Well-Balanced Force Field ff03CMAP for Folded and Disordered Proteins

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ABSTRACT: Molecular dynamics simulation as an important complement of experiment is widely used to study protein structures and functions. However, previous studies indicate that the current force fields cannot, simultaneously, provide accurate descriptions of folded proteins and intrinsically disordered proteins (IDPs). Therefore, a correction maps (CMAP)-optimized force field based on the Amber ff03 force field (termed ff03CMAP herein) was developed for a balanced sampling of folded proteins and IDPs. Extensive validations of short peptides, folded proteins, disordered proteins, and fast-folding proteins show that simulated chemical shifts, J-coupling constants, order parameters, and residual dipolar couplings (RDCs) with the ff03CMAP force field are in very good agreement with nuclear magnetic resonance measurements and are more accurate than other ff03-series force fields. The influence of solvent models was also investigated. It was found that the combination of ff03CMAP/TIP4P-Ew is suitable for folded proteins, and that of ff03CMAP/TIP4P-D is better for disordered proteins. These findings confirm that the newly developed force field ff03CMAP can improve the balance of conformer sampling between folded proteins and IDPs.

INTRODUCTION

Both folded proteins and disordered proteins are related to important biological processes. Folded proteins are easier to study because they are ordered and stable. But disordered proteins also need exploring. In eukaryotes, more than 30% of proteins contain disordered regions with more than 50 consecutive residues. Proteins with disordered regions or overall intrinsically disordered proteins (IDPs) have been proved to have important biological functions, such as molecular recognition, molecular assembly, protein modification, and so on. Furthermore, IDPs are associated with many human diseases, such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, cancer, and cardiovascular disease, to name a few. IDPs are more flexible and unstable with little secondary structures than structured or ordered proteins. “Intrinsically disordered” implies a sequence-dependent nature in IDPs that tend to lack of ordered structures. Many experimental methods have been utilized to study IDPs, such as electron paramagnetic resonance, X-ray diffraction, nuclear magnetic resonance (NMR), and small-angle X-ray scattering (SAXS).

Because of their important biological functions, IDPs have become common topics in molecular dynamics studies in recent years. Standard protein force fields were developed based on lots of parameters from folded proteins, and with the development of techniques, significant discrepancies are shown in the comparison between the MD simulation and experimental measurements. Due to limitation for none of the tested force fields such as f99SB/TIP3P, C36m, C22*, TIP3P, f03ws, and f99SB/TIP4P-D, simultaneously provided accurate descriptions of folded proteins and disordered proteins. A set of special-purpose force fields has been developed for simulating IDPs, such as ff99IDP, ff14IDPs, ff14IDPSFF, ff03ws, RSFF2, a99SB-disp, CHARMM-M36IDPSFF, and so on. In addition, the D. E. Shaw group also modified the dispersion interaction of the TIP4P water model (TIP4P-D) to improve the quality of IDP simulations. However, it remains elusive to reach a good balance between ordered and disordered states with either standard or special-purpose force fields.

ff03 is a new-generation Amber force field that has become widely used in biomolecular simulation studies. Based on ff03, the best group modified backbone dihedral potentials in the context of the TIP4P/2005 water model. Their efforts lead to three ff03 variants: ff03*, ff03w, and ff03ws. These modifications were shown to partially improve the performance of conformer sampling of IDPs and folded proteins. The
Figure 1. Test system from short peptides to proteins. Five representative folded proteins such as third immunoglobulin binding domain of protein G (GB), α/β, free bovine pancreatic trypsin inhibitor (BPTI, α/β), cold-shock protein from the hyperthermophilic bacterium Thermotoga maritima (CspTm, α/β), and ubiquitin of human (α/β) and chicken brain α spectrin repeat 17 (SPR17, α/β). Nine typical disordered protein, including 19 length peptide of hen egg-white lysozyme (HEWL19), phosphorylated SRSF1 (RS), HIV-1 Rev ARM peptide (HIVRev), 40 length amyloid-β-peptides (AB40), 42 length amyloid-β-peptides (AB42), activation domain of the nuclear hormone receptor coactivator (ACTR), an aspartic proteinase inhibitor for Saccharomyces cerevisiae (IA3), p53 N-terminal transactivation domain (p53N), and tau protein fragment (Tauf4). Three fast-folding proteins including 15-residue helix-forming peptide Ac-(AAQAA)3-NH2 (AAQAA3), β-hairpin B1 domain of protein G (GB1), and Chignolin, a 10 residue-folded peptide designed by segment statistics (CLN025).
where \(P_i^{DB}\) is the population of the \(i\)th grid in the database benchmark and \(P_i^{MD}\) is the population of the \(i\)th grid in the MD simulation.

