

1 Rescaling protein-protein 2 interactions improves Martini 3 for 3 flexible proteins in solution

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12 **Abstract** Multidomain proteins with flexible linkers and disordered regions play
13 important roles in many cellular processes, but characterizing their conformational
14 ensembles is difficult. We have previously shown that the coarse-grained model,
15 Martini 3, produces too compact ensembles in solution, that may in part be remedied
16 by strengthening protein–water interactions. Here, we show that decreasing the
17 strength of protein–protein interactions leads to improved agreement with
18 experimental data on a wide set of systems. We show that the ‘symmetry’ between
19 rescaling protein–water and protein–protein interactions breaks down when studying
20 interactions with or within membranes; rescaling protein–protein interactions better
21 preserves the binding specificity of proteins with lipid membranes, whereas rescaling
22 protein–water interactions preserves oligomerization of transmembrane helices. We
23 conclude that decreasing the strength of protein–protein interactions improves the
24 accuracy of Martini 3 for IDPs and multidomain proteins, both in solution and in the
25 presence of a lipid membrane.

26

27 Introduction

28 Intrinsically disordered proteins (IDPs), folded proteins with long disordered tails, and
29 multidomain proteins with folded domains connected by flexible linkers, are character-
30 ized by their high level of conformational dynamics. Molecular dynamics (MD) simula-
31 tions provide a valuable tool for studying IDPs and multidomain proteins, as they can be
32 used to determine full conformational ensembles at atomic resolution (*Thomsen and*
33 *Lindorff-Larsen, 2022*). However, there are two central challenges that must be over-
34 come for MD simulations to provide a useful description of such systems: the force field
35 describing all the bonded and non-bonded interactions between atoms in the system
36 must be sufficiently accurate *and* the conformational space of the protein must be suffi-

37 ciently sampled (*Bottaro and Lindorff-Larsen, 2018*).

38 One way to address the challenge of sufficient sampling is to use coarse-grained (CG)
39 MD simulations in which groups of atoms are represented as single beads (*Ingólfsson*
40 *et al., 2014*). Martini is a widely used CG model in which 2–4 non-hydrogen atoms are
41 represented by a single bead (*Marrink et al., 2007; Monticelli et al., 2008*). An attractive
42 aspect of Martini is its modular structure and high degree of transferability, which allows
43 the simulation of complex systems containing several different classes of biomolecules.
44 The current version of Martini, Martini 3, shows improvements over previous versions in
45 areas such as molecular packing, transmembrane helix interactions, protein aggregation,
46 and DNA base pairing (*Souza et al., 2021*).

47 We have previously shown that Martini 3 simulations of IDPs produce overly compact
48 conformational ensembles, resulting in poor agreement with small-angle X-ray scattering
49 (SAXS) and paramagnetic relaxation enhancement (PRE) experiments (*Thomasen et al.,*
50 *2022*). Using an approach inspired by previous work on assessing and rebalancing non-
51 bonded interactions in Martini (*Stark et al., 2013; Javanainen et al., 2017; Berg et al.,*
52 *2018; Berg and Peter, 2019; Alessandri et al., 2019; Larsen et al., 2020; Benayad et al.,*
53 *2021; Majumder and Straub, 2021; Lamprakis et al., 2021; Martin et al., 2021*) and atom-
54 istic force fields (*Best et al., 2014*), we found that agreement with SAXS and PRE data could
55 be significantly improved by uniformly increasing the strength of non-bonded Lennard-
56 Jones interactions between protein and water beads by ~10% (*Thomasen et al., 2022*).
57 This was also shown to be the case for three multidomain proteins, hnRNPA1, hisSUMO-
58 hnRNPA1, and TIA1; however, due to the small sample size and the similarity between
59 these three proteins, it remains an open question whether the approach generalizes to
60 other multidomain proteins.

61 Our previous work was concerned with the properties of proteins in aqueous solu-
62 tion in the absence of other classes of biomolecules. Intuitively, increasing the strength of
63 protein-water interactions should affect the affinity between proteins and other biomolecules.
64 As a prototypical example, one would expect that increasing protein-water interactions
65 would decrease the affinity of proteins for lipid membranes, since the interaction is tuned
66 by the relative affinity of proteins for water vs. the membrane environment. The extent
67 to which our previously described force field modification affects protein-membrane in-
68 teractions, however, remains unclear. There is increasing evidence that IDPs and disor-
69 dered regions play important physiological roles at lipid membranes (*Kjaergaard and*
70 *Kragelund, 2017; Zeno et al., 2018; Das and Eliezer, 2019; Fakhree et al., 2019; Cornish*
71 *et al., 2020*), and so it is important to understand better how force field changes that
72 improve the description of disordered proteins in solution affect their interactions with
73 membranes. In this context, it is important to note that unmodified Martini 3 has been
74 quite successful at reproducing the specific membrane interactions for peripheral mem-
75 brane proteins, as we previously showed (*Srinivasan et al., 2021*).

76 For previous versions of Martini, problems with overestimated protein-protein inter-
77 actions have been corrected either by increasing the strength of protein-water interac-
78 tions (*Berg et al., 2018; Berg and Peter, 2019; Larsen et al., 2020; Martin et al., 2021*)
79 or by decreasing the strength of interactions between protein beads (*Stark et al., 2013;*
80 *Javanainen et al., 2017; Benayad et al., 2021*). We hypothesize that for proteins in so-
81 lution, the two force field corrections likely have similar effects, simply rebalancing the
82 relative energies associated with hydration versus self-interaction. However, in the case

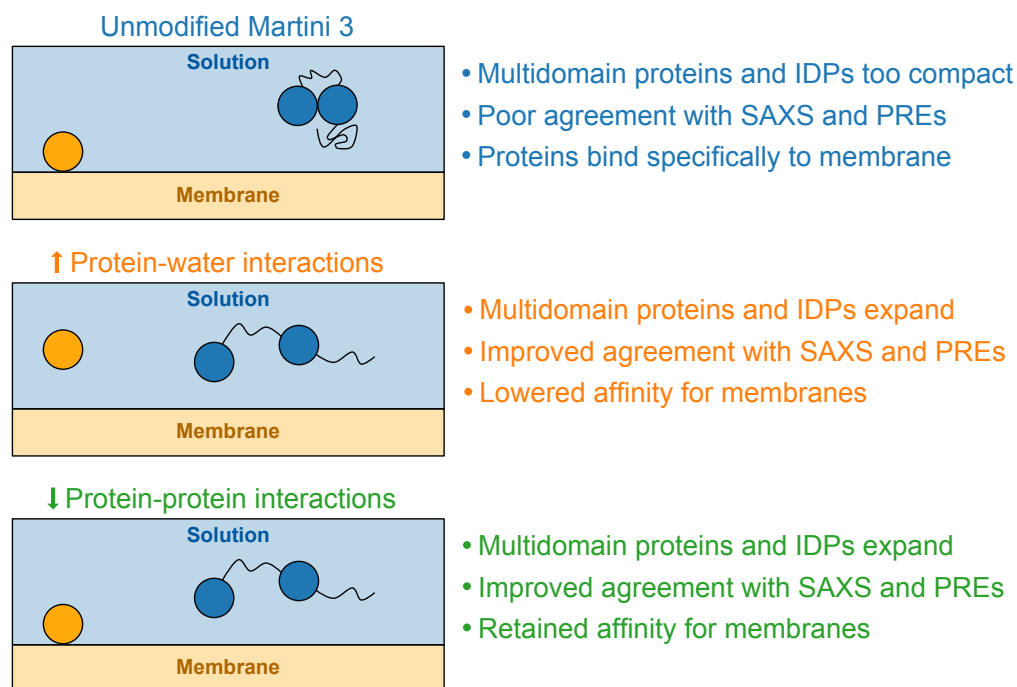


Figure 1. Expected effects of proposed force field modifications. Schematic overview showing the expected effects of rescaling protein-water and protein-protein interactions in Martini 3. Overestimated compactness of soluble IDPs and multidomain proteins and specific membrane interactions for peripheral membrane proteins have previously been reported (*Srinivasan et al., 2021; Thomassen et al., 2022*).

83 of mixed systems, for example with proteins, water, and membranes, we might expect
84 clearer differences between these approaches. For example, decreasing the strength of
85 protein-protein interactions may better retain the affinity between proteins and other
86 molecules as originally parameterized, while increased protein-water interactions may
87 lower this affinity (Fig. 1). Thus, it remains an open question whether this specificity is
88 retained when protein-water interactions are increased, and whether rescaling protein-
89 protein interactions provides equivalent or improved agreement with experimental ob-
90 servations, both in comparison with unmodified Martini 3 and Martini 3 with rescaled
91 protein-water interactions. We note that Martini 3 has already been shown to provide
92 good agreement with free energies of dimerization for transmembrane proteins (*Souza*
93 *et al., 2021*), so the major focus of this work is to rebalance the interactions of proteins
94 in solution to improve the agreement with experiments.

95 Here, we expand upon our previous work to address these questions. First, we have
96 expanded the set of multidomain proteins to include 15 proteins for which SAXS data
97 have previously been collected (Fig. 2). Using this five-times larger set of proteins, we
98 show that, as was the case for IDPs, increasing the strength of protein-water interactions
99 by 10% improves the agreement with SAXS data. We further show that decreasing the
100 strength of non-bonded interactions between protein beads by 12% leads to a compara-
101 ble improvement in agreement with SAXS and PRE data for IDPs and multidomain pro-
102 teins in solution, but better preserves the specificity of protein-membrane interactions
103 for peripheral membrane proteins. In contrast, we find that rescaling protein-protein in-

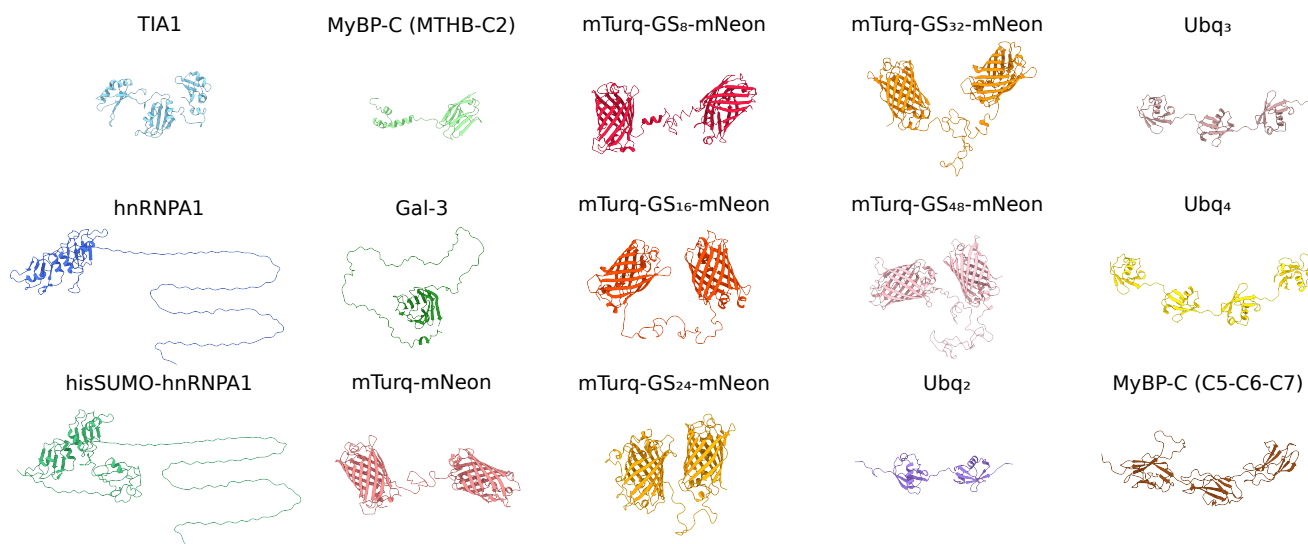


Figure 2. Starting structures for simulations of multidomain proteins. Starting structures of multidomain proteins used for Martini simulations. See the Methods section for a description of the source of the structures and how they were assembled.

