

1 Improving Martini 3 for disordered 2 and multidomain proteins

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8 Abstract

9 Coarse-grained molecular dynamics simulations are a useful tool to determine conformational
10 ensembles of proteins. Here, we show that the coarse-grained force field Martini 3
11 underestimates the global dimensions of intrinsically disordered proteins (IDPs) and multidomain
12 proteins when compared with small angle X-ray scattering (SAXS) data, and that increasing the
13 strength of protein-water interactions favours more expanded conformations. We find that
14 increasing the strength of interactions between protein and water by ca. 10% results in improved
15 agreement with the SAXS data for IDPs and multi-domain proteins. We also show that this
16 correction results in a more accurate description of self-association of IDPs and folded proteins
17 and better agreement with paramagnetic relaxation enhancement data for most IDPs. While
18 simulations with this revised force field still show deviations to experiments for some systems
19 our results suggest that it is overall a substantial improvement for coarse-grained simulations of
20 soluble proteins.

22 Introduction

23 Intrinsically disordered proteins (IDPs) are proteins that do not fold into a single well-defined struc-
24 ture, but rather sample a range of conformations (*Wright and Dyson, 1999*). Similarly, multidomain
25 proteins consisting of folded domains connected by flexible linkers or intrinsically disordered re-
26 gions (IDRs) are conformationally dynamic, as the folded domains can reorient with respect to each
27 other (*Delaforge et al., 2016*). Molecular dynamics (MD) simulations are a useful tool for structural
28 characterization of IDPs and multidomain proteins. Using integrative methods, MD simulations
29 can be used to determine conformational ensembles of IDPs and multidomain proteins in accor-
30 dance with experimental data (*Thomassen and Lindorff-Larsen, 2022*). Successful application of
31 MD simulations relies on accurate force fields and adequate sampling of protein conformations
32 (*Bottaro and Lindorff-Larsen, 2018*).

33 Coarse-grained MD simulations, where groups of atoms are represented by single beads, allow
34 for efficient sampling of IDP and multidomain protein conformations (*Ingólfsson et al., 2014*). One
35 of the most widely used coarse-grained force fields for biomolecular systems is Martini (*Marrink
36 et al., 2007; Monticelli et al., 2008*). Martini maps two to four non-hydrogen atoms to one bead and
37 is mainly parameterized against thermodynamic partitioning data. While Martini has been used
38 successfully to study a wide range of biomolecular systems, earlier versions of the force field have
39 been found to underestimate the global dimensions of flexible multidomain proteins (*Larsen et al.,
40 2020; Martin et al., 2021; Jussupow et al., 2020*) and overestimate protein-protein interactions
41 (*Stark et al., 2013; Berg and Peter, 2019; Alessandri et al., 2019; Benayad et al., 2021; Majumder
42 and Straub, 2021; Lamprakis et al., 2021*). In order to favor more expanded conformations of

43 multidomain proteins, we have previously used an approach based on increasing the strength of
44 non-bonded interactions between protein and water beads (*Larsen et al., 2020; Martin et al., 2021*),
45 improving the agreement with SAXS experiments. Similarly, others have decreased the strength of
46 non-bonded interactions between protein beads to improve the accuracy of IDP phase partitioning
47 (*Benayad et al., 2021*) and protein-protein interactions (*Stark et al., 2013*).

48 A new version of the Martini force field, Martini 3, was recently released, featuring a rebalanc-
49 ing of non-bonded interaction terms and addition of new bead types (*Souza et al., 2021*). Martini 3
50 shows improved accuracy for a wide range of systems in biology and materials science and a high
51 level of transferability. Improved areas include molecular packing, transmembrane helix interac-
52 tions, protein aggregation, and DNA base-pairing (*Souza et al., 2021; Lamprakis et al., 2021*). Here,
53 we have tested the ability of Martini 3 to reproduce the global dimensions of IDPs and multidom-
54 main proteins. We find that simulations with Martini 3 on average underestimate the radius of
55 gyration (R_g) by $\approx 30\%$, and suggest a rescaling factor for increased protein-water interactions that
56 improves agreement with small angle X-ray scattering (SAXS) data and alleviates problems with
57 overestimated protein-protein interactions.

58 Results and Discussion

59 We chose a set of twelve IDPs and three multidomain proteins to cover a range of different systems
60 with available SAXS data (*Riback et al., 2017; Cordeiro et al., 2019; Mylonas et al., 2008; Riback et al.,*
61 *2017; Ahmed et al., 2021; Martin et al., 2020; Johnson et al., 2017; Gomes et al., 2020; Kjaergaard*
62 *et al., 2010; Jephthah et al., 2019; Fagerberg et al., 2020; Sonntag et al., 2017; Martin et al., 2021*)
63 and ran MD simulations of each protein using the Martini 3 force field. For all proteins, we found
64 that the ensemble generated with Martini 3 was too compact when comparing the average R_g from
65 the simulation with the R_g calculated from SAXS data using Guinier analysis (Fig. 1a-b, e). A direct
66 comparison with the experimental SAXS data also revealed deviations beyond the level expected
67 by experimental errors (Fig. 1c-d).

68 For atomistic force fields, it has previously been shown that increasing the protein-water inter-
69 actions will favour expanded conformations of IDPs, resulting in more accurate global dimensions
70 (*Best et al., 2014*). Inspired by this approach, we increased the strength of protein-water interac-
71 tions by rescaling the ϵ parameter in the non-bonded Lennard-Jones potentials between all protein
72 and water beads by a rescaling factor, λ . For all proteins, increased protein-water interactions ($\lambda > 1$)
73 resulted in an increased R_g and improved agreement with SAXS data as measured by the reduced
74 χ^2 (χ_r^2). To determine an optimal value of λ for IDPs, we scanned six λ -values from 1.04 to 1.14
75 for each IDP. Based on the χ_r^2 to SAXS data and agreement between R_g calculated from ensemble
76 coordinates and R_g calculated from experimental SAXS profiles we chose $\lambda = 1.10$ as a balance
77 between improving agreement with experiments and keeping the force field as close as possible
78 to the original (Fig. 1a-c). We performed the same analysis for the three multidomain proteins
79 with flexible linkers, including also $\lambda = 1.02$. These had optimal values of λ around 1.06 (Fig. 1d-e),
80 suggesting that the optimal value may be different for folded domains and IDPs.