Quantification in the Evaluation for Force Fields. To quantitatively compare different \(ff03\) variants for folded proteins and disordered proteins with experimental measurements, we utilized the normalized force-field score.\(^9\) For folded proteins, eq 2 was used to calculate the average normalized RMSD from each class of the experimental data as follows

\[
\text{folded protein } FF_{\text{score}} = \frac{1}{N} \sum_{i=1}^{N} \frac{FF_{\text{rmsd}}}{\text{rmsd}_{\text{norm}}}
\]

where \(N\) is the number of classes of experimental measurements, \(FF_{\text{rmsd}}\) is the RMSD of the \(i\)th class for simulated and experimental values, and \(\text{rmsd}_{\text{norm}}\) is the lowest RMSD of \(i\)th class in all force fields. According to this metric, \(FF_{\text{score}}\) is always greater than or equal to 1, and 1 is the best score theoretically, which means that this force field perfectly reproduces the experimental data. For the disordered protein, we divided the experimental measurements into two groups because there are fewer experimental measurements than the folded proteins, for which there are chemical shifts and other NMR measurements. If the experimental data for both chemical shifts and NMR measurements are available, \(FF_{\text{score}}\) is calculated with eq 3.

\[
\text{disordered protein } FF_{\text{score}} = \frac{\text{CS}_{\text{score}} + \text{NMR}_{\text{score}}}{2}
\]

and if there is only a chemical shift, the \(FF_{\text{score}}\) is calculated with eq 4.

\[
\text{disordered protein } FF_{\text{score}} = \frac{\text{CS}_{\text{score}}}{2}
\]

where \(\text{CS}_{\text{score}}\) and \(\text{NMR}_{\text{score}}\) are calculated the same as the score of class in folded protein \(FF_{\text{score}}\).

Calculation of Experimental Observables. Backbone chemical shifts were calculated by SHIFTX2 for \(C_{\alpha}, C_{\beta}, C, N, H_{\alpha}\), and \(HN\) atom types.\(^{53}\) Backbone scalar coupling constants were calculated using published Karplus relations for \(3J_{HNH_{\alpha}}\), \(3J_{HNC}\), \(3J_{NC}\), \(3J_{CC}\), \(3J_{HNC_{\alpha}}\), \(3J_{HNC_{\beta}}\), \(1J_{CN_{\alpha}}\), \(1J_{CN_{\beta}}\), and \(2J_{CC_{\gamma}}\) and side-chain scalar coupling constants with Karplus relations for \(3J_{CC_{\gamma}}\) and \(3J_{NC_{\gamma}}\).\(^{63}\) Backbone residual dipolar couplings (RDCs) were calculated using PALES with a local alignment window of 15 residues.\(^{64,65}\) Backbone amide and the side-chain methyl axis \(S^2\) order parameters were calculated with the direct method described in Trbovic et al.\(^{66}\) Small-angle X-ray scattering (SAXS) curves were calculated using the FoXS package.\(^{67}\) \(C_{\alpha}\) RMSD and radius of gyration (\(R_g\)) were calculated using CPPTRAJ in AmberTools.\(^{40}\) Conformational clustering was performed with the kClust program in the MMTSB tool.\(^{68}\) MDTraj, a python package, was also used for miscellaneous calculations.\(^{69}\) The PyMOL molecular visualization system was used to show three-dimensional structures for all proteins.\(^{70}\) All experimental measurements are listed in Table S2.

Figure 2. \(FF\) scores for short peptide, disordered proteins, and folded proteins. Average scores are also shown for folded proteins and disordered proteins. Fifteen tested systems included 1 short peptide, 5 folded proteins, and 9 disordered proteins, which are sorted by the residue length in each category.
addition, an obvious energy barrier exists between the handed helix distribution, except for MET, GLY, and LEU. In the benchmark database, we found that there is almost no left-optimization, the lowest RMSp is less than 0.064%, as shown among 20 amino acids. In contrast, after 10 cycles of TIP3P more accurate sampling of the conformers for short peptide, IDPs, and fold proteins are shown in Figure 2.