104 teractions decreases the propensity of transmembrane helices to dimerize, whereas this
 105 propensity is mostly unchanged when rescaling protein-water interactions.

106 Results

107 Analysis of an expanded set of multidomain proteins

108 Previously, we tested Martini 3 using a set of three multidomain proteins, TIA1, hnRNPA1,
 109 and hisSUMO-hnRNPA1, for which SAXS data have been measured (*Sonntag et al., 2017*;
 110 *Martin et al., 2021*). Given the similarity of the three proteins (all three are RNA-binding
 111 proteins, and the two latter differ only in the addition of a hisSUMO-tag), we wished to
 112 expand the set of proteins with mixed regions of order and disorder to include a wider
 113 range of sizes and domain architectures. We searched the literature for such proteins
 114 with reported SAXS data and identified 12 proteins that we added to our set (Fig. 2): the
 115 tri-helix bundle of the m-domain and the C2 domain of myosin-binding protein C (MyBP-
 116 C_{MTHB-C2}) (*Michie et al., 2016*); the C5, C6, and C7 domains of myosin-binding protein C
 117 (MyBP-C_{C5-C6-C7}) (*Nadvi et al., 2016*); linear di- tri- and tetraubiquitin (Ubq₂, Ubq₃, Ubq₄)
 118 (*Jussupow et al., 2020*); the two fluorescent proteins mTurquoise2 and mNeonGreen con-
 119 nected by a linker region with the insertion of 0, 8, 16, 24, 32, or 48 GS repeats (mTurq-
 120 GS_X-mNeon) (*Moses et al., 2024*); and Galectin-3 (Gal-3) (*Lin et al., 2017*). Apart from
 121 Gal-3, these proteins all contain at least two distinct folded domains, connected by link-
 122 ers of different lengths and composition; three proteins (Gal-3, hnRNPA1, and hisSUMO-
 123 hnRNPA1) also contain a long disordered region attached to a folded domain. Collectively,
 124 we will refer to this set as multidomain proteins, though we note that Gal-3 only contains
 125 a single folded domain.

126 We have previously shown that Martini 3 produces conformational ensembles that
 127 are more compact than found experimentally for a set of 12 IDPs and for the three mul-

128 tidomain proteins TIA1, hnRNPA1, and hisSUMO-hnRNPA1, and that rescaling ϵ in the
129 Lennard-Jones potential between all protein and water beads by a factor $\lambda_{PW}=1.10$ re-
130 sulted in more expanded ensembles that substantially improved the agreement with
131 SAXS data (*Thomassen et al., 2022*). Using our much larger set of multidomain proteins,
132 we examined whether Martini 3 generally produces too compact conformational ensem-
133 bles of multidomain proteins, and whether our modified force field with rescaled protein-
134 water interactions would generalize to the expanded set of proteins. We ran Martini 3
135 simulations of the 12 new multidomain proteins with unmodified Martini 3 and with
136 $\lambda_{PW}=1.10$ and calculated SAXS intensities from the simulations. We found that, on av-
137 erage across the 15 proteins, increasing the strength of protein-water interactions by
138 $\lambda_{PW}=1.10$ substantially improved the direct agreement with the experimental SAXS data,
139 as quantified by the reduced χ^2 , χ_r^2 (Fig. 3). For only one of the 15 proteins, MyBP-
140 $C_{MTHB-C2}$, the modified force field gave rise to reduced agreement with the SAXS data.
141 This result shows that our previously proposed modification of protein-water interac-
142 tions in Martini 3, which was optimized to improve the global dimensions of IDPs, also
143 provides a general improvement in the global dimensions of multidomain proteins.

144 Rescaling protein-protein interactions

145 Inspired by previous work on earlier versions of the Martini force field, (*Stark et al.,*
146 *2013; Javanainen et al., 2017; Benayad et al., 2021*), we next examined whether rescaling
147 protein-protein interactions instead of protein-water interactions would provide a similar
148 or further improvement in the agreement with the experimental data. To do so, we ran
149 Martini 3 simulations for the set of 12 IDPs with SAXS data available that we had studied
150 previously (*Thomassen et al., 2022*) and the new set of 15 multidomain proteins. In these
151 simulations, we rescaled ϵ in the Lennard-Jones potential between all protein beads by
152 a factor λ_{PP} . We scanned different values of this parameter, and found $\lambda_{PP}=0.88$ to pro-
153 vide the best agreement with experiments (Fig. S1). We found that this level of rescaling
154 protein-protein interactions ($\lambda_{PP}=0.88$) provides a comparable improvement in the agree-
155 ment with the experimental data as rescaling protein-water interactions by $\lambda_{PW}=1.10$ for
156 both multidomain proteins (Fig. 3) and IDPs (Fig. 4).

157 To further test the effect of rescaling protein-protein interactions by $\lambda_{PP}=0.88$ and
158 compare with the approach of rescaling protein-water interactions, we ran simulations of
159 five IDPs with intramolecular PRE data available: the LCD of hnRNPA2 (*Ryan et al., 2018*),
160 the LCD of FUS (*Monahan et al., 2017*), α -synuclein (*Dedmon et al., 2005*), full-length tau
161 (hTau40) (*Mukrasch et al., 2009*), and osteopontin (OPN) (*Platzner et al., 2011*), and cal-
162 culated PRE data from the simulations (Fig. 4e). Again, $\lambda_{PP}=0.88$ provided a comparable
163 level of agreement with the PRE data as we previously found using $\lambda_{PW}=1.10$ (*Thomassen*
164 *et al., 2022*). Specifically, the agreement with the PRE data improved for all proteins ex-
165 cept the hnRNPA2 LCD (Fig. 4e).

166 To further characterize the symmetry between rescaling protein-water and protein-
167 protein interactions, we compared the ensembles produced with the two rescaling ap-
168 proaches, and with unmodified Martini 3, using the distribution of R_g (Fig. S2-3) and a
169 principal component analysis (PCA) based on the pairwise distances between backbone
170 beads (Fig. S4-5). This analysis confirmed that the two rescaling approaches produce
171 ensembles which are highly similar when compared with unmodified Martini 3. We con-
172 clude that decreasing the strength of protein-protein interactions by $\lambda_{PP}=0.88$ provides

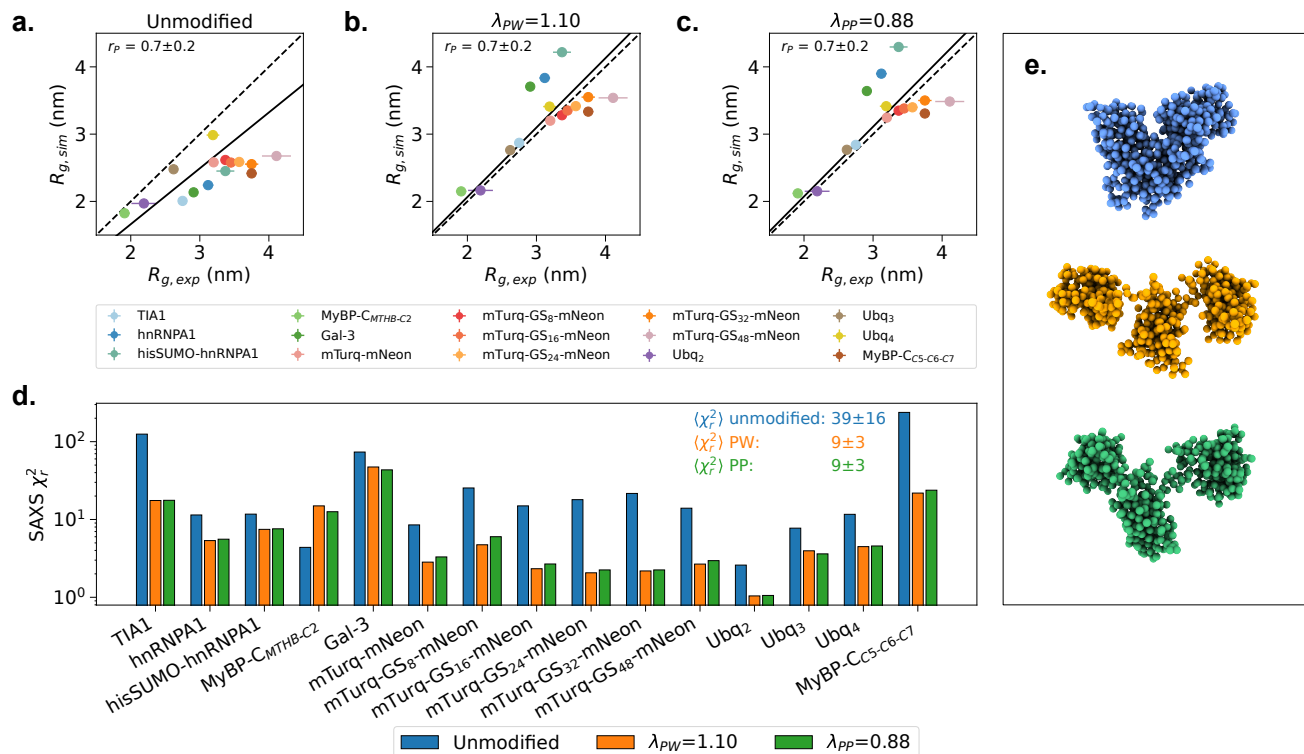


Figure 3. Agreement between simulations and SAXS data for multidomain proteins. R_g calculated from simulations plotted against R_g determined from Guinier fits to the SAXS data for **a** simulations with unmodified Martini 3, **b** simulations with protein-water interactions in Martini 3 rescaled by $\lambda_{PW}=1.10$, and **c** simulations with protein-protein interactions in Martini 3 rescaled by $\lambda_{PP}=0.88$. The diagonal is shown as a dashed line and a linear fit with intercept 0 weighted by experimental errors is shown as a solid line. Pearson correlation coefficients (r_p) with standard errors from bootstrapping are shown on the plots. **d.** Reduced χ^2 to experimental SAXS intensities given by SAXS intensities calculated from unmodified Martini 3 simulations (blue) and Martini 3 simulations with protein-water interactions rescaled by $\lambda_{PW}=1.10$ (orange) or protein-protein interactions rescaled by $\lambda_{PP}=0.88$ (green). Mean and standard error of the mean over all proteins are shown on the plot. Note the logarithmic scale for χ^2_r . **e.** Representative conformation of TIA1 with an R_g corresponding to the average R_g in (blue) simulations with unmodified Martini 3, (orange) simulations with protein-water interactions in Martini 3 rescaled by $\lambda_{PW}=1.10$, and (green) simulations with protein-protein interactions in Martini 3 rescaled by $\lambda_{PP}=0.88$. Simulations of hnRNPA1, hisSUMO-hnRNPA1 and TIA1 with $\lambda_{PW}=1.10$ were taken from *Thomasen et al. (2022)*.