81 We examined whether the too compact IDP structures in Martini 3 could be amended by simpler
82 changes to the simulation setup instead of rescaling the protein-water interactions. To test
83 whether including long-range electrostatics would favor more expanded conformations, we ran
84 simulations of Histatin-5 and α -synuclein with Particle Mesh Ewald (PME) electrostatics (Fig. S2).
85 The radii of gyration were, however very similar to those obtained using the standard reaction-field
86 method, with some small differences for the longer protein (α -synuclein) when it was more ex-
87 panded. To examine whether a lack of transient secondary structure in the simulations compared
88 to experiments caused the compaction, we ran simulations of ACTR restraining it to form a helical
89 structure in two regions that transiently sample helices in solution (*Kjaergaard et al., 2010*). This
90 did not solve the problem either (Fig. S2). Finally, to examine whether the observed IDP compaction
91 was a result of a lack of bulk solvent in our simulations, we ran simulations of α -synuclein in a very
92 large box ($d = 34.1$ nm), but the results were essentially the same as in the smaller ($d = 24.1$ nm) box

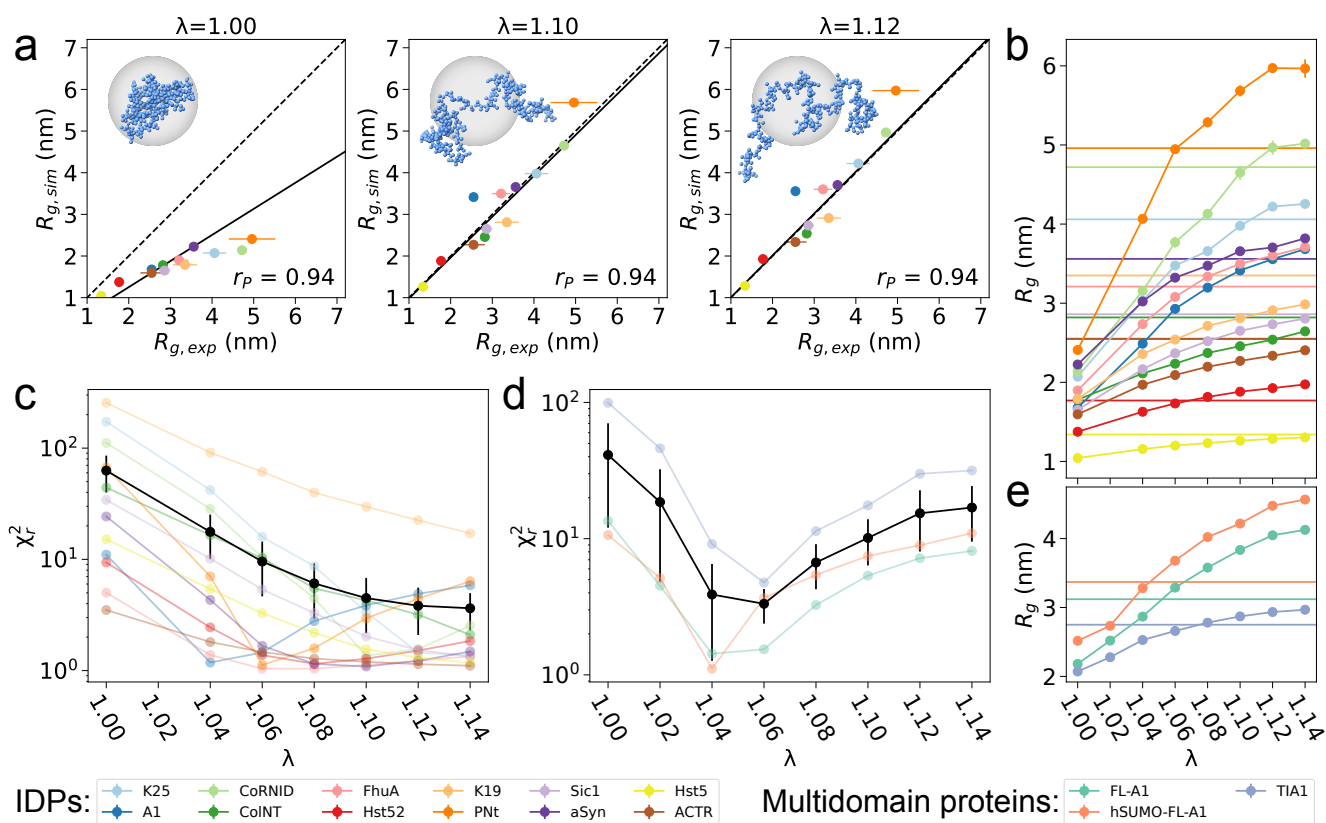


Figure 1. Increased protein-water interactions improve the agreement with SAXS data for IDPs and multidomain proteins

a. Average R_g from MD simulations with three different rescaling factors for the protein-water interactions (λ) plotted against experimental R_g from Guinier analysis of SAXS data for a set of twelve IDPs. Error bars for the experimental values were determined in the Guinier fit, and those for the simulations (here and elsewhere) were determined by block error analysis (Flyvbjerg and Petersen, 1989). A linear fit with intercept 0 weighted by experimental errors is shown as a solid line. The Pearson correlation coefficient (r_p) is shown. The insert shows structures of Tau K25 (Mylonas et al., 2008) with the average R_g found for each λ . See Fig. S1 for similar plots for other values of λ .

b. Average R_g from MD simulations over a range of λ -values for a set of twelve IDPs. Experimental values from Guinier analysis of SAXS data are shown as horizontal lines.

c-d. Reduced χ_r^2 between SAXS profiles calculated from MD simulations and experimental SAXS profiles for a range of λ -values for a set of twelve IDPs (c) and three multidomain proteins (d). Average χ_r^2 is shown in black with standard error of the mean as error bars (note the log scale).

e. Average R_g from MD simulations over a range of λ -values for three multidomain proteins. Experimental values from Guinier analysis of SAXS data are shown as horizontal lines. Data and scripts are available via github.com/KULL-Centre/papers/tree/main/2021/Martini-Thomasen-et-al

93 (Fig. S2). Given that the compaction of the IDPs was not substantially affected by these changes,
94 we continued with our approach of increasing protein-water interactions.

