nucleic Acids. *J. Chem. Theory Comput*  
8  
9  
10 **2018**, *14* (4), 2084-2108.

11  
12  
13  
14  
15 63. Simmonett, A. C.; Pickard, F. C. t.; Ponder, J. W.; Brooks, B. R., An Empirical  
16  
17  
18 Extrapolation Scheme for Efficient Treatment of Induced Dipoles. *J Chem Phys* **2016**,  
19  
20  
21  
22 *145* (16), 164101-164101.

23  
24  
25  
26 64. Woessner, D. E., Nuclear Spin Relaxation in Ellipsoids Undergoing Rotational  
27  
28  
29 Brownian Motion. *J Chem Phys* **1962**, *37* (3), 647-654.

30  
31  
32  
33  
34 65. Lange, A.; Castaneda, C.; Hoeller, D.; Lancelin, J.-M.; Fushman, D.; Walker, O.,  
35  
36  
37 Evidence for Cooperative and Domain-Specific Binding of the Signal Transducing  
38  
39  
40 Adaptor Molecule 2 (Stam2) to Lys(63)-Linked Diubiquitin. *J Biol Chem* **2012**, *287* (22),  
41  
42  
43  
44 18687-18699.

45  
46  
47  
48 66. Walker, O.; Varadan, R.; Fushman, D., Efficient and Accurate Determination of the  
49  
50  
51 Overall Rotational Diffusion Tensor of a Molecule from 15N Relaxation Data Using  
52  
53  
54  
55 Computer Program Rotdif. *J Magn Reson* **2004**, *168* (2), 336-345.

1  
2  
3  
4 67. Berlin, K.; Longhini, A.; Dayie, T. K.; Fushman, D., Deriving Quantitative Dynamics  
5  
6  
7 Information for Proteins and Rnas Using Rotdif with a Graphical User Interface. *J Biomol*  
8  
9  
10 *NMR* **2013**, *57*(4), 333-52.

11  
12  
13  
14  
15 68. Knapp, B.; Ospina, L.; Deane, C. M., Avoiding False Positive Conclusions in  
16  
17  
18 Molecular Simulation: The Importance of Replicas. *J. Chem. Theory Comput* **2018**, *14*  
19  
20  
21 (12), 6127-6138.

22  
23  
24  
25  
26 69. Wong, V.; Case, D. A., Evaluating Rotational Diffusion from Protein MD Simulations.  
27  
28  
29 *J Phys Chem B* **2008**, *112*(19), 6013-6024.

30  
31  
32  
33  
34 70. Linke, M.; Köfinger, J.; Hummer, G., Rotational Diffusion Depends on Box Size in  
35  
36  
37 Molecular Dynamics Simulations. *J Phys Chem Lett* **2018**, *9*(11), 2874-2878.

38  
39  
40  
41 71. Yeh, I.-C.; Hummer, G., System-Size Dependence of Diffusion Coefficients and  
42  
43  
44 Viscosities from Molecular Dynamics Simulations with Periodic Boundary Conditions. *J*  
45  
46  
47 *Phys Chem B* **2004**, *108*(40), 15873-15879.

1  
2  
3  
4 72. Case, D. A., Calculations of NMR Dipolar Coupling Strengths in Model Peptides. *J*  
5  
6  
7 *Biomol NMR* **1999**, *15* (2), 95-102.

8  
9  
10  
11 73. Fushman, D.; Varadan, R.; Assfalg, M.; Walker, O., Determining Domain Orientation  
12  
13  
14 in Macromolecules by Using Spin-Relaxation and Residual Dipolar Coupling  
15  
16  
17  
18 Measurements. *Prog Nucl Magn Reson Spectrosc* **2004**, *44* (3-4), 189-214.

19  
20  
21  
22 74. González, M. A.; Abascal, J. L. F., The Shear Viscosity of Rigid Water Models. *J*  
23  
24  
25  
26 *Chem Phys* **2010**, *132* (9), 096101.

27  
28  
29  
30 75. Zapletal, V.; Mládek, A.; Melková, K.; Louša, P.; Nomilner, E.; Jaseňáková, Z.;  
31  
32  
33  
34 Kubáň, V.; Makovická, M.; Laníková, A.; et al., Choice of Force Field for Proteins  
35  
36  
37 Containing Structured and Intrinsically Disordered Regions. *Biophys J* **2020**, *118* (7),  
38  
39  
40  
41 1621-1633.



TOC Graphic.