173 an equally good alternative to rescaling protein-water interactions for IDPs and multido-
174 main proteins in solution.

175 **Protein self-association in solution**

176 The observation that multidomain proteins in solution are too compact in Martini 3 sim-
177 ulations suggests that interactions between folded protein domains may be overesti-
178 mated, at least at the high effective concentration within a single chain. To explore this
179 further, we examined the effect of rescaling protein-protein interactions on the inter-
180 actions between folded proteins in trans. To this aim, we ran MD simulations of two
181 protein systems that should undergo transient homodimerization, ubiquitin and villin
182 HP36, which we also used in our previous work (*Thomassen et al., 2022*). Ubiquitin self-
183 associates with a K_d of 4.9 ± 0.3 mM based on NMR chemical shift perturbations (*Liu*
184 *et al., 2012*) and villin HP36 self-associates with a $K_d > 1.5$ mM based on NMR diffusion
185 measurements (*Brewer et al., 2005*). We ran MD simulations of two copies of the pro-
186 teins with $\lambda_{pp}=0.88$ and calculated the fraction of the time that the proteins were bound
187 (Fig. 5a). For both proteins $\lambda_{pp}=0.88$ resulted in decreased self-association, and again
188 we found that $\lambda_{pp}=0.88$ gave comparable results to our previously published simulations
189 with $\lambda_{pW}=1.10$ (*Thomassen et al., 2022*). Comparing the simulations with the expected
190 fraction bound based on the experimentally determined K_d values, we found that ubiq-
191 uitin self-association is likely slightly overestimated with unmodified Martini 3 and slightly
192 underestimated with $\lambda_{pp}=1.10$ and $\lambda_{pp}=0.88$. For villin HP36, all three force fields gave
193 rise to a fraction bound within the expected range. While the overestimated compaction
194 of multidomain proteins suggest that interactions between folded domains may be too
195 strong in Martini 3, our results on the self-association of ubiquitin and villin HP36 do not
196 provide a clear indication that this is the case.

197 To further investigate the effect of rescaling protein-protein interactions on protein
198 self-association, we performed simulations of four IDP systems, which we also used in
199 our previous work (*Thomassen et al., 2022*). Specifically, we ran simulations with $\lambda_{pp}=0.88$
200 of two copies of α -synuclein, hTau40, or p15PAF, which should not self-associate under
201 the given conditions based on PRE (*Dedmon et al., 2005; Mukrasch et al., 2009*) or size-
202 exclusion chromatography-multiangle laser-light scattering (SEC-MALLS) data (*De Biasio*
203 *et al., 2014*), as well as two copies of the FUS LCD, which should transiently interact under
204 the given conditions based on PRE data (*Monahan et al., 2017*). We then calculated the
205 fraction of time that the proteins were bound in the simulations (Fig. 5a). Again $\lambda_{pp}=0.88$
206 gave comparable results to our previously published simulations with $\lambda_{pW}=1.10$. The re-
207 sults show that unmodified Martini 3 overestimates the self-association of IDPs, and that
208 both rescaling approaches result in lowered self-association and therefore better agree-
209 ment with experiments. However, none of the force fields give rise to a clear distinction
210 between the FUS LCD and the three IDPs which should not self-associate, suggesting that
211 Martini 3 does not properly capture specificity in IDP-IDP interactions.

212 To investigate further how well specific interactions between copies of the FUS LCD
213 were captured, we calculated intermolecular PRE data from our simulations for direct
214 comparison with the experimental PRE data (*Monahan et al., 2017*) (Fig. 5b-c). Simula-
215 tions with $\lambda_{pp}=0.88$ and $\lambda_{pW}=1.10$ produce similar PREs when calculated with the spin-
216 labels at residue 16 and residue 142, but we observed some discrepancy between the
217 two force fields for PREs calculated with the spin-label at residue 86. These differences

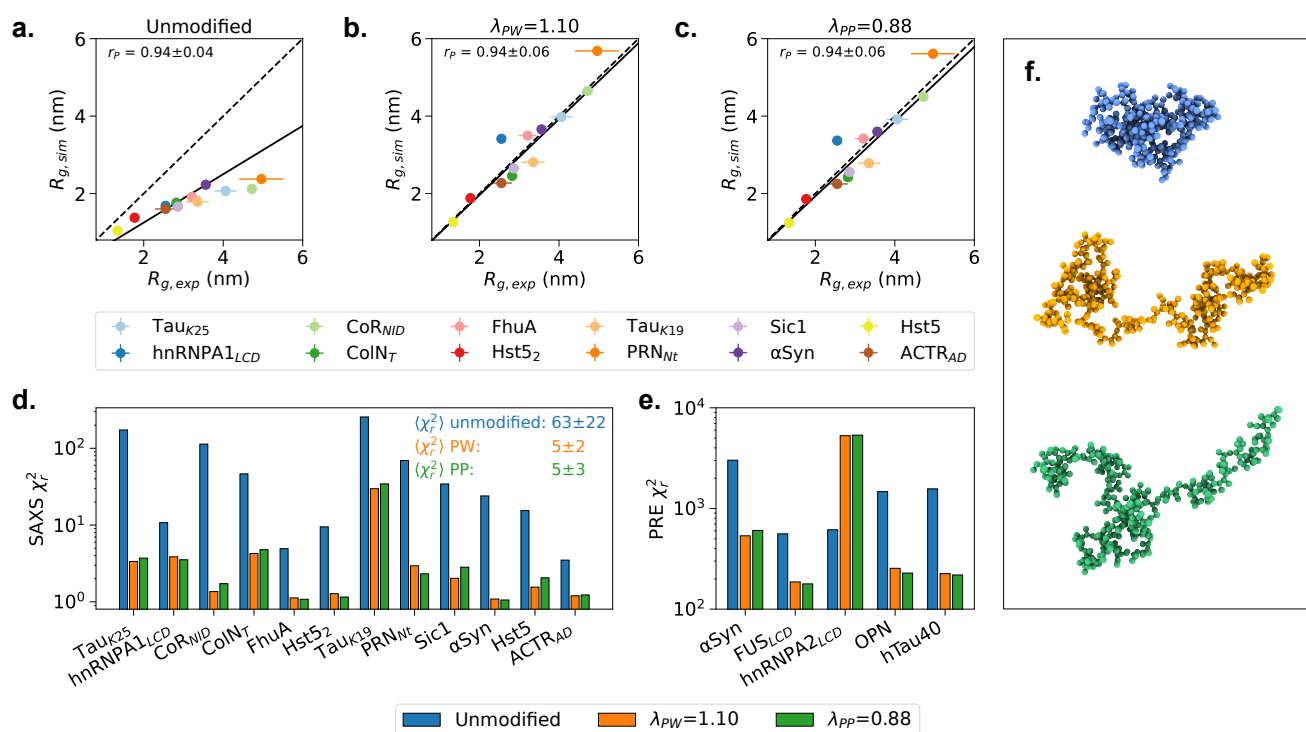


Figure 4. Agreement between simulations and SAXS or PRE data for IDPs. R_g calculated from simulations plotted against R_g determined from Guinier fits to the SAXS data for **a** simulations with unmodified Martini 3, **b** simulations with protein-water interactions in Martini 3 rescaled by $\lambda_{PW}=1.10$, and **c** simulations with protein-protein interactions in Martini 3 rescaled by $\lambda_{PP}=0.88$. The diagonal is shown as a dashed line and a linear fit with intercept 0 weighted by experimental errors is shown as a solid line. Pearson correlation coefficients (r_p) with standard errors from bootstrapping are shown on the plots. **d.** Reduced χ^2 to experimental SAXS intensities given by SAXS intensities calculated from unmodified Martini 3 simulations (blue) and Martini 3 simulations with protein-water interactions rescaled by $\lambda_{PW}=1.10$ (orange) or protein-protein interactions rescaled by $\lambda_{PP}=0.88$ (green). Mean and standard error of the mean over all proteins are shown on the plot. Note the logarithmic scale for χ^2_r . **e.** Reduced χ^2 to experimental PRE NMR data given by unmodified Martini 3 simulations (blue) and Martini 3 simulations with protein-water interactions rescaled by $\lambda_{PW}=1.10$ (orange) or protein-protein interactions rescaled by $\lambda_{PP}=0.88$ (green). Note the logarithmic scale for χ^2_r . **f.** Representative conformation of Tau_{K25} with an R_g corresponding to the average R_g in (blue) simulations with unmodified Martini 3, (orange) simulations with protein-water interactions in Martini 3 rescaled by $\lambda_{PW}=1.10$, and (green) simulations with protein-protein interactions in Martini 3 rescaled by $\lambda_{PP}=0.88$. All simulations with $\lambda_{PW}=1.10$ were taken from *Thomassen et al. (2022)*.

218 could be due to a true difference between the force fields, but may also be due to lack of
219 convergence on the protein-protein contacts, as the bound state is not very populated in
220 the simulations. Both $\lambda_{PP}=0.88$ and $\lambda_{PW}=1.10$ show slight improvement over unmodified
221 Martini 3 based on the χ_r^2 to the experimental PRE data, but none of the force fields fully
222 capture the variation in interactions across the sequence. For example, interactions with
223 the N-terminal region seem to be underestimated with the rescaled force fields based
224 on the PRE data with the spin-label at residue 16, while the interactions with the cen-
225 tral region seem to be overestimated with the unmodified force field based on the PRE
226 data with the spin-label at residue 86. The interpretation of the results is complicated by
227 the fact that the rotational correlation time, τ_c , providing the best fit to the experimental
228 data is lower for the unmodified force field (1 ns), than for $\lambda_{PW}=1.10$ (8 ns) and $\lambda_{PP}=0.88$
229 (9 ns), suggesting that the fit of τ_c is absorbing some of the true difference between the
230 force fields. Overall, the comparison with intermolecular PRE data for the FUS LCD is
231 consistent with an improvement in the overall strength of IDP-IDP interactions, but a
232 remaining lack of interaction specificity with the rescaled force fields. The results also
233 show that rescaling protein-protein interactions gives as good or better agreement with
234 the intermolecular PRE data when compared with our previous approach of rescaling
235 protein-water interactions.