95 To further investigate the effect of rescaling protein-water interactions, we performed a num-
96 ber of tests comparing the original force field ($\lambda=1$) to the force field with increased protein-water
97 interactions ($\lambda=1.10$ and 1.12). First, we tested the effect on the intrachain interactions in IDPs
98 by comparing paramagnetic relaxation enhancement (PRE) data calculated from simulations of α -
99 synuclein, the FUS low-complexity domain (LCD), the LCD in hnRNP A2, full-length tau (htau40),
100 and osteopontin (OPN) to PRE experiments (*Dedmon et al., 2005; Monahan et al., 2017; Ryan et al.,*
101 *2018; Mukrasch et al., 2009; Platzer et al., 2011*). We found that increasing the strength of protein-
102 water interactions improved the agreement with PRE data as quantified by the χ_r^2 for all proteins
103 except A2 LCD (Fig. 2a).

104 Next, we tested the effect of rescaling protein-water interactions on interchain IDP-IDP interac-
105 tions. We simulated two copies of the FUS LCD at conditions matching interchain PRE experiments
106 (*Monahan et al., 2017*) and calculated interchain PREs from the simulations for comparison. Again,
107 increasing protein-water interactions improved the agreement with PRE data (Fig. 2b). These re-
108 sults suggest that increasing the strength of protein-water interactions results in more accurate
109 intra- and interchain interactions for IDPs.

110 As a negative test of IDP-IDP interactions, we ran simulations with two copies of IDPs that were
111 not expected to interact substantially. We chose α -synuclein, htau40, and p15_{pafl}, which should not
112 interact under the given simulation conditions based on experimental evidence (*Dedmon et al.,*
113 *2005; Mukrasch et al., 2009; Platzer et al., 2011*). Our results show that the original force field sub-
114 stantially overestimated IDP-IDP interactions, predicting a high population of the bound state. For
115 all proteins, increasing protein-water interactions by $\lambda=1.10$ and 1.12 reduced the population of
116 the bound state to below 10%, improving the agreement with experiment (Fig. 2c). We note that it
117 was not in all cases possible to converge the populations of the bound and unbound states in sim-
118 ulations with the unmodified force field, as the complexes stayed bound for very long (examples
119 in Figs. S6 and S7). However, our simulations were started from the unbound state, so we expect
120 that a lack of convergence would result in underestimation of the bound state population at $\lambda=1$.
121 For $\lambda=1.10$ and 1.12 , several binding and unbinding events were sampled, and the distribution of
122 the fraction bound over independent simulations was narrower (Fig. 2c).

123 For comparison, we also calculated the population of the bound state in our simulations of
124 the FUS LCD, which should associate to a measurable extent based on PRE experiments (*Monahan*
125 *et al., 2017*). However, FUS LCD had a population of the bound state similar to the IDPs that should
126 not self-associate (Fig. 2c). Since the agreement with interchain PREs was improved for FUS LCD
127 after increasing protein-water interactions, it may be that the bound state population of FUS LCD
128 is accurate, while the bound state of the non-interacting IDPs is still slightly overestimated after
129 increasing protein-water interactions. Nevertheless, the affinities of IDP self-association are much
130 improved.

131 Finally, we investigated the effect of rescaling protein-water interactions on interactions be-
132 tween folded proteins. Inspired by previous simulations (*Best et al., 2014; Berg and Peter, 2019*)
133 and nuclear magnetic resonance (NMR) experiments (*Brewer et al., 2005; Liu et al., 2012*), we sim-
134 ulated two copies of the villin headpiece HP36 (villin HP36) and two copies of ubiquitin, and calcu-
135 lated the populations of the bound state (Fig. 2c). Simulations with Martini 3 appeared to overesti-
136 mate ubiquitin homodimerization when compared with the dissociation constant $K_d = 4.9 \pm 0.3$ mM
137 determined by NMR chemical shift perturbations (*Liu et al., 2012*), but rescaling the strength of
138 protein-water interactions by $\lambda=1.10$ improved the agreement with the experimental affinity. We
139 note that the interactions observed in the simulations were not specific to the homodimerization
140 site determined by NMR (*Liu et al., 2012*) (Fig. S8). Although salt-dependent aggregation behavior
141 was shown to be qualitatively improved for villin HP36 in the Martini 3 publication (*Souza et al.,*
142 *2021*), homodimerization of villin HP36 also appeared to be overestimated with the unmodified
143 force field. Based on diffusion coefficients determined by NMR, villin HP36 homodimerization