Table 2. FF Scores of Five Folded Proteins for Six ff03-Series Force Fields

<table>
<thead>
<tr>
<th>protein</th>
<th>ff03/TIP3P</th>
<th>ff03w/TIP3P</th>
<th>ff03w/TIP4P2005</th>
<th>ff03CMAP/TIP4P-Ew</th>
<th>ff03CMAP/TIP4P-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB3</td>
<td>1.185</td>
<td>1.212</td>
<td>1.246</td>
<td>1.209</td>
<td>1.001</td>
</tr>
<tr>
<td>BPTI</td>
<td>1.078</td>
<td>1.241</td>
<td>1.088</td>
<td>1.171</td>
<td>1.173</td>
</tr>
<tr>
<td>CspTm</td>
<td>1.376</td>
<td>1.674</td>
<td>1.718</td>
<td>1.663</td>
<td>1.000</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>1.231</td>
<td>1.506</td>
<td>1.249</td>
<td>1.769</td>
<td>1.003</td>
</tr>
<tr>
<td>SPR17</td>
<td>1.026</td>
<td>1.041</td>
<td>1.052</td>
<td>1.219</td>
<td>1.019</td>
</tr>
</tbody>
</table>

Chemical shifts are in ppm, J-coupling constants are in Hz and the scores are unitless.

## RESULTS AND DISCUSSION

### CMAP Optimization

Ten cycles of CMAP optimization were performed for each amino acid. In the first cycle (CMAP0), the initial 0/0 distribution was obtained from the standard ff03 force field, where the lowest RMSp is 0.234% among 20 amino acids. In contrast, after 10 cycles of optimization, the lowest RMSp is less than 0.064%, as shown in Figure S2. Comparison of the distributions of CMAP0 and the benchmark database, we found that there is almost no left-handed helix distribution, except for MET, GLY, and LEU. In addition, an obvious energy barrier exists between the β-sheet region and the α-helix region, hence it would be difficult to sample both types of structures. After optimization, these limitations are no longer present. The parameters for the best RMSp for each amino acid were selected as the final CMAP values. These parameters and structural factors were integrated with the standard ff03 force field to generate the new force field ff03CMAP.

### Evaluation of ff03-Series Force Fields

We assessed the performance of ff03-series force fields in reproducing the experimental data. The same conditions were used in all MD simulations among all tested force fields. The FF scores for short peptides, IDPs, and fold proteins are shown in Figure 2 and specific values are listed in Supporting Information Table S3. Figure S2 suggests that the combination of ff03CMAP/TIP4P-D agrees the best with experiments for short peptide and IDP. In addition, the combination of ff03CMAP/TIP4P-Ew leads to the best agreement with experiment for folded proteins. In summary, the use of ff03CMAP can yield more accurate sampling of the conformers for short peptide, IDP, and folded proteins.

### Short Peptide Ala5

Table 1 shows the RMSd’s of secondary chemical shifts, J-coupling constants, and force-field score for Ala5. There are 6 types of secondary chemical shifts and 7 types of J-coupling constants. For the CS score, the performance of ff03CMAP/TIP4P-Ew is much better than all other force fields. However, the performance of ff03*2/TIP3P is the best for the NMR score. If we combine CS and NMR scores (i.e., overall FF score), ff03-driven force fields are significantly improved over the origin ff03 and the ff03CMAP/TIP4P-Ew is the best. The detail RMSd’s of secondary chemical shifts and J-coupling constants are shown in Figures S3 and S4.

### Folded Proteins

To evaluate the stability of folded proteins when modeled with ff03CMAP, five representative folded proteins were simulated: GB3 (α), BPTI (α/β), CspTm (all-β), ubiquitin (α/β), and SPR17 (all-α). The initial structures are extracted from PDB and the simulation time is 1 μs for each system.

Table 2 shows the FF scores of the five tested proteins for all ff03-series force fields. It is obvious that the FF score for the combination of ff03CMAP/TIP4P-Ew is the best among all tested force fields and the value close to 1, except for BPTI. This suggests that ff03CMAP/TIP4P-Ew indeed can be used to simulate folded proteins, and we also found that the original ff03 force field performs better than other revised ff03 force fields. It is no surprise that the performance of ff03CMAP/TIP4P-D is a little worse than that of ff03CMAP/TIP4P-Ew because the TIP4P-D water model would destabilize the folded states of proteins as reported.15 The details of the FF score composition for five proteins are shown in Tables S4–S8.