236 **Rescaling protein-water interactions for backbone beads only**

237 While the overall agreement with SAXS experiments was improved for almost all pro-
238 teins when rescaling protein-protein or protein-water interactions, some proteins were
239 still too expanded or compact with respect to the experimental R_g , suggesting that some
240 sequence-specific effects on compaction were not fully captured. We reasoned that
241 sequence-specific effects on the ensemble properties would possibly be better captured
242 if we rescaled only the interactions between the protein backbone and water; this ap-
243 proach could lead to the desired expansion of the proteins while retaining the inter-
244 actions of the amino acid side chains as originally parameterized. We therefore per-
245 formed simulations of our set of IDPs and multidomain proteins in which we rescaled
246 ϵ in the Lennard-Jones potential between all protein backbone and water beads by a fac-
247 tor λ_{PW-BB} , scanning different values of this parameter, and found $\lambda_{PW-BB}=1.22$ to provide
248 the best agreement with experiments (Fig. S1). However, the simulations of the IDPs and
249 multidomain proteins with $\lambda_{PW-BB}=1.22$ showed similar agreement with experiments as
250 when rescaling all protein-water interactions or protein-protein interactions (Fig. S6-7),
251 and the ensembles resulting from the three rescaling approaches were similar based
252 on R_g distributions (Fig. S2-3) and analyses of the pairwise distances between backbone
253 beads (Fig. S4-5). Given that rescaling of only protein backbone-water interactions did
254 not show any substantial improvement with respect to the previous approaches, and
255 that the strong interactions between the protein backbone and water may have unde-
256 sirable effects on the behaviour of the hydration shell, we decided not to pursue this
257 approach further.

258 **Amino acid side chain analogues**

259 We wished to further investigate the symmetry between rescaling of protein-water and
260 protein-protein interactions using simulations of oil/water partitioning, as this was a cen-
261 tral approach in the original parameterization of non-bonded interactions in Martini. In-

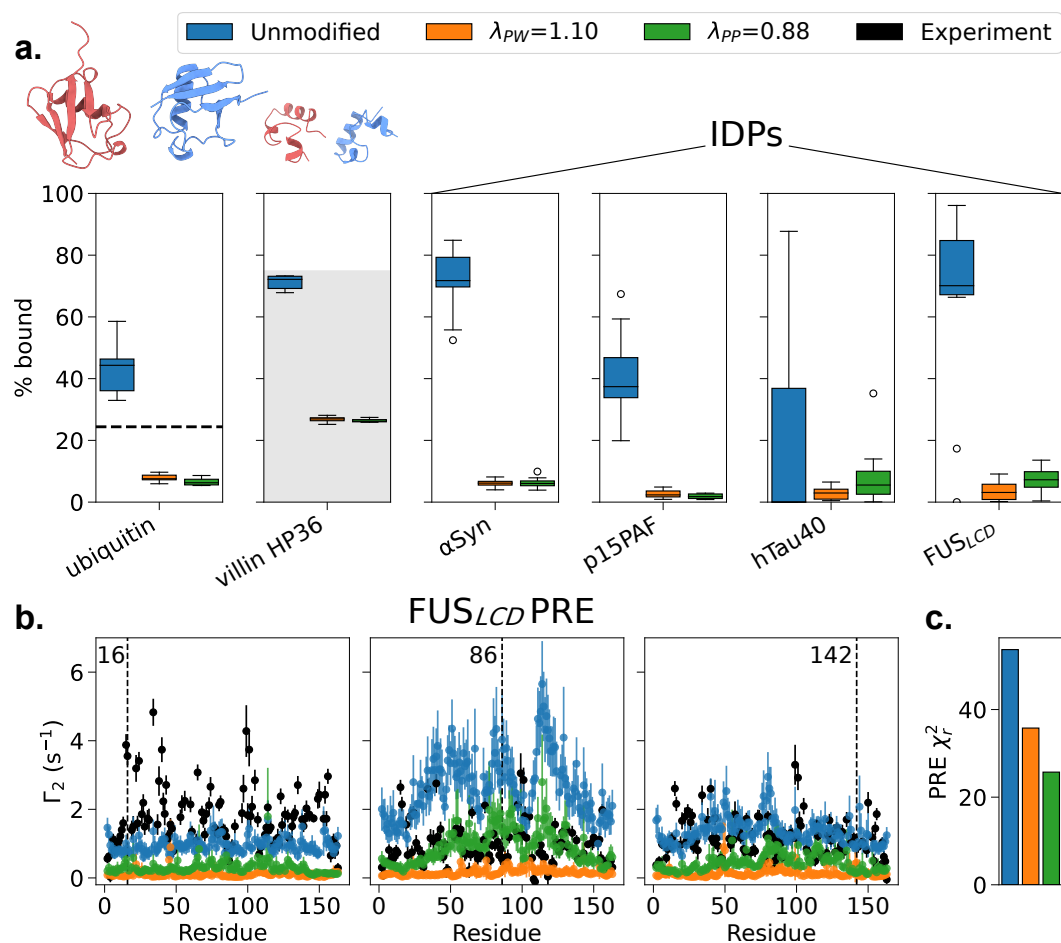


Figure 5. Protein self-association. **a.** Fraction bound calculated from MD simulations of two copies of the folded proteins ubiquitin and villin HP36, and two copies of the IDPs α -synuclein p15PAF, hTau40, and FUS LCD, with unmodified Martini 3 (blue), Martini 3 with protein-water interactions rescaled by $\lambda_{PW}=1.10$ (orange) (taken from *Thomasen et al. (2022)*), and Martini 3 with protein-protein interactions rescaled by $\lambda_{PP}=0.88$ (green). Box plots show the results of 10 replica simulations. The bound fraction in agreement with $K_d = 4.9$ mM for ubiquitin self-association is shown as a dashed line (*Liu et al., 2012*). The bound fraction 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*). α -synuclein p15PAF, and hTau40 should not self-associate under the given conditions based on PRE (*Dedmon et al., 2005; Mukrasch et al., 2009*) or SEC-MALLS (*De Biasio et al., 2014*) data, while FUS LCD should transiently self-associate based on PRE data (*Monahan et al., 2017*). Boxplots show the first quartile, median, and third quartile; whiskers extend from the box to the farthest data point lying within 1.5 times the inter-quartile range from the box, and points outside the whiskers are shown individually. **b.** Interchain PREs calculated from the simulations of two copies of FUS LCD from panel a and comparison with experimental PREs (black) (*Monahan et al., 2017*). PREs are shown for the three spin-label sites at residues 16, 86, and 142 marked with dashed black lines. Rotational correlation time τ_c was selected individually for each λ to minimize χ_r^2 . Error bars represent the standard error of the mean. **c.** χ_r^2 between calculated and experimental PRE data for two copies of FUS LCD shown in panel b.

262 spired by the initial parameterization of Martini proteins, we performed simulations of
263 the cyclohexane/water partitioning of amino acid side chain analogues (*Monticelli et al.,*
264 *2008*). As rescaling protein-protein interactions should not substantially affect the inter-
265 actions of amino acids with water or cyclohexane, we ran a single set of simulations to
266 represent both unmodified Martini 3 and $\lambda_{PP}=0.88$, as well as a set of simulations with
267 protein-water interactions rescaled by $\lambda_{PW}=1.10$. We calculated the transfer free energy
268 from cyclohexane to water, $\Delta G_{\text{CHEX-W}}$, from our simulations and compared them with
269 experimentally determined $\Delta G_{\text{CHEX-W}}$ -values (Fig. S8) (*Radzicka and Wolfenden, 1988;*
270 *Monticelli et al., 2008*). The results show that rescaling protein-water interactions by
271 $\lambda_{PW}=1.10$ slightly increases partitioning to the water phase, as would be expected, but the
272 effect is small when compared with the overall discrepancy between simulation and ex-
273 periment. The two rescaling approaches also provide comparable Pearson correlations
274 with the experimental $\Delta G_{\text{CHEX-W}}$ -values ($r_{\text{Pearson}} = 0.94 \pm 0.03$ and $r_{\text{Pearson}} = 0.95 \pm 0.03$ for
275 $\lambda_{PP}=0.88$ and $\lambda_{PW}=1.10$ respectively). We conclude that the results from the oil/water par-
276 titioning simulations do not clearly favour one rescaling approach over the other. How-
277 ever, the results illustrate that changes in the non-bonded interactions which have a very
278 modest effect on small molecule partitioning may have a much larger effect on protein-
279 protein interactions and the properties of flexible proteins, highlighting the importance
280 of a direct comparison with experiments that report on protein structure.

281 Simulations of the dimerization of side chain analogues have previously been used
282 to shed light on similarities and differences across force fields (*de Jong et al., 2012*). We
283 therefore also performed simulations of the self-association of Phe-Phe, Tyr-Phe, Tyr-Tyr,
284 Lys-Asp, and Arg-Asp side chain analogues. Here $\lambda_{PP}=0.88$ and $\lambda_{PW}=1.10$ both result in a
285 small decrease in self-association as measured by the fraction of time bound throughout
286 the simulations (Fig. S9). The two rescaling approaches also give comparable free energy
287 profiles along the center-of-mass (COM) distance, despite the fact that $\lambda_{PP}=0.88$ results in
288 a rebalancing of the Coulomb and Lennard-Jones potentials in the Lys-Asp and Arg-Asp
289 interactions. Comparing with experimentally measured affinities shows that Martini 3
290 correctly ranks Arg-Asp interactions as stronger than Lys-Asp, and this behaviour is pre-
291 served with both $\lambda_{PP}=0.88$ and $\lambda_{PW}=1.10$ (*Springs and Haake, 1977*). The simulations are
292 also in reasonable agreement with the fraction bound expected from the experimental
293 affinities (*Springs and Haake, 1977*), but perhaps slightly overestimate the strength of
294 the interactions. While the ranking of Tyr-Tyr, Tyr-Phe, and Phe-Phe is consistent with
295 previous analyses of Martini (*de Jong et al., 2012*) and show Phe-Phe to be the strongest
296 in all three versions of Martini 3, analysis of experimental data of disordered proteins
297 suggest that Tyr-Tyr interactions should be stronger than Tyr-Phe or Phe-Phe (*Bremer*
298 *et al., 2021; Tesei et al., 2021b*). Similarly, measurements of vapour pressure show that
299 benzene-phenol interactions are stronger than benzene-benzene interactions (*Christian*
300 *and Tucker, 1982*). These results suggest that a rebalancing of aromatic-aromatic inter-
301 actions in Martini 3 may be necessary to better capture sequence-specific effects in IDPs
302 and multidomain proteins.

303 **Protein-membrane interactions**

304 In the simulations described above, we found that the effects of increasing protein-water
305 interactions or decreasing protein-protein interactions were very similar. We, however,
306 hypothesized that these two force field modifications could have substantially different

307 effects on systems in which proteins interact with other classes of molecules that are
308 not protein or water. We expected that increased protein-water interactions would re-
309 sult in lower affinity for other molecules, which bind in competition with solvation, while
310 decreased protein-protein interactions would not affect the affinity to the same extent,
311 barring any effects of altering the conformational ensemble.