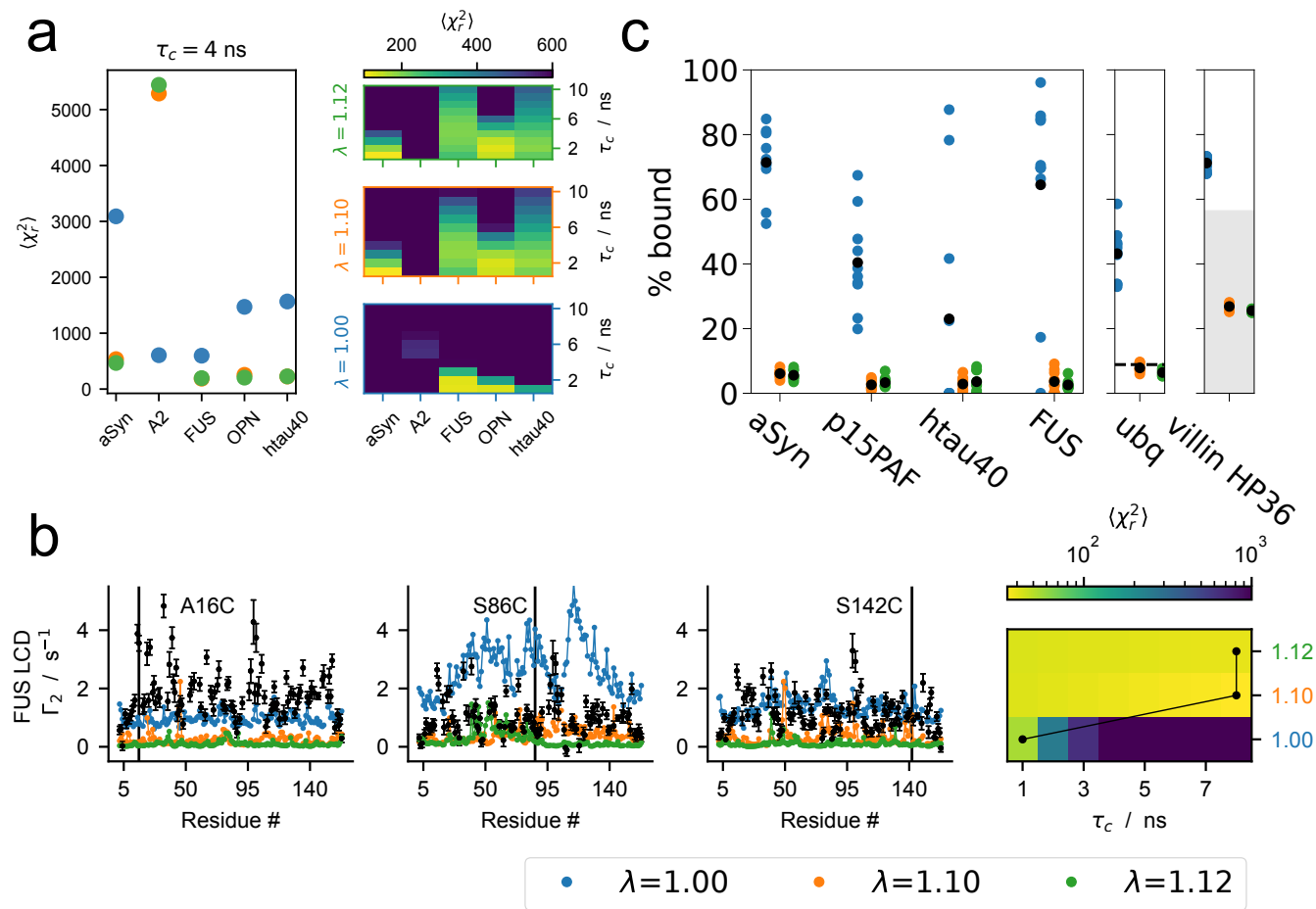


Figure 2. Effect of increased protein-water interactions on intrachain contacts and protein-protein interactions

a. Agreement between intrachain PREs calculated from MD simulations with different protein-water interaction rescaling factors λ and experimental PREs for the five IDPs α -synuclein, A2 LCD, FUS LCD, OPN, and httau40 measured by χ_r^2 . Left panel shows results with $\tau_c = 4$ ns. Right panel shows that the results are consistent across a range of τ_c -values (see also Figs. S3 and S4). **b.** Interchain PREs calculated from MD simulations with different λ -values of two copies of FUS LCD and comparison with experimental PREs (black). PREs are shown for three spin-label sites marked with black lines. Rotational correlation time τ_c was selected individually for each λ to minimize χ_r^2 . For results at $\tau_c = 6$ ns, see Fig. S5 **c.** Fraction bound calculated from MD simulations of two copies of the IDPs α -synuclein, p15_{PAF}, httau40, and FUS LCD and the folded proteins ubiquitin and villin HP36 with different protein-water interaction rescaling factors λ . The results from ten replica simulations are shown as colored points with the average value shown in black. The fraction bound in agreement with $K_d = 4.9$ mM for ubiquitin self-association is shown as a dashed line (Liu *et al.*, 2012). The fraction bound in agreement with a $K_d > 1.5$ mM for villin HP36 self-association is shown as a shaded gray area (Brewer *et al.*, 2005).

144 should have a $K_d > 1.5$ mM (*Brewer et al., 2005*), but the population of the bound state was higher
145 than expected for this affinity. After rescaling protein-water interactions by $\lambda = 1.10$ and 1.12, the
146 populations of the bound state were in agreement with $K_d > 1.5$ mM. Thus, increased protein-water
147 interactions also seem to improve the affinities of protein-protein interactions for folded proteins,
148 although the lack of specificity in these interactions may skew the results, as observed for ubiquitin.

149 Conclusions

150 Our results show that simulations with the Martini 3 force field result in underestimated global
151 dimensions of IDPs and multidomain proteins, and that rescaling the Lennard-Jones potentials for
152 protein-water interactions by a factor $\lambda = 1.10$ improves agreement with SAXS experiments. Addi-
153 tionally, this improves the agreement with PRE data for all but one of the tested IDPs, suggest-
154 ing improved accuracy of intra- and interchain interactions. Our results also show that Martini 3
155 greatly overestimates IDP homodimerization, indicating that IDP-IDP interactions are too strong,
156 but increasing protein-water interactions leads to a more accurate balance. The same is true for
157 homodimerization of the folded proteins ubiquitin and villin HP36. For multidomain proteins con-
158 taining flexible linkers or IDRs, a rescaling factor of $\lambda = 1.06$ seems to be sufficient to result in good
159 agreement with the SAXS data, although this is based on a smaller set of proteins. We note that
160 the agreement with experiments at $\lambda = 1.10$, determined as the optimal value for IDPs, is also better
161 than at $\lambda = 1$ for multidomain proteins.