To quantify the fluctuation in simulations, Cα and PDs of three folded proteins are shown in Figure 3. For CspTm and...
ubiquitin, the RMSDs in the \textit{ff}03CMAP/TIP4PEw simulations are small and stable, which is consistent with the FF score. However, the RMSD’s for SPR17 rise over 4 Å after 700 ns in the \textit{ff}03CMAP/TIP4PEw simulation. The RMSDs of \textit{ff}03ws for three folded proteins fluctuate quite significantly, implying less stable folded states for the tested proteins.

More detailed analyses were conducted for ubiquitin to compare the performances of these force fields. Secondary chemical shifts and backbone scalar coupling constants of ubiquitin (Figures S10 and S11) suggest that the \textit{ff}03CMAP/TIP4PEw simulation agrees the best with the experimental data. The same can be said for the side-chain scalar coupling constants as shown in Tables S18 and S19. RDCs of backbone N–HN, Ca–Ha, Ca–C, C–N, and C–HN were also calculated as shown in Figure S12. Similar to chemical shifts and scalar coupling constants, the performance of the \textit{ff}03CMAP/TIP4PEw simulation also agrees among the best, along with the \textit{ff}03 simulation, while the \textit{ff}03ws simulation agrees the worse. The order parameters for backbone amide and side-chain methyl groups are shown in Figure 4 and Table S20, respectively. The order parameters of the loop regions in the \textit{ff}03CMAP/TIP4PD and \textit{ff}03ws simulation are much lower than those from other force fields, which suggest that these two force fields overestimate disordered characters for this system, especially on some loop regions. Except for backbone order parameters, \textit{ff}03CMAP/TIP4PEw exhibited similar behavior to experiment for side-chain order parameters (Table S20).

The RMSDs of the secondary chemical shift, J-coupling, order parameters, and RDCs are gathered in Table 3. The summary indicates that \textit{ff}03CMAP/TIP4PEw performs excellently in reproducing all available experimental measurements, and its FF score is very close to 1. However, the FF score of \textit{ff}03CMAP/TIP4PD is more than 1.5, which suggests that the TIP4P-D water model is unsuitable for the simulation of folded proteins.

To further evaluate the stability of \textit{ff}03CMAP for folded proteins, the dominant conformers of ubiquitin from six \textit{ff}03-series force fields. (A) CspTm, (B) ubiquitin, and (C) SPR17.
series force fields are retrieved and shown in Figure 5. It was found that the top three clusters in the ff03 simulation occupy 100.00% of the snapshots. All of the conformers include a high percentage of helical structures. Top 5 clusters in the ff03* simulation also occupy 100.00% of the snapshots with partially nonhelical structures. In the ff03w simulation, only 2 clusters were found, and the conformers are highly structured. In the ff03w simulation, the top 8 clusters only occupy 78.70% of the snapshots. In the ff03CMAP/TIP4Pew simulation, there is only one cluster and 4 clusters in the ff03CMAP/TIP4PD simulation. Additional conformation clustering was also conducted for the GB3, BPTI, CspTm, and SPR17 simulations (Supporting Information Figures S27−S30). These conformer clusters indicate that ff03CMAP/TIP4Pew, ff03, and ff03w may be the better ff03 choices for folded protein MD simulation.

**Intrinsically Disordered Proteins.** We tested 9 typical disordered proteins with 19−124 residues. The FF scores of Table 3. RMSDs of Secondary Chemical Shifts, J-Coupling Constants, RDCs, S² Parameters and FF Scores of Ubiquitin for Six ff03-Series Force Fields