312 To examine the effect of rescaling the Lennard-Jones interaction parameters on the
313 affinity of proteins for different biomolecules, we chose to investigate protein interac-
314 tions with lipid membranes. We had two main motivations for this choice: first, protein-
315 membrane interactions have been thoroughly characterized using Martini (*Yamamoto*
316 *et al., 2015*; *Naughton et al., 2016*; *Srinivasan et al., 2021*); second, Martini has from
317 its early development days in particular been focused on lipid membranes and protein-
318 membrane interactions (*Marrink and Tieleman, 2013*; *Herzog et al., 2016*; *Javanainen*
319 *et al., 2017*).

320 We therefore performed simulations of peripheral proteins in the presence of lipid
321 bilayers, using both unmodified Martini 3 and the two modified versions, $\lambda_{PP}=0.88$ and
322 $\lambda_{PW}=1.10$, following a protocol we have previously described (*Srinivasan et al., 2021*). In
323 short, we ran unbiased MD simulations starting with the protein at a minimum distance
324 of 3 nm away from the bilayer. Over the course of the MD simulation, the proteins in-
325 teract, often transiently and reversibly, with the membrane (Fig. S10-11), and membrane
326 binding was quantified as previously described (*Srinivasan et al., 2021*) based on defining
327 bound states when the minimum distance was lower than or equal to 0.7 nm.

328 To characterize the effect of our rescaling protocol on a broad set of protein-membrane
329 interactions, we selected a diverse set of proteins: (i) one negative control, hen egg-white
330 lysozyme, which is highly soluble in water and is not expected to interact specifically with
331 the membrane in the absence of negatively charged phospholipids (*Howard et al., 1988*);
332 (ii) three peripheral membrane proteins consisting of a single folded domain (Phospholi-
333 pase2, Arf1 in its GTP-bound state, and the C2 domain of Lactadherin) for which we pre-
334 viously characterized the membrane-binding behaviour (*Srinivasan et al., 2021*); (iii) two
335 membrane-binding multidomain proteins: PTEN (1–351), containing a N-terminal Phos-
336 phatase domain and C2 domain that are known to be sufficient for membrane binding,
337 and the Talin FERM domain, that has multiple sub-domains (F0 to F3) and binds to mem-
338 branes through specific phosphoinositol(4,5)phosphate (PIP2) binding sites present in its
339 F2 and F3 subdomains (*Buhr et al., 2023*); (iv) two intrinsically disordered regions (IDRs)
340 that have been characterized as membrane-binding regions: the N-terminal IDR of TRPV4
341 (*Goretzki et al., 2023*) and a short C-terminal motif (CTM) of Complexin (*Snead et al.,*
342 *2014*). For the two IDRs, simulations in solution with both $\lambda_{PP}=0.88$ and $\lambda_{PW}=1.10$ result
343 in expanded ensembles and a larger average value of R_g compared to unmodified Mar-
344 tini 3 (Fig. S12).

345 As hypothesized, the different force field modifications have different effects on protein-
346 membrane interactions (Fig. 6). In particular, we find that simulations with decreased
347 protein-protein interactions ($\lambda_{PW}=0.88$) provide a similar degree of protein-membrane
348 interaction when compared with unmodified Martini-3. In contrast, simulations with an
349 increased strength of protein-water interactions ($\lambda_{PW}=1.10$) show significantly reduced
350 membrane affinity and binding for all proteins, almost always leading to a complete
351 lack of interactions between the protein and the lipid bilayer. Given that $\lambda_{PW}=1.10$ and
352 $\lambda_{PP}=0.88$ provide a comparably good description of IDPs and multidomain proteins in so-

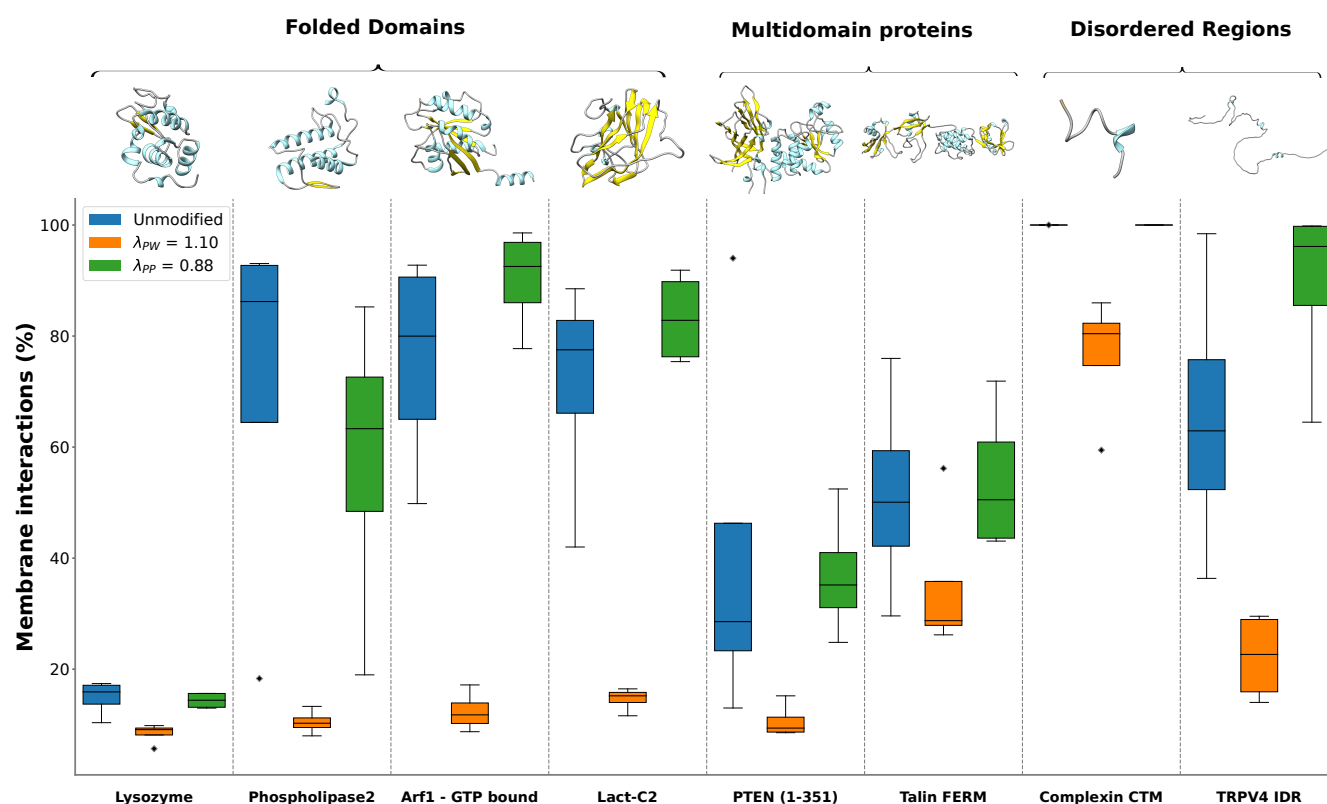


Figure 6. Protein-membrane interactions. MD simulations (four replicas, each 3 μ s long) were performed for peripheral membrane proteins, multidomain proteins, and intrinsically disordered regions with appropriate membrane composition (see Methods for details). Simulations were performed with unmodified Martini 3 (blue), protein-water interactions in Martini 3 rescaled by $\lambda_{PW}=1.10$ (orange), and protein-protein interactions in Martini 3 rescaled by $\lambda_{PP}=0.88$ (green). For each system, the corresponding atomistic structure of the protein is shown on top. Boxplots show the first quartile, median, and third quartile; whiskers extend from the box to the farthest data point lying within 1.5 times the inter-quartile range from the box, and points outside the whiskers are shown individually.

353 lution, and that $\lambda_{PP}=0.88$ more accurately retains the specificity and strength of protein-
 354 membrane interactions as originally parameterized in Martini 3, we suggest that $\lambda_{PP}=0.88$
 355 is overall a more robust and transferable modification to Martini 3.

356 Capturing effects of sequence changes

357 Having selected $\lambda_{PP}=0.88$ as the preferred force field modification for proteins in solution,
 358 we next examined to what extent this force field could capture more subtle sequence
 359 effects in IDPs and multidomain proteins.

360 The $\lambda_{PP}=0.88$ force field provides the same Pearson correlations between experimen-
 361 tal and simulation R_g as unmodified Martini 3, initially suggesting that there is no im-
 362 provement in capturing relative protein-specific differences in R_g (Fig. 3 and 4). To test
 363 this for a series of similar proteins with systematic differences in sequence and structure,
 364 we selected the mTurq-GS $_X$ -mNeon proteins, for which the R_g should increase systemat-
 365 ically with linker length. We calculated the Pearson correlation between simulation and
 366 experimental R_g -values for these proteins with the different force fields (Fig. 7a), and

367 found that the simulations with unmodified Martini 3 only provide a small separation
368 of the R_g -values as a function of linker length, and therefore give a Pearson correlation
369 coefficient with a high degree of uncertainty based on bootstrapping ($r_{Pearson}=0.6\pm 0.6$),
370 while the simulations with rescaled interactions allow for a clearer separation of R_g as
371 a function of linker length ($r_{Pearson}=0.9\pm 0.1$ for both $\lambda_{PW}=1.10$ and $\lambda_{PP}=0.88$). This result
372 suggests that rescaling protein-water or protein-protein interactions allows for a higher
373 sensitivity of ensemble properties to subtle changes in protein sequence and structure,
374 such as differences in interdomain linker length.

375 To further investigate the ability of Martini 3 with rescaled protein-protein interac-
376 tions to capture more subtle sequence effects in IDPs, we performed simulations of six
377 variants of the LCD of hnRNPA1, which have varied composition of charged and aromatic
378 residues while retaining the length of the wild-type sequence (*Bremer et al., 2022*), using
379 unmodified Martini 3 and Martini 3 with $\lambda_{PP}=0.88$. We also performed simulations with
380 $\lambda_{PP}=0.92$, as this provided the optimal agreement with SAXS data for wild-type hnRNPA1.
381 We compared the R_g calculated from the simulations with R_g values measured by SAXS
382 for the six variants and wild-type. As expected based on the results presented above,
383 we found that unmodified Martini 3 substantially underestimates the R_g of all variants
384 (Fig. 7b). While modifying protein-protein interactions by $\lambda_{PP}=0.88$ gives the best results
385 on average across all proteins we studied, it leads to a slight overestimation of the R_g
386 for the wild-type and variants of the LCD from hnRNPA1 (Fig. 7b). If we instead select
387 $\lambda_{PP}=0.92$ as the value of λ_{PP} that gives the best result for the wild-type hnRNPA1 LCD
388 (among the values that we examined) we—per construction—find a more accurate level
389 of expansion across the variants. Equally important, we found that unmodified Martini 3
390 does not accurately capture the variation in R_g associated with the sequence variation
391 ($r_{Pearson}=-0.1\pm 0.5$), while simulations with $\lambda_{PP}=0.92$ and $\lambda_{PP}=0.88$ result in a more accu-
392 rate estimate of the effect of the sequence variation on the R_g values ($r_{Pearson}=0.7\pm 0.3$
393 and $r_{Pearson}=0.9\pm 0.2$ respectively). This result suggests that decreasing the strength of
394 protein-protein interactions in Martini 3 improves the sensitivity of IDP ensemble prop-
395 erties to sequence variation.