162 For systems with no additional information available, we suggest setting $\lambda = 1.10$. If one wishes
163 to modify the original force field as little as possible, λ can be set to 1.06 for multidomain proteins,
164 although $\lambda = 1.10$ shows improvement over the original Martini 3 force field for all systems tested
165 here and may be a good overall compromise across different systems. As an alternative approach,
166 a λ -value can be chosen specifically for the system of interest if the level of compaction has been
167 probed experimentally. However, this does not necessarily entail optimizing the λ -value for every
168 condition of interest. For example, we have previously selected a single λ -value for simulations of
169 full-length hnRNPA1 (A1) (with a beta version of Martini 3) based on SAXS data at one salt concen-
170 tration, and studied the effect of salt on the level of compaction by keeping the λ -value fixed but
171 varying the salt concentration (*Martin et al., 2021*). A similar approach may be useful to transfer
172 λ -values between proteins with related sequence properties, for example in mutagenesis studies.

173 We note that our approach of rescaling protein-water interactions does not perfectly capture
174 sequence-dependent differences in IDP compaction. This is evidenced by the observation that the
175 optimal value of λ correlates with the relative expansion of the IDP and that there is less sequence-
176 dependent separation of R_g between different IDPs with $\lambda = 1.10$ than with the original force field
177 (Fig. S9). Thus, a possible explanation for the worsened agreement with intrachain PREs for A2
178 LCD is that it is a relatively compact IDP (*Ryan et al., 2018*), and $\lambda = 1.10$ may result in an overly
179 expanded ensemble. This may also be the reason why our set of multidomain proteins seems
180 to require a lower value of λ than IDPs do; two of the multidomain proteins in the set are vari-
181 ants of full-length A1 for which the isolated IDR requires only $\lambda = 1.04$ for optimal agreement with
182 SAXS. Thus, full-length A1 may be a similar outlier due to the properties of its IDR. Additionally,
183 although the accuracy of IDP-IDP affinities is improved, the modified force field does not seem to
184 accurately distinguish between IDPs that should and should not self-associate, as FUS LCD showed
185 a similar level of self-association to IDPs that should not self-associate. Despite these limitations to
186 our approach, the modified version of Martini 3 with protein-water interactions rescaled by $\lambda = 1.10$
187 shows clear improvement over the original force field in capturing the global dimensions, interac-
188 tions, and affinities of IDPs. We stress that this modification to the force field is only tested for
189 proteins in solution, and may not be applicable to all classes of biomolecules in Martini. We also
190 note that additional experimental studies that quantify weak, transient interactions between both
191 highly soluble and more interaction-prone IDPs would be very useful to address these issues.

192 The issues discussed above illustrate that rescaling all protein-water interactions by a single fac-
193 tor λ is a somewhat ad-hoc approach to improve Martini 3, as the modified force field does not fully

194 capture the sequence-dependent physico-chemical properties of proteins. Some of these issues
195 could potentially be addressed by a more detailed reparameterization of Martini, for example by
196 modifying the interactions of specific bead types or amino acids separately. To investigate which
197 specific bead types or amino acids could require modified interaction potentials, we determined
198 the correlation between the optimal value of λ for each protein and the bead type composition
199 (Fig. S10) as well as other sequence metrics (Fig. S11). However, this approach did not uncover any
200 clear strategy for reparameterizing specific bead types or amino acids. An alternative approach
201 to favor more expanded conformations of IDPs could be the addition of IDR-specific backbone
202 dihedrals, similar to the secondary structure-specific dihedrals already implemented in Martini.
203 However, our results indicate that addition of dihedral potentials to capture for example transient
204 secondary structure in IDPs would not solve the problems with overestimated compaction.

205 The functions of some IDPs and multidomain proteins depend on their ability to form biomolec-
206 ular condensates (*Boeynaems et al., 2018*), often involving the formation of transient and multiva-
207 lent protein-protein interactions and liquid-liquid phase separation (LLPS). Generally, the propen-
208 sity of an IDP to undergo LLPS is correlated with its single-chain compactness (*Choi et al., 2020*).
209 A modified version of Martini 2.2 with decreased protein-protein interactions has already been
210 shown to improve the description of LLPS of an IDP (*Benayad et al., 2021*), and Martini 3 has also
211 been used to study salt-dependent condensate formation (*Tsanai et al., 2021*). We expect that
212 increased protein-water interactions, yielding improved accuracy of the global dimensions of IDPs
213 and weakened IDP-IDP interactions, will be useful in future applications of Martini 3 to study the
214 role of IDPs in biomolecular condensates as well as their single-chain conformations and dynamics.

215 Methods

216 IDP simulations

217 We selected a set of twelve IDPs of varied sequence, with lengths between 24 and 334 amino acid
218 residues and with SAXS data available: the N-terminal region of pertactin (Pnt) (*Riback et al., 2017*),
219 the NR interaction domain of N-CoR (CoRNID) (*Cordeiro et al., 2019*), two deletion mutants of Tau
220 (K19 and K25) (*Mylonas et al., 2008*), the 'plug' domain from a TonB-dependent receptor (FhuA) (*Rib-
221 ack et al., 2017*), α -synuclein (aSyn) (*Ahmed et al., 2021*), the low-complexity domain of hnRNP A1
222 (A1) (*Martin et al., 2020*), the T-domain of colicin N (ColNT) (*Johnson et al., 2017*), Sic1 (*Gomes et al.,
223 2020*), the activation domain of ACTR (ACTR) (*Kjaergaard et al., 2010*), Histatin-5 (Hst5) (*Jephthah
224 et al., 2019*) and a tandem repeat of Histatin-5 (Hst52) (*Fagerberg et al., 2020*) (Table 1).