<table>
<thead>
<tr>
<th></th>
<th>ff03/TIP3P</th>
<th>ff03*/TIP3P</th>
<th>ff03w/TIP4P2005</th>
<th>ff03CMAP/TIP4Pew</th>
<th>ff03CMAP/TIP4PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cα</td>
<td>0.481</td>
<td>0.716</td>
<td>0.507</td>
<td>0.723</td>
<td>0.485</td>
</tr>
<tr>
<td>Cβ</td>
<td>0.816</td>
<td>0.885</td>
<td>0.825</td>
<td>1.069</td>
<td>0.770</td>
</tr>
<tr>
<td>C</td>
<td>0.607</td>
<td>0.702</td>
<td>0.611</td>
<td>0.789</td>
<td>0.609</td>
</tr>
<tr>
<td>N</td>
<td>2.050</td>
<td>2.450</td>
<td>2.188</td>
<td>2.625</td>
<td>2.142</td>
</tr>
<tr>
<td>HA</td>
<td>0.145</td>
<td>0.185</td>
<td>0.141</td>
<td>0.234</td>
<td>0.106</td>
</tr>
<tr>
<td>HDN</td>
<td>0.329</td>
<td>0.349</td>
<td>0.326</td>
<td>0.407</td>
<td>0.298</td>
</tr>
<tr>
<td>3JHNHα</td>
<td>1.341</td>
<td>1.487</td>
<td>1.484</td>
<td>1.597</td>
<td>1.196</td>
</tr>
<tr>
<td>3JHNC</td>
<td>0.684</td>
<td>0.725</td>
<td>0.623</td>
<td>0.986</td>
<td>0.410</td>
</tr>
<tr>
<td>2JCN</td>
<td>0.643</td>
<td>0.734</td>
<td>0.671</td>
<td>0.701</td>
<td>0.633</td>
</tr>
<tr>
<td>3JHDC</td>
<td>0.681</td>
<td>0.734</td>
<td>0.701</td>
<td>0.852</td>
<td>0.564</td>
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<tr>
<td>2JHDC</td>
<td>0.465</td>
<td>0.567</td>
<td>0.494</td>
<td>0.588</td>
<td>0.415</td>
</tr>
<tr>
<td>2JHDN</td>
<td>2.669</td>
<td>2.717</td>
<td>2.628</td>
<td>3.030</td>
<td>2.225</td>
</tr>
<tr>
<td>2JHNC</td>
<td>0.984</td>
<td>1.056</td>
<td>0.977</td>
<td>1.229</td>
<td>0.856</td>
</tr>
<tr>
<td>3JHNC</td>
<td>0.464</td>
<td>0.555</td>
<td>0.437</td>
<td>0.682</td>
<td>0.361</td>
</tr>
<tr>
<td>3JHNCβ</td>
<td>0.650</td>
<td>0.973</td>
<td>0.717</td>
<td>0.936</td>
<td>0.419</td>
</tr>
<tr>
<td>3JHDC</td>
<td>0.490</td>
<td>0.592</td>
<td>0.501</td>
<td>0.557</td>
<td>0.428</td>
</tr>
<tr>
<td>Sα2</td>
<td>0.074</td>
<td>0.104</td>
<td>0.081</td>
<td>0.149</td>
<td>0.069</td>
</tr>
<tr>
<td>Sαw²</td>
<td>0.279</td>
<td>0.260</td>
<td>0.247</td>
<td>0.289</td>
<td>0.174</td>
</tr>
<tr>
<td>RDC</td>
<td>0.174</td>
<td>0.249</td>
<td>0.187</td>
<td>0.325</td>
<td>0.166</td>
</tr>
<tr>
<td>CSα</td>
<td>1.092</td>
<td>1.321</td>
<td>1.107</td>
<td>1.512</td>
<td>1.012</td>
</tr>
<tr>
<td>backbone 3J</td>
<td>1.225</td>
<td>1.359</td>
<td>1.228</td>
<td>1.562</td>
<td>1.004</td>
</tr>
<tr>
<td>side-chain 3J</td>
<td>1.348</td>
<td>1.853</td>
<td>1.440</td>
<td>1.768</td>
<td>1.000</td>
</tr>
<tr>
<td>backbone S²</td>
<td>1.070</td>
<td>1.510</td>
<td>1.175</td>
<td>2.156</td>
<td>1.000</td>
</tr>
<tr>
<td>side-chain S²</td>
<td>1.603</td>
<td>1.493</td>
<td>1.418</td>
<td>1.657</td>
<td>1.000</td>
</tr>
<tr>
<td>backbone RDC</td>
<td>1.048</td>
<td>1.500</td>
<td>1.127</td>
<td>1.958</td>
<td>1.000</td>
</tr>
<tr>
<td>FFα</td>
<td>1.231</td>
<td>1.506</td>
<td>1.249</td>
<td>1.769</td>
<td>1.003</td>
</tr>
</tbody>
</table>

Chemical shifts are in ppm, J-coupling constants and RDC are in Hz, and the scores and S² parameters are unitless.