396 **Comparison with high-resolution ensembles**

397 Next, we aimed to test the effect of our proposed force field modification by compar-
398 ing our Martini 3 simulations with ensembles produced by higher resolution models.
399 First, we compared our simulations of α -synuclein with extensive atomistic MD simula-
400 tions produced with state-of-the-art force fields. We used an ensemble similarity metric
401 based on dimensionality reduction of the pairwise RMSD between ensemble conformers
402 (*Lindorff-Larsen and Ferkinghoff-Borg, 2009; Tiberti et al., 2015*) to quantitatively com-
403 pare our unmodified and $\lambda_{PP}=0.88$ Martini 3 simulations with atomistic simulations per-
404 formed with the Amber03ws and Amber99SB-disp force fields (*Robustelli et al., 2018*).
405 We note that both Amber03ws and Amber99SB-disp produce ensembles of α -synuclein
406 which are too compact when compared with R_g from SAXS (*Ahmed et al., 2021*), while
407 the ensemble from our Martini 3 simulation with $\lambda_{PP}=0.88$ is more expanded, in excellent
408 agreement with SAXS. In spite of this discrepancy, the ensemble comparison shows that
409 rescaling protein-protein interactions in Martini 3 by $\lambda_{PP}=0.88$ increases the similarity to
410 the atomistic simulations with both Amber03ws and Amber99SB-disp (Table 1). Interest-
411 ingly, the $\lambda_{PP}=0.88$ Martini 3 simulation is more similar to both atomistic simulations than

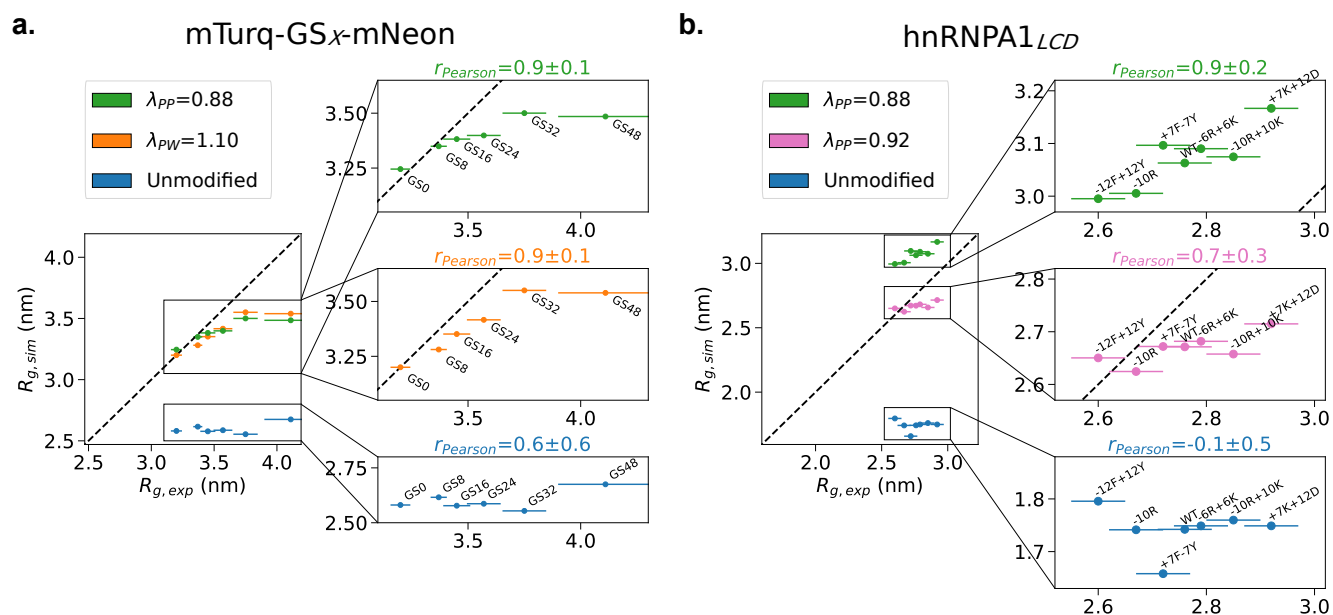


Figure 7. Radii of gyration of mTurq-mNeon and hnRNPA1_{LCD} variants. **a.** R_g calculated from simulations with unmodified Martini 3 (blue), Martini 3 with protein-water interactions rescaled by $\lambda_{PW}=1.10$ (orange), and Martini 3 with protein-protein interactions rescaled by $\lambda_{PP}=0.88$ (green) are plotted against R_g determined by SAXS for mTurquoise2 and mNeonGreen connected by a linker region with the insertion of 0, 8, 16, 24, 32, or 48 GS repeats (*Moses et al., 2024*). **b.** R_g calculated from simulations with unmodified Martini 3 (blue) and Martini 3 with protein-protein interactions rescaled by $\lambda_{PP}=0.92$ (pink) or $\lambda_{PP}=0.88$ (green) are plotted against R_g determined by SAXS for wild-type hnRNPA1_{LCD} and six sequence variants with varied composition of charged and aromatic residues (*Bremer et al., 2022*). We show a zoom-in for each of the force fields along with the given Pearson correlation coefficient with standard error from bootstrapping.

	Martini 3	Martini 3 $\lambda_{pp}=0.88$	A03ws	A99SB-disp
Martini 3	0.0	0.043	0.049	0.065
Martini 3 $\lambda_{pp}=0.88$		0.0	0.039	0.040
A03ws			0.0	0.048
A99SB-disp				0.0

Table 1. Comparison between Martini and atomistic simulations of α -synuclein. Comparison of unmodified Martini 3 and Martini 3 $\lambda_{pp}=0.88$ simulations of α -synuclein with a 20 μ s simulation with the Amber03ws force field and a 73 μ s simulation with the Amber99SB-disp force field from *Robustelli et al. (2018)*. The values shown are the Jensen-Shannon divergence calculated with the DRES approach in Encore (*Lindorff-Larsen and Ferkinghoff-Borg, 2009; Tiberti et al., 2015*) based on the Ca RMSD between structures in the two ensembles. The lower bound is 0, corresponding to two identical ensembles, and the upper bound is $\ln(2)$ (~ 0.69).

	Martini 3	Martini 3 $\lambda_{pp}=0.88$	Expt. ensemble
Martini 3	0.0	0.030	0.087
Martini 3 $\lambda_{pp}=0.88$		0.0	0.077
Expt. ensemble			0.0

Table 2. Comparison between Martini simulations and an experimentally derived ensemble of hnRNPA1. Comparison of unmodified Martini 3 and Martini 3 $\lambda_{pp}=0.88$ simulations of hnRNPA1 with an atomistic ensemble from *Ritsch et al. (2022)* generated based on DEER, PRE, and SAXS data (Protein Ensemble Database PED00212). The values shown are the Jensen-Shannon divergence calculated with the DRES approach in Encore (*Lindorff-Larsen and Ferkinghoff-Borg, 2009; Tiberti et al., 2015*) based on the Ca RMSD between structures in the two ensembles. The lower bound is 0, corresponding to two identical ensembles, and the upper bound is $\ln(2)$ (~ 0.69).

412 the atomistic simulations are to each other, suggesting that the agreement is within the
413 expected variation between force fields.

414 Next we wished to perform a similar test for a multidomain protein. We used the
415 same approach to quantify the similarity between our unmodified and $\lambda_{pp}=0.88$ Mar-
416 tini 3 simulations of hnRNPA1 with an ensemble that was generated based on data from
417 double electron-electron resonance (DEER) electron paramagnetic resonance, PRE, and
418 SAXS experiments (*Ritsch et al., 2022*). Again, the comparison shows that the Martini 3
419 simulation with $\lambda_{pp}=0.88$ is more similar to the experimentally derived atomistic ensem-
420 ble (Table 2). The results from these two test cases suggest that our proposed force field
421 modification of $\lambda_{pp}=0.88$ also improves the agreement with higher resolution simulations
422 and experimentally derived ensemble models.

423 Protein self-association in the membrane

424 To test the effect of rescaling protein-protein and protein-water interactions on protein
425 interactions in a lipid membrane environment, we performed simulations of the homo-
426 dimerization of the transmembrane domain of both EphA1 and ErbB1 from the recep-
427 tor tyrosine kinase (RTK) domain family, which were used as test systems for Martini 3
428 (*Souza et al., 2021*). RTKs are a well-studied protein class for protein-protein interac-
429 tions in a membrane environment and, for both proteins, experimental free energies
430 of association have been determined by Förster resonance energy transfer (FRET) (*Chen*

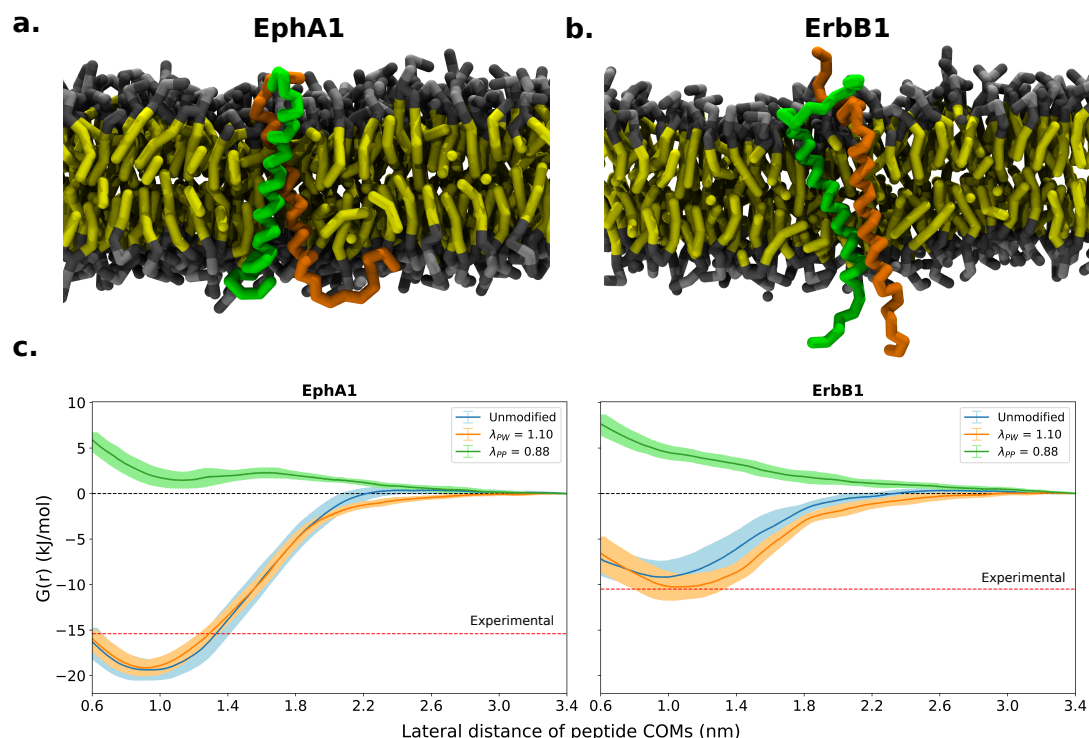


Figure 8. Transmembrane protein self-association. **a-b.** Snapshots of **a.** EphA1 and **b.** ErbB1 simulation systems. The two copies of the protein are shown in green and orange. Lipid heads are shown in gray and lipid tails are shown in yellow. **c.** Potential of mean force profiles for the transmembrane domains of EphA1 and ErbB1 were calculated from simulations with unmodified Martini 3 (blue), Martini 3 with protein-water interactions rescaled by $\lambda_{PW}=1.10$ (orange), and Martini 3 with protein-protein interactions rescaled by $\lambda_{PP}=0.88$ (green). Profiles were aligned to zero at the plateau region at $r=3.4$ nm, indicated by dashed black line. The dashed red line corresponds to experimental values of association free energy (ΔG) from FRET experiments for EphA1 and ErbB1. The error represents the standard deviation of four profiles calculated from 2 μ s blocks.