225 We performed all MD simulations using Gromacs 2020.3 (*Abraham et al., 2015*) and the Martini
226 3.0 force field (*Souza et al., 2021*) or adapted force fields with rescaled protein-water interactions.
227 Proteins were coarse-grained using the Martinize2 python script, placed in a dodecahedral box
228 using Gromacs and solvated using the Insane python script (*Wassenaar et al., 2015*). Initial box
229 sizes were chosen by using starting structures from simulations in *Tesei et al. (2021b)* correspond-
230 ing to the 95th percentile of R_g -distributions and using Gromacs *editconf* with the flag *-d 4.0*. Box
231 sizes were later increased if necessary. NaCl concentration was set to match the conditions in SAXS
232 experiments and to neutralize the system. No secondary structure or elastic network model was
233 assigned with Martinize2 for IDPs and IDRs (see below for tests on ACTR). Energy minimization was
234 performed using steepest descent for 10,000 steps with a 30 fs timestep. The Lennard-Jones po-
235 tentials between all protein and water beads were rescaled by a factor λ . Seven values of λ were
236 tested for each system: 1.00 (original force field), 1.04, 1.06, 1.08, 1.10, 1.12 and 1.14. The systems
237 were equilibrated for 10 ns with a 2 fs timestep using the Velocity-Rescaling thermostat (*Bussi et al.,
238 2007*) and Parinello-Rahman barostat (*Parinello and Rahman, 1981*). Production simulations were
239 run for between 27 μ s and 40 μ s with a 20 fs timestep using the Velocity-Rescaling thermostat and
240 Parinello-Rahman barostat. The temperature was set to match conditions in SAXS experiments
241 and the pressure was set to 1 bar. Non-bonded interactions were treated with the Verlet cut-off
242 scheme. The cut-off for Van der Waals interactions was set to 1.1 nm. Coulomb interactions were

243 treated using the reaction-field method with a 1.1 nm cut-off and dielectric constant of 15. Frames
244 were saved every 1 ns. Periodic boundary conditions were treated with Gromacs *trjconv* with the
245 flags *-pbc whole -center*. Simulation convergence was assessed using block-error analysis (*Flyvbjerg*
246 *and Petersen, 1989*) of the R_g using the BLOCKING code (<https://github.com/fpesceKU/BLOCKING>).
247 Simulations were backmapped to all-atom using a modified (*Larsen et al., 2020*) version of the
248 Backward algorithm (*Wassenaar et al., 2014*), in which simulation runs are excluded and energy
249 minimization is shortened to 200 steps.

250 We also ran MD simulations of five IDPs with paramagnetic relaxation enhancement (PRE) data
251 available: the low-complexity domain of FUS (FUS) (*Monahan et al., 2017*), the low-complexity
252 domain of hnRNPA2 (A2) (*Ryan et al., 2018*) aSyn (*Dedmon et al., 2005*), full-length tau (htau40)
253 (*Mukrasch et al., 2009*) and osteopontin (OPN) (*Platzer et al., 2011*). For these proteins we set
254 the NaCl concentration and temperature to match the conditions in PRE experiments. Simulations
255 were otherwise set up and run using the same protocol as above.

256 **Multidomain protein simulations**

257 We selected a set of three multidomain proteins with SAXS data available: full-length hnRNPA1
258 (FL-A1), full-length hnRNPA1 with an N-terminal His-SUMO tag (hSUMO-FL-A1) and TIA-1 (Table 2).
259 SAXS data and initial structures of FL-A1 and hSUMO-FL-A1 were taken from *Martin et al. (2021)*.
260 These structures were built based on the structures of SUMO1 (PDB: 1A5R) (*Bayer et al., 1998*) and
261 the RRM1 and RRM2 domains (PDB: 1HA1) (*Shamoo et al., 1997*). The initial structure of TIA-1 was
262 taken from *Larsen et al. (2020)* and SAXS data was taken from *Sonntag et al. (2017)*. The structure
263 was built based on the structures of RRM1 (PDB 5O2V) (*Sonntag et al., 2017*), RRM2 (PDB: 5O3J)
264 (*Sonntag et al., 2017*) and the RRM2-RRM3 complex (PDB: 2MJN) (*Wang et al., 2014*).

265 Simulations of multidomain proteins were set up and run using the same protocol as for the
266 IDP simulations with a few exceptions: (i) Secondary structure was assigned with DSSP (*Kabsch and*
267 *Sander, 1983*) in Martinize2. Disordered regions were assigned as coil. (ii) An elastic network model
268 was applied with Martinize2 to keep folded domains intact. Interdomain elastic restraints and the
269 elastic restraints in disordered regions and linker regions were removed. The elastic restraints
270 consisted of a harmonic potential of 700 kJ mol⁻¹ nm⁻² between backbone beads within a 0.9 nm
271 cut-off. (iii) Dihedrals between side chains and backbone beads were added based on the initial
272 structures with the *-scfix* flag in Martinize2. These dihedrals were removed for disordered regions
273 and linker regions. (iv) $\lambda=1.02$ was also tested. Simulations of FL-A1 and TIA1 were run for 40 μ s.
274 Simulations of hSUMO-FL-A1 were run for 15.9-19.4 μ s.

275 **Simulations of dimerization of folded proteins**

276 Initial structures of ubiquitin were taken from *Vijay-Kumar et al. (1987)* (PDB: 1UBQ). Initial struc-
277 tures of villin HP36 were taken from *McKnight et al. (1997)* (PDB: 1VII). Simulations of folded pro-
278 teins were set up and run using the same protocol as for IDP simulations with a few exceptions:
279 (i) Two copies of ubiquitin were placed in a cubic box with 14.92 nm sides, giving a protein con-
280 centration of 1 mM. Two copies villin HP36 were placed in a cubic box with 7.31 nm sides, giving
281 a protein concentration of 8.5 mM. (ii) Secondary structure was assigned with DSSP (*Kabsch and*
282 *Sander, 1983*) in Martinize2. (iii) An elastic network model was applied with Martinize2. The elastic
283 restraints consisted of a harmonic potential of 700 kJ mol⁻¹ nm⁻² between backbone beads within
284 a 0.9 nm cut-off. For ubiquitin, we removed elastic restraints from the C-terminus (residue 72-76)
285 to allow for flexibility (*Lindorff-Larsen et al., 2005*). (iv) Dihedrals between side chains and back-
286 bone beads were added based on the initial structures with the *-scfix* flag in Martinize2. We ran
287 simulations testing three different values of λ : 1.00, 1.10, and 1.12. For each value of λ , we ran ten
288 replicas of 40 μ s each.