**Figure 5.** Conformation clustering of simulation for ubiquitin. The figure shows the top eight clusters at most with dominant conformations and percentage for ff03 (A), ff03* (B), ff03w (C), ff03CMAP/TIP4Pew (D), ff03CMAP/TIP4PD (E), and ff03ws (F).
the six ff03-series force fields are listed in Table 4. Except for HEWL19 and HIVRev, the FF scores of ff03CMAP/TIP4P-D simulations are the lowest, and most of them are very close to 1, indicating very good agreement with the experiment. Although the FF scores of ff03CMAP/TIP4P-D for HEWL19 and HIVRev are not the best, the differences with the best performing force fields are not significant. This suggests that ff03CMAP/TIP4P-D can reproduce good conformers of tested IDPs. The details of the FF score composition for each tested system are shown in Tables S9–S17.

It is interesting to note that all ff03 revisions improve over the original ff03 in IDP simulations, as they are all designed to reproduce the properties of IDPs. As expected, the CMAP method can provide accurate descriptions of IDPs as in previous developments.1–13,16,72 In addition, the TIP4P-D water model is demonstrated again to be suitable for IDP simulations.73 To understand the influence of solvent models, three IDPs were simulated with ff03/TIP4P-D (Tables S12, S14, and S15). The results show that the TIP4P-D water model indeed partly improves the performance of the tested IDP conformers. However, the results are still much worse than those of ff03CMAP/TIP4P-D, suggesting that the CMAP improvement in ff03CMAP/TIP4P-D simulations plays a key role in reproducing the IDP conformers.

To further illustrate the properties of ff03CMAP force fields, we calculated the average RMSDs for different experimental measurements of IDPs (Table 5). We found that the performance of ff03CMAP/TIP4P-D is the best for all experimental observables, and ff03CMAP/TIP4P-Ew also performs reasonably well. It is noticeable that ff03CMAP significantly improved the quality of simulated Ca and N secondary chemical shifts and JHNH, scalar coupling constant, which are closely related to backbone dihedrals. It is clear that the CMAP method can be used to correct the dihedral distributions, and the TIP4P-D water model further refines the interactions between protein and water, leading to excellent observed performance in the ff03CMAP/TIP4P-Ew simulations for IDPs.

For IDP simulations, underestimation of Rg is a common limitation for generic protein force fields.9,18 We calculated Rg distributions for all tested IDPs (Figure 6). The four force fields with four-site water models can sample a wider range of Rg distributions and larger Rg mean values, especially for ff03CMAP/TIP4P-D and ff03w. Conformers are more compact in force fields in the TIP3P water model such as ff03 and ff03*. We compared the experimental Rg and simulated Rg of three IDPs. The analysis indicates that the ff03CMAP force field and ff03w overestimate the Rg of Aβ40 and RS. The average Rg of ff03CMAP/TIP4P-Ew is also very small and close to the experimental value. For ACTR, only the Rg in the ff03CMAP/TIP4P-D simulation is located within the experimental range and other force fields significantly underestimate it.

We also calculated residual helicity for ACTR, whose results are shown in Figure 7. The figure indicates that ff03CMAP/TIP4P-D shows the best agreement with the experimental data among these force fields.

Besides the above overall assessments, we next use a classical example of IDPs, RS, in the following discussion to illustrate the performance of these force fields. To evaluate the backbone and side-chain sampling for RS, we compared secondary chemical shifts and scalar coupling constants. Figure 8 shows the secondary chemical shifts and backbone scalar coupling constants for six ff03-series force fields. Tables S21 and S22 list detailed data used in analysis. The chemical shifts and scalar coupling constants calculated from the new revised ff03 force fields are much closer to the experimental data than those from the original ff03 force field, and the RMSDs between the simulated and experimental values from ff03CMAP/TIP4P-Ew and ff03CMAP/TIP4P-D combinations are smaller than those from the other ff03-series force fields (Table 4). We also calculated the backbone N–HN, Ca–Hα, and Ca–C RDCs.
with a local alignment window of 15 residues (Figure 9),
whose performance is similar to that of chemical shifts and coupling constants, with both $ff03$CMAP/TIP4Pew and $ff03$CMAP/TIP4PD giving a lower $Q$-factor of RDCs (calculated by PALES64) with smaller standard deviations than the other tested force fields.