431 *et al., 2009; Artemenko et al., 2008*). Our results show that unmodified Martini 3 and
 432 Martini 3 with $\lambda_{PW}=1.10$ produce comparable potentials of mean force (PMFs) (Fig. 8),
 433 resulting in overestimated ΔG of association by ~ 4 kJ/mol for EphA1 and reasonable agree-
 434 ment with the experimental ΔG for ErbB1, consistent with the results from *Souza et al.*
 435 (2021). Rescaling protein-protein interactions by $\lambda_{PP}=0.88$ results in a complete loss of
 436 self-association as the PMF profiles becomes repulsive for both proteins (Fig. 8). These re-
 437 sults suggest that, while unmodified Martini 3 and Martini 3 with $\lambda_{PW}=1.10$ may slightly
 438 overestimate protein-protein interactions in the membrane environment, $\lambda_{PP}=0.88$ re-
 439 sults in a substantial underestimation of protein-protein interactions in the membrane,
 440 and is likely not a suitable force field modification for studying oligomerization of trans-
 441 membrane protein systems.

442 Discussion

443 We have previously shown that simulations with Martini 3 underestimate the global di-
 444 mensions of IDPs, and that increasing the strength of protein-water interactions by 10%
 445 results in more expanded ensembles and substantially improves the agreement with

446 SAXS data (*Thomasen et al., 2022*). Here, we expanded this approach to a set of 15 mul-
447 tidomain proteins for which SAXS data have been recorded. Our results show that Mar-
448 tini 3 on average provides too compact ensembles of these multidomain proteins, and
449 that, as was the case for IDPs, rescaling protein-water interactions by 10% substantially
450 improves the agreement with SAXS data. We also show that decreasing the strength of in-
451 teractions between protein beads by 12% results in the same expansion of the ensembles
452 and improved agreement with experiments. We also tested the effect of increasing the
453 strength of interactions between only the protein backbone beads and water, but did not
454 find that this provides any further improvement in the agreement with the experimental
455 data. While the different rescaling approaches provide essentially the same results for
456 proteins in solution, we show that rescaling protein-protein interactions is the preferable
457 option in order to best retain the specificity and strength of protein-membrane interac-
458 tions as originally parameterized in Martini 3. We note, however, that this change to the
459 force field leads to decreased dimerization of proteins within a membrane environment,
460 and a significant underestimation of free energies of dimerization. Therefore, we sug-
461 gest that decreasing the strength of protein-protein interactions by 12% is suitable for
462 systems with flexible proteins in solution and in proximity to membranes, but likely not
463 for systems with specific protein-protein interactions in the membrane. An important
464 outcome of our work is also the curation of a set of multidomain proteins with available
465 SAXS data and starting structures for simulations, which can be used for future research
466 in force field assessment and development.

467 One of the challenges when running Martini 3 simulations of multidomain proteins
468 is selecting which regions to keep folded with the elastic network model and which re-
469 gions to leave unrestrained. In this work, we manually selected the folded domains in
470 the structures using domain annotations and intuition. It is, however, difficult to know
471 *a priori* whether distinct domains should act as single structural modules due to specific
472 interactions or move freely with respect to one another. Recently, it has been proposed
473 to use the pairwise alignment error output from AlphaFold2 predictions to assign auto-
474 matically the elastic network restraints (*Jussupow and Kaila, 2023*). In future work, this
475 may provide a more accurate distinction between domains that should be relatively rigid
476 or dynamic with respect to each other. Additionally, replacing the elastic network model
477 with a more flexible structure-based model (*Go, 1983*) may provide the ability to sample
478 both the bound and unbound state in cases where folded domains have specific interac-
479 tions (*Poma et al., 2017*). In stronger and more specific interdomain interactions, the res-
480 olution of Martini 3 may also play a more important role. For example, water-mediated
481 hydrogen-bonding networks would not be captured with the 4-to-1 mapping of water
482 beads. As most of the proteins presented in this work likely do not have very specific
483 interactions between domains, the lack of structured water is presumably not an issue.

484 Although the simple approach of decreasing the strength of protein-protein interac-
485 tions uniformly by 12% shows an improvement over unmodified Martini 3 in reproducing
486 the global dimensions of IDPs and multidomain proteins, we note that the agreement
487 with the SAXS data is still not perfect ($\chi_r^2 > 1$ in most cases), and there are systematic out-
488 liers with respect to the experimental R_g values. Although some of the system-specific
489 deviations could potentially be alleviated by e.g. more accurately assigning and model-
490 ing the restraints on the folded domains, the overall deviation from the experimental
491 data suggests that a more fundamental rebalancing of non-bonded interactions, and

perhaps also CG mapping scheme, is necessary to describe the behaviour of IDPs and multidomain proteins within the Martini framework. Again, we suggest that the data we have collected here will be useful to test any such changes, and the results obtained with $\lambda_{pp}=0.88$ are a useful point of reference for other force field modifications. The increased sensitivity to sequence perturbations observed for the hnNRPA1 sequence variants and the series of mTurq-GS_X-mNeon proteins also suggests that $\lambda_{pp}=0.88$ could provide a good starting point for rebalancing protein interactions at the amino acid or bead level to improve the specificity in weaker protein-protein interactions.

For other types of systems, it has been suggested that the non-bonded interactions in Martini 3 must be rescaled to a different extent to reach agreement with experimental observations. For example, modifying protein-water interactions in Martini 3 affects the propensity of the disordered LCD of FUS to form condensates in a way that appears to depend on the salt concentration (Zerze, 2023), while the insertion of transmembrane helices into the phospholipid bilayer may require decreased protein-water interactions (Claveras Cabezudo et al., 2023). Additionally, unmodified Martini 3 has been shown to provide accurate free energies of dimerization for transmembrane proteins (Souza et al., 2021). Our results show that this behavior is preserved when rescaling protein-water interactions, whereas decreasing the strength of protein-protein interactions is likely not suitable for systems with specific protein self-association in the membrane. In light of these results, it seems that uniformly rescaling non-bonded interactions may not be able to provide a universally transferable protein model within the Martini framework, and that a more detailed rebalancing of interactions or CG mapping scheme is necessary. Future work could, for example, examine the combined effects of more modest rescaling of protein-protein and protein-water interactions, or focus on secondary-structure dependent force field parameters as recently proposed for another CG force field (Yamada et al., 2023).

Overall, however, our results demonstrate that for soluble proteins decreasing the non-bonded interactions between all protein beads by 12% leads to a more accurate balance of interactions while retaining the specificity of protein-membrane interactions. We foresee that our protocol will be a useful starting point to investigate the interactions of IDPs with lipid membranes using chemically transferable MD simulations, and that these investigations will further provide insights into possible strategies on future force field development efforts. Since CG simulations also play an important role in integrative structural biology (Thomassen and Lindorff-Larsen, 2022), we also expect that these developments will enable an even tighter link between simulations and experiments to study large and complex biomolecular assemblies.

Methods

IDP simulations

We performed MD simulations of a set of 12 IDPs with SAXS data available (Table 3) and five IDPs with intramolecular PRE data available (Table 4) (Tesei et al., 2021b; Thomassen et al., 2022) using Gromacs 2020.3 (Abraham et al., 2015). We ran simulations with the Martini 3.0 force field (Souza et al., 2021) with the well-depth, ϵ , in the Lennard-Jones potential between all protein beads rescaled by a factor λ_{pp} or with ϵ in the Lennard-Jones potential between all protein backbone and water beads rescaled by a factor λ_{PW-BB} .

Protein	N_R	SAXS R_g (nm)	T (K)	c_s (M)	SAXS ref.
Hst5	24	1.34 ± 0.05	293	0.15	<i>Jepthah et al. (2019)</i>
Hst5 ₂	48	1.77 ± 0.049	298	0.15	<i>Fagerberg et al. (2020)</i>
ACTR _{AD}	71	2.55 ± 0.27	278	0.2	<i>Kjaergaard et al. (2010)</i>
Sic1	92	2.86 ± 0.14	293	0.2	<i>Gomes et al. (2020)</i>
ColN _T	98	2.82 ± 0.034	277	0.4	<i>Johnson et al. (2017)</i>
Tau _{K19}	99	3.35 ± 0.29	288	0.15	<i>Mylonas et al. (2008)</i>
hnRNPA1 _{LCD}	137	2.55 ± 0.1	296	0.05	<i>Martin et al. (2020)</i>
α Syn	140	3.56 ± 0.036	293	0.2	<i>Ahmed et al. (2021)</i>
FhuA	144	3.21 ± 0.22	298	0.15	<i>Riback et al. (2017)</i>
Tau _{K25}	185	4.06 ± 0.28	288	0.15	<i>Mylonas et al. (2008)</i>
CoR _{NID}	271	4.72 ± 0.12	293	0.2	<i>Cordeiro et al. (2019)</i>
PRN _{Nt}	334	4.96 ± 0.56	298	0.15	<i>Riback et al. (2017)</i>

Table 3. IDPs with available SAXS data. Number of amino acid residues (N_R), experimental R_g , temperature (T), and salt concentration (c_s) used in simulations, and the reference for the SAXS data used.

536 We generated CG structures using Martinize2 based on initial all-atom structures corre-
537 sponding to the 95th percentile of the R_g -distributions from simulations in *Tesei et al.*
538 *(2021b)*. Secondary structure and elastic restraints were not assigned for IDPs. Struc-
539 tures were placed in a dodecahedral box using Gromacs editconf and solvated, with NaCl
540 concentrations corresponding to the ionic strength used in SAXS or PRE experiments, us-
541 ing the Insane python script (*Wassenaar et al., 2015*). The systems were equilibrated for
542 10 ns with a 2 fs time step using the Velocity-Rescaling thermostat (*Bussi et al., 2007*)
543 and Parinello-Rahman barostat (*Parrinello and Rahman, 1981*). Production simulations
544 were run for 40 μ s with a 20 fs time step using the Velocity-Rescaling thermostat (*Bussi*
545 *et al., 2007*) and Parinello-Rahman barostat (*Parrinello and Rahman, 1981*). The simula-
546 tion temperature was set to match the SAXS or PRE experiment, and the pressure was set
547 to 1 bar. Non-bonded interactions were treated with the Verlet cut-off scheme. A cut-off
548 of 1.1 nm was used for van der Waals interactions. A dielectric constant of 15 and cut-off
549 of 1.1 nm were used for Coulomb interactions. Simulation frames were saved every 1 ns.
550 Molecule breaks from crossing the periodic boundaries were treated with Gromacs trj-
551 conv using the flags: -pbc whole -center. Convergence of the simulations was assessed by
552 block-error analysis (*Flyvbjerg and Petersen, 1989*) of R_g calculated from simulation co-
553 ordinates using the blocking code from: <https://github.com/fpesceKU/BLOCKING>. All CG
554 trajectories were back-mapped to all-atom structures using a simplified version (*Larsen*
555 *et al., 2020*) of the Backward algorithm (*Wassenaar et al., 2014*), in which simulation runs
556 are excluded and the two energy minimization runs are shortened to 200 steps.