289 **Simulation of dimerization of IDPs**

290 Simulations of two copies of FUS, aSyn, htau40, and p15_{pa_f} were set up and run using the same
291 protocol as for IDP simulations. To match conditions in reference experiments (*Monahan et al.,*
292 *2017; Dedmon et al., 2005; Mukrasch et al., 2009; De Biasio et al., 2014*), the proteins were placed
293 in cubic boxes with 40.5, 25.51, 48.02, and 34.15 nm side lengths respectively, giving total protein
294 concentrations of 50, 200, 30, and 83.4 μM . We ran simulations testing three different values of λ :
295 1.00, 1.10, and 1.12, with ten replicas for each λ . Simulations of FUS, aSyn, htau40, and p15_{pa_f} were
296 run for 11.5-21.0, 40, 7.7-12.7, and 16.2-35.8 μs respectively.

297 **IDP simulations to test the effect of long-range electrostatics, secondary structure,** 298 **and more bulk solvent**

299 To test whether inclusion of long-range electrostatics affects the compaction of IDPs, we ran sim-
300 ulations of Hst5 and aSyn using Particle Mesh Ewald (PME) electrostatics with a Fourier spacing of
301 0.16 nm, cubic interpolation, and a real-space cut-off of 1.1 nm. These simulations were otherwise
302 set up and run using the same protocol as for other IDPs. Simulations of Hst5 were run for 20 μs
303 and simulations of aSyn were run for 8-9.6 μs .

304 To test whether inclusion of secondary structure affects the compaction of IDPs, we ran simu-
305 lations of ACTR with helix dihedrals applied to two transient helices at positions Glu28-Ser40 and
306 Leu47-Leu54 (*Kjaergaard et al., 2010*). Assignment of helix dihedrals was performed with Mar-
307 tinize2. These simulations were otherwise set up and run using the same protocol as for other
308 IDPs.

309 To test whether overestimated IDP compaction is the result of a lack of bulk solvent, we ran
310 simulations of aSyn in a much larger box ($d=34.1$ nm). These simulations were otherwise set up
311 and run using the same protocol as for other IDPs.

312 **Calculating the radius of gyration**

313 We calculated the R_g from the coarse-grained trajectories using Gromacs *gyrate* (*Abraham et al.,*
314 *2015*). Figure S12 shows the distribution of R_g for the 12 IDPs. We used block-error analysis (*Flyv-
315 jerg and Petersen, 1989*) to estimate the error on the averages. Experimental R_g and corresponding
316 error bars were calculated from SAXS profiles by Guinier analysis using ATSAS AUTORG with default
317 settings (*Petoukhov et al., 2007*).

318 **SAXS calculations**

319 After each trajectory had been backmapped to all-atom resolution, we extracted 15000 frames
320 (evenly distributed in the time-series) to calculate SAXS profiles using Pepsi-SAXS (*Grudin et al.,*
321 *2017*). To avoid potential problems of overfitting the parameters for the contrast of the hydration
322 layer ($\delta\rho$) and the displaced solvent (r_0) (if these are fitted individually to each structure) we used
323 values that have previously been shown to provide good agreement with experiment for flexible
324 proteins (*Pesce and Lindorff-Larsen, 2021*). Values for the intensity of the forward scattering ($I(0)$)
325 and the constant background (*cst*) were fitted globally with least-squares regression weighted by
326 the experimental errors using the Scikit-learn python library (*Pedregosa et al., 2011*).

327 To quantify the agreement between experimental SAXS profiles and those calculated from sim-
328 ulations, we calculated the reduced χ^2 :

$$\chi_r^2 = \frac{1}{m} \sum_q^m \frac{(I_q^{CALC} - I_q^{EXP})^2}{\sigma(BIFT)_q^2} \quad (1)$$

329 Here m is the number of data points, I_q^{CALC} and I_q^{EXP} are the averaged calculated SAXS inten-
330 sity and the experimental SAXS intensity at scattering angle q , and $\sigma(BIFT)_q$ is the error for the
331 experimental intensity at scattering angle q corrected according to: $\sigma(BIFT)_q = \sigma_q \sqrt{\chi_{r,BIFT}^2}$, where
332 σ_q is the experimental error and $\chi_{r,BIFT}^2$ quantifies the agreement between the experimental SAXS

333 data and the model SAXS profile calculated from the pair distance distribution function obtained
334 through the Bayesian Indirect Fourier Transform algorithm (BIFT) (*Hansen, 2000*). This approach
335 has been shown to lead to improved error estimates for experimental SAXS profiles (*Larsen and*
336 *Pedersen, 2021*) and, here, made it possible to compare more directly and average over the χ_r^2
337 from the different systems. The BIFT algorithm optimizes the hyperparameter D_{max} (maximum dis-
338 tance between scattering particles in the system) starting from an initial estimate. To set this initial
339 estimate we, for each protein, used the largest value of D_{max} observed over all simulations with
340 different values of λ .

341 PRE calculations

342 We used the DEER-PREdict software (*Tesei et al., 2021a*) to calculate PRE NMR data for three pro-
343 teins (Table 3) from all-atom backmapped trajectories. DEER-PREdict implements a model-free
344 formalism (*Iwahara et al., 2004*) combined with a rotamer library approach to describe the MTSL
345 spin-label probe (*Polyhach et al., 2011*). We assumed an effective correlation time of the spin label,
346 τ_l , of 100 ps and scanned the molecular correlation time, τ_c , from 1 to 20 ns in increments of 1 ns.
347 Additionally, to calculate PRE intensity ratios, we assumed a transverse relaxation rate for the dia-
348 magnetic protein of 10 s^{-1} and approximated the total INEPT time of the HSQC measurement to
349 10 ms (*Battiste and Wagner, 2000*). We calculated intermolecular PRE rates from two-chain simu-
350 lations treating one chain as spin-labeled and the other as ^{15}N -labeled. We averaged the PRE rates
351 obtained for the two combinations of spin-labeled and ^{15}N -labeled chain. Agreement between
352 calculated and experimental PREs was quantified by the reduced χ^2 over all spin-label sites:

$$\chi_r^2 = \frac{1}{N_{labels} N_{res}} \sum_j^{N_{labels}} \sum_i^{N_{res}} \left(\frac{Y_{ij}^{exp} - Y_{ij}^{calc}}{\sigma_{ij}^{exp}} \right)^2 \quad (2)$$

353 Where N_{labels} and N_{res} are the number of spin-labels and residues, Y_{ij}^{exp} and Y_{ij}^{calc} are the experi-
354 mental and calculated PRE rates for label j and residue i , and σ_{ij}^{exp} is the experimental error of the
355 PRE rate for label j and residue i .