Next, FF scores were used to compare the performance of all force fields (listed in Table 6) in RS simulations. The RMSDs of the original $ff03$ force field are the largest and the FF score is larger than 4. The $ff03$CMAP/TIP4PD combination gives the best agreement with all experimental measurements and the FF scores are around 1.1.

Finally, we computed the ensemble-averaged SAXS curves for RS and fitted with the experimental curve (Figure 10). The $\chi^2$ value was used to evaluate the quality of the fitted result to the given experimental SAXS profile as shown in the literature.74 Our analysis shows that the $\chi^2$ of $ff03$CMAP/TIP4Pew is the smallest among 6 tested $ff03$-series force fields. This suggests that $ff03$CMAP/TIP4Pew can reproduce the SAXS properties for RS, while the $ff03$CMAP/TIP4PD combination leads to conformers that are too expansive.

To further illustrate the conformer sampling efficiency, kClust was used to cluster conformers according to $\phi$ angle and Ca RMSD. Representative conformers and their occupations are shown in Figures S31−S39. The results indicate that both $ff03$CMAP and $ff03ws$ can sample more flexible and diverse disordered conformers, while the representative conformers in the $ff03$ simulation contain several short helices with tight packing. The convergence of conformer sampling is another important issue for IDP simulations. We used the biphasic decay model to evaluate the convergence time scales for IDP simulations. It is interesting to note that $ff03$CMAP simulations have smaller

Figure 6. $R_g$ distribution of the simulation and experimental data for disordered proteins. Simulated and experimental $R_g$ distribution for (A) Aβ40, (B) RS, and (C) ACTR, and simulated $R_g$ distribution for (D) HEWL19, (E) HIVRev, (F) Aβ42, (G) 1A3, (H) pS3N, and (I) tauF4. Mean $R_g$ is shown in dash lines for $ff03$ (red), $ff03^*$ (brown), $ff03ws$ (light green), $ff03ws$ (cyan), $ff03$CMAP (blue), $ff03$CMAP/T4D (violet), and $ff03$/T4D (mauve). Experimental values are displayed as black solid lines. The black dash lines mean the standard error of experimental values.

Figure 7. Helical propensities observed in simulations of ACTR. Experimental values are displayed as black lines predicted from experimental NMR chemical shifts using the program $\delta2d$.3

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Ab Initio Folding of Fast-Folding Proteins. We performed REMD for three typical fast-folding proteins, such as 16-residue two β-sheets GB1, small β-hairpin-forming protein CLN025, and helical 15-mer AAQAA3. The decay half times, which suggests that ff03CMAP simulations converge earlier than other ff03-series simulations.

Figure 8. Secondary chemical shifts and backbone scalar coupling constants of simulation and experimental data for RS. Simulated and experimental secondary chemical shifts for (A) Cα and (B) C, backbone scalar coupling constants for (C) J_{HNHα}, (D) J_{CCγ} and (E) J_{HCαC}.

Table 6. RMSD of Secondary Chemical Shifts, J-Coupling Constants, RDC and FF Score of RS for Six ff03-Series Force Fields

<table>
<thead>
<tr>
<th></th>
<th>ff03/TIP3P</th>
<th>ff03*/TIP3P</th>
<th>ff03w/TIP4P2005</th>
<th>ff03ws/TIP4P2005</th>
<th>ff03CMAP/TIP4PEw</th>
<th>ff03CMAP/TIP4PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.813</td>
<td>0.502</td>
<td>0.484</td>
<td>0.385</td>
<td>0.239</td>
<td>0.193</td>
</tr>
<tr>
<td>C</td>
<td>1.199</td>
<td>0.333</td>
<td>0.296</td>
<td>0.307</td>
<td>0.354</td>
<td>0.373</td>
</tr>
<tr>
<td>J_{HNHα}</td>
<td>1.777</td>
<td>0.987</td>
<td>0.974</td>
<td>0.981</td>
<td>0.407</td>
<td>0.491</td>
</tr>
<tr>
<td>J_{CCγ}</td>
<td>1.625</td>
<td>1.407</td>
<td>1.420</td>
<td>1.438</td>
<td>1.084</td>
<td>1.195</td>
</tr>
<tr>
<td>J_{HCαC}</td>
<td>4.190</td>
<td>2.462</td>
<td>2.511</td>
<td>2.395</td>
<td>1.774</td>
<td>1.824</td>
</tr>
<tr>
<td>J_{HCαC}</td>
<td>0.302</td>
<td>0.314</td>
<td>0.271</td>
<td>0.278</td>
<td>0.330</td>
<td>0.311</td>
</tr>
<tr>
<td>RDC</td>
<td>1.425</td>
<td>0.908</td>
<td>0.939</td>
<td>0.841</td>
<td>0.788</td>
<td>0.716</td>
</tr>
<tr>
<td>CS_{norm}</td>
<td>6.720</td>
<td>1.862</td>
<td>1.753</td>
<td>1.514</td>
<td>1.217</td>
<td>1.128</td>
</tr>
<tr>
<td>NMRscore</td>
<td>2.186</td>
<td>1.451</td>
<td>1.448</td>
<td>1.394</td>
<td>1.063</td>
<td>1.081</td>
</tr>
<tr>
<td>FFscore</td>
<td>4.453</td>
<td>1.657</td>
<td>1.600</td>
<td>1.454</td>
<td>1.140</td>
<td>1.105</td>
</tr>
</tbody>
</table>

Chemical shifts are in ppm, J-coupling constants, and RDCs are in Hz.
melting curves in the ff03CMAP/TIP4PEw simulations are shown in Figure 11. The melting curves show that GB1 and CLN025 can be ab initio folded when modeled with ff03CMAP/TIP4PEw. However, a few folded structures were observed in the REMD simulation of AAQAA3. To study whether the ab initio folding of helical structures can be improved by modifying CMAP parameters, we updated a new set of CMAP parameters by only decreasing the parameters in the $\alpha_h$ region with the revised force field termed as ff03CMAP2. Our REMD simulation shows that ff03CMAP2/TIP4PEw performs significantly better in helix folding. In the meantime, ff03CMAP2/TIP4PEw can maintain almost the same melting curves for sheet and hairpin fast-folding proteins. For IDP systems, ff03CMAP2/TIP4PEw performs slightly worse compared with the previous version but still better than other ff03-driven force fields.

**CONCLUSIONS**

The backbone dihedral term for all 20 amino acids was optimized to improve the performance of the current force field. TIP4P-Ew and TIP4P-D are combined with the newly developed force field ff03CMAP to simulate different types of proteins. Extensive tests of the typical short peptide, folded proteins, disordered proteins, and fast-folding proteins show that the simulated chemical shifts, $J$-coupling, order parameters, and RDC with the ff03CMAP force fields are in quantitative agreement with those from the NMR experiment and are more accurate than other ff03-series force fields. The influences of solvent models were also investigated. The results indicate that ff03CMAP/TIP4PEw for folded proteins and ff03CMAP combined with TIP4P-D was suitable for disordered proteins (ff03CMAP/TIP4PEw also shows good performance in IDPs). Therefore, these findings confirm that the newly developed force field ff03CMAP can improve the balance and efficiency of conformer sampling between intrinsically folded proteins and disordered proteins. Although the ff03CMAP force field has the limitation of folding helix structures, this can be improved by adjusting CMAP parameters, which is the ff03CMAP2.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jctc.9b00623.

Half-time convergence and definition of AAQAA3 helicity are listed in the supplementary file; simulation conditions for all protein or peptide systems tested in this research (Table S1); experimental measurements used in this work (Table S2); the FF score of six ff03-series force fields for 15 tested systems (Table S3); detailed comparison between the simulation and experimental data for each force field for each system we tested (Tables S4–S17); side-chain calculations (coupling constants and order parameters) of ubiquitin and RS (Tables S18–S22); average half-time of IDP simulation for six ff03-series force fields (Table S23); count of amino acids in coil database (Figure S1); RMSp between simulation and benchmark for CMAP optimization of 20 amino acids (Figure S2); the detailed information of secondary chemical shifts, coupling constants, and RDC for each force field for each system we tested (Figures S27–S39); and the biphasic exponential decay model fitting for disordered proteins (Figure S40) (PDF).

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**Notes**

The authors declare no competing financial interest.

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