356 Dimerization calculations

357 We analyzed the population of the bound and unbound states of ubiquitin, villin HP36, FUS, α -
358 synuclein, httau40, and p15_{pafl} homodimers in our simulations (Table 4). We calculated the mini-
359 mum distance between any beads in the two proteins over the trajectory using Gromacs *mindist*
360 (*Abraham et al., 2015*). The fraction bound was defined as the fraction of frames where the mini-
361 mum distance was below 0.8 nm.

362 For simulations of ubiquitin, the fraction bound was also calculated using the minimum distance
363 only between beads in the binding site (residue 8, 13, 44, 45, 46, 49, 67, 68, 70, 71, and 73) defined by
364 NMR chemical shift perturbations (*Liu et al., 2012*). This greatly reduced population of the bound
365 state, showing that Martini3 did not capture the specificity of the interaction. For ubiquitin and
366 villin HP36 dimerization, we calculated what the fraction bound should be at the concentration in
367 our simulations based on the K_d -values of 4.9 mM and 1.5 mM respectively (*Liu et al., 2012; Brewer*
368 *et al., 2005*). The fraction bound was calculated as:

$$f_b = \frac{2C_p + K_d - \sqrt{4K_d C_p + K_d^2}}{2C_p} \quad (3)$$

369 where f_b is the fraction bound, C_p is the concentration of one of the copies of the protein in the
370 simulation box, and K_d is the dissociation constant.

371 Data availability

372 Scripts and data are available via [github.com/KULL-Centre/papers/tree/main/2021/Martini-Thomasen-](https://github.com/KULL-Centre/papers/tree/main/2021/Martini-Thomasen-et-al)
373 [et-al](https://github.com/KULL-Centre/papers/tree/main/2021/Martini-Thomasen-et-al)

Table 1. IDP simulations for SAXS and R_g calculations: Number of amino acid residues (N_R), box size (d), experimental R_g , simulation temperature (T), and salt concentration in the simulation (c_s).

Protein	N_R	d (nm)	SAXS R_g (nm)	T (K)	c_s (M)	SAXS ref.
Hst5	24	13.7	1.34 ± 0.05	293	0.15	<i>Jephthah et al. (2019)</i>
(Hst5) ₂	48	17.4	1.77 ± 0.049	298	0.15	<i>Fagerberg et al. (2020)</i>
ACTR	71	18.9	2.55 ± 0.27	278	0.2	<i>Kjaergaard et al. (2010)</i>
Sic1	92	21.4	2.86 ± 0.14	293	0.2	<i>Gomes et al. (2020)</i>
CoINT	98	20.5	2.82 ± 0.034	277	0.4	<i>Johnson et al. (2017)</i>
K19	99	20.4	3.35 ± 0.29	288	0.15	<i>Mylonas et al. (2008)</i>
A1	137	23.5	2.55 ± 0.1	296	0.05	<i>Martin et al. (2020)</i>
α Syn	140	24.1	3.56 ± 0.036	293	0.2	<i>Ahmed et al. (2021)</i>
FhuA	144	23.9	3.21 ± 0.22	298	0.15	<i>Riback et al. (2017)</i>
K25	185	27.4	4.06 ± 0.28	288	0.15	<i>Mylonas et al. (2008)</i>
CoRNID	271	30.5	4.72 ± 0.12	293	0.2	<i>Cordeiro et al. (2019)</i>
PNT	334	33.2	4.96 ± 0.56	298	0.15	<i>Riback et al. (2017)</i>

Table 2. Multidomain protein simulations for SAXS and R_g calculations: Number of amino acid residues (N_R), box size (d), experimental R_g , simulation temperature (T), and salt concentration in the simulation (c_s).

Protein	N_R	d (nm)	SAXS R_g (nm)	T (K)	c_s (M)	SAXS ref.
TIA1	275	15.9	2.75 ± 0.031	300	0.1	<i>Sonntag et al. (2017)</i>
FL-A1	314	25.6	3.12 ± 0.022	300	0.15	<i>Martin et al. (2021)</i>
hSUMO-FL-A1	433	28.4	3.37 ± 0.014	300	0.1	<i>Martin et al. (2021)</i>

Table 3. IDP simulations for single-chain PRE calculations: Number of amino acid residues (N_R), box size (d), experimental R_g , simulation temperature (T), and salt concentration in the simulation (c_s).

Protein	N_R	d (nm)	T (K)	c_s (M)	PRE ref.
α Syn	140	24.1	283	0.125	<i>Dedmon et al. (2005)</i>
A2	155	22.8	298	0.005	<i>Ryan et al. (2018)</i>
FUS	163	23.4	298	0.15	<i>Monahan et al. (2017)</i>
OPN	220	23.9	298	0.15	<i>Platzer et al. (2011)</i>
htau40	441	34.7	278	0.1	<i>Mukrasch et al. (2009)</i>

Table 4. Protein dimerization simulations: Number of amino acid residues (N_R), box size (d), experimental R_g , simulation temperature (T), and salt concentration in the simulation (c_s).

Protein	N_R	d (nm)	T (K)	c_s (M)	PRE or affinity ref.
α Syn	140x2	25.51	283	0.125	<i>Dedmon et al. (2005)</i>
FUS	163x2	40.5	298	0.15	<i>Monahan et al. (2017)</i>
p15PAF	76x2	34.15	298	0.15	<i>De Biasio et al. (2014)</i>
htau40	441x2	48.02	278	0.1	<i>Mukrasch et al. (2009)</i>
ubq	76x2	14.9	303	0.11	<i>Liu et al. (2012)</i>
villin HP36	36x2	7.31	298	0.15	<i>Brewer et al. (2005)</i>

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