557 **Multidomain protein structures**

558 We performed MD simulations of a set of 15 multidomain proteins with SAXS data avail-
559 able (Table 5). We built the initial structure of MyBP-C_{MTHB-C2} based on the NMR structure
560 containing both domains (PDB: 5K6P) (*Michie et al., 2016*). We built the structures of the
561 linear polyubiquitin chains, Ubq₂, Ubq₃, and Ubq₄, based on the crystal structure of the
562 open conformation of Ubq₂ (PDB: 2W9N) (*Komander et al., 2009*). For Ubq₃ and Ubq₄, the

Protein	N_R	T (K)	c_s (M)	PRE ref.
α Syn	140	283	0.125	<i>Dedmon et al. (2005)</i>
hnRNPA2 _{LCD}	155	298	0.005	<i>Ryan et al. (2018)</i>
FUS _{LCD}	163	298	0.15	<i>Monahan et al. (2017)</i>
OPN	220	298	0.15	<i>(Platzer et al., 2011)</i>
hTau40	441	278	0.1	<i>(Mukrasch et al., 2009)</i>

Table 4. IDPs with available PRE data. Number of amino acid residues (N_R), temperature (T), and salt concentration (c_s) used in simulations, and the reference for the PRE data used.

Protein	N_R	SAXS R_g (nm)	T (K)	c_s (M)	SAXS ref.
MyBP-C _{MTHB-C2}	137	1.91 ± 0.08	277	0.15	<i>Michie et al. (2016)</i>
Ubq ₂	162	2.2 ± 0.18	293	0.33	<i>Jussupow et al. (2020)</i>
Ubq ₃	228	2.62 ± 0.02	293	0.33	<i>Jussupow et al. (2020)</i>
Gal-3	250	2.91 ± 0.06	303	0.04	<i>Lin et al. (2017)</i>
TIA1	275	2.75 ± 0.05	300	0.1	<i>Sonntag et al. (2017)</i>
Ubq ₄	304	3.19 ± 0.09	293	0.33	<i>Jussupow et al. (2020)</i>
hnRNPA1	314	3.12 ± 0.08	300	0.15	<i>Martin et al. (2021)</i>
MyBP-C _{C5-C6-C7}	328	3.75 ± 0.08	298	0.28	<i>Nadvi et al. (2016)</i>
hisSUMO-hnRNPA1	433	3.4 ± 0.13	300	0.1	<i>Martin et al. (2021)</i>
mTurq-mNeon	470	3.20 ± 0.04	293	0.15	<i>Moses et al. (2024)</i>
mTurq-GS ₈ -mNeon	486	3.37 ± 0.04	293	0.15	<i>Moses et al. (2024)</i>
mTurq-GS ₁₆ -mNeon	502	3.45 ± 0.06	293	0.15	<i>Moses et al. (2024)</i>
mTurq-GS ₂₄ -mNeon	518	3.57 ± 0.08	293	0.15	<i>Moses et al. (2024)</i>
mTurq-GS ₃₂ -mNeon	534	3.8 ± 0.1	293	0.15	<i>Moses et al. (2024)</i>
mTurq-GS ₄₈ -mNeon	566	4.1 ± 0.21	293	0.15	<i>Moses et al. (2024)</i>

Table 5. Multidomain proteins with available SAXS data. Number of amino acid residues (N_R), experimental R_g , temperature (T), and salt concentration (c_s) used in simulations, and the reference for the SAXS data used.

563 linker regions between the original and extended structures were remodelled using Mod-
564 eller (*Šali and Blundell, 1993*). We built the initial structure of Gal-3 based on the crystal
565 structure of the folded C-terminal domain (PDB: 2NMO) (*Collins et al., 2007*) and the IDR
566 from the AlphaFold structure of full-length Gal3 (AF-P17931-F1) (*Jumper et al., 2021; Tun-*
567 *yasuvunakool et al., 2021*). We built the structure of MyBP-C_{C5-C6-C7} based on the NMR
568 structure of the C5 domain (PDB: 1GXE) (*Idowu et al., 2003*), and the AlphaFold structure
569 of the full-length MyBP-C (AF-Q14896-F1) (*Jumper et al., 2021; Tunyasuvunakool et al.,*
570 *2021*). We inserted missing residues in the NMR structure of the C5 domain using Mod-
571 eller (*Šali and Blundell, 1993*). For the mTurq-GS_x-mNeon constructs, we used structures
572 from Monte-Carlo simulations in *Moses et al. (2024)* as starting structures for our simu-
573 lations. To validate the starting structures, we calculated the RMSD between the two
574 fluorescent protein domains and corresponding crystal structures (mTurquoise2 (PDB:
575 4AR7) (*von Stetten et al., 2012*) and mNeonGreen (PDB: 5LTR) (*Clavel et al., 2016*)) using
576 PyMOL align, which gave an RMSD of 0.2-0.3 Å.

577 **Multidomain protein simulations**

578 We ran MD simulations of the set of multidomain proteins using Gromacs 2020.3 (*Abra-*
579 *ham et al., 2015*). We ran simulations with the Martini 3.0 force field (*Souza et al., 2021*),
580 as well as several modified versions of Martini 3.0 in which the well-depth, ϵ , in the
581 Lennard-Jones potential between all protein and water beads was rescaled by a factor
582 λ_{PW} , ϵ in the Lennard-Jones potential between all protein beads was rescaled by a factor
583 λ_{PP} , or ϵ in the Lennard-Jones potential between all protein backbone and water beads
584 was rescaled by a factor λ_{PW-BB} . We assigned secondary structure-specific potentials
585 using DSSP (*Kabsch and Sander, 1983*) and Martinize2. The secondary structure of all
586 residues in linkers and IDRs were manually assigned to coil, turn, or bend. We applied
587 an elastic network model using Martinize2 consisting of harmonic potentials with a force
588 constant of $700 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ between all backbone beads within a cut-off distance of
589 0.9 nm. We removed the elastic network potentials in all linkers and IDRs and between
590 folded domains, so only the structures of individual folded domains were restrained (Ta-
591 ble S1). Dihedral and angle potentials between sidechain and backbone beads were as-
592 signed using the -scfix flag in Martinize2, but removed in all linkers and IDRs. Structures
593 were placed in a dodecahedral box using Gromacs editconf and solvated, with NaCl con-
594 centrations corresponding to the ionic strength used in SAXS experiments, using the In-
595 sane python script (*Wassenaar et al., 2015*). The systems were equilibrated for 10 ns
596 with a 2 fs time step using the Berendsen thermostat and Berendsen barostat (*Berend-*
597 *sen et al., 1984*). Production simulations were run for at least 40 μs with a 20 fs time step
598 using the Velocity-Rescaling thermostat (*Bussi et al., 2007*) and Parinello-Rahman baro-
599 stat (*Parrinello and Rahman, 1981*). The simulation temperature was set to match the
600 corresponding SAXS experiment and the pressure was set to 1 bar. Non-bonded interac-
601 tions were treated with the Verlet cut-off scheme. A cut-off of 1.1 nm was used for van
602 der Waals interactions. A dielectric constant of 15 and cut-off of 1.1 nm were used for
603 Coulomb interactions. Simulation frames were saved every 1 ns. Molecule breaks from
604 crossing the periodic boundaries were treated with Gromacs trjconv using the flags: -
605 pbc whole -center. Convergence of the simulations was assessed by block-error analysis
606 (*Flyvbjerg and Petersen, 1989*) of R_g calculated from simulation coordinates using the
607 blocking code from: <https://github.com/fpesceKU/BLOCKING>. All CG trajectories were
608 back-mapped to all-atom structures using a simplified version (*Larsen et al., 2020*) of
609 the Backward algorithm (*Wassenaar et al., 2014*), in which simulation runs are excluded
610 and the two energy minimization runs are shortened to 200 steps.

611 **Simulations of protein self-association in solution**

612 We ran MD simulations of two copies of the two folded proteins ubiquitin and villin HP36,
613 and the four IDPs FUS_{LCD}, α -synuclein, hTau40, and p15PAF, as previously described
614 (*Thomassen et al., 2022*), using the Martini 3.0 force field (*Souza et al., 2021*) with the
615 well-depth, ϵ , in the Lennard-Jones potential between all protein beads rescaled by a fac-
616 tor $\lambda_{PP}=0.88$. We used PDB ID 1UBQ (*Vijay-Kumar et al., 1987*) and PDB ID 1VII (*McKnight*
617 *et al., 1997*) as starting structures for ubiquitin and villin HP36, respectively. The simu-
618 lations were set up and run using the same protocol as for IDP simulations. Two copies
619 of ubiquitin, villin HP36, FUS_{LCD}, α -synuclein, hTau40, and p15PAF were placed in cubic
620 boxes with side lengths 14.92, 7.31, 40.5, 25.51, 48.02, and 34.15 nm giving protein con-
621 centrations of 1000, 8500, 50, 200, 30, and 83.4 μM respectively. NaCl concentrations and

Protein	N_R	d (nm)	T (K)	c_s (M)	c_p (μ M)	Self-association ref.
α Syn	140x2	25.51	283	0.125	200	<i>Dedmon et al. (2005)</i>
FUS _{LCD}	163x2	40.5	298	0.15	50	<i>Monahan et al. (2017)</i>
p15PAF	111x2	34.15	298	0.15	83.4	<i>De Biasio et al. (2014)</i>
hTau40	441x2	48.02	278	0.1	30	<i>Mukrasch et al. (2009)</i>

Table 6. IDPs with available self-association data. Number of amino acid residues (N_R), cubic box side lengths (d), simulation temperature (T), salt concentration (c_s), initial protein concentration (c_p) used in simulations, and the reference for the self-association data.

Protein	N_R	d (nm)	K_d (mM)	T (K)	c_s (M)	c_p (mM)	Self-association ref.
Villin HP36	36x2	7.31	>1.5	298	0.15	8.5	<i>Brewer et al. (2005)</i>
Ubq	76x2	14.92	4.9 ± 0.3	303	0.11	1.0	<i>Liu et al. (2012)</i>

Table 7. Folded proteins with available self-association data. Number of amino acid residues (N_R), cubic box side lengths (d), experimental K_d for self-association, temperature (T), salt concentration (c_s), initial protein concentration (c_p) used in simulations, and the reference for the self-association data.

622 temperatures were set according to the corresponding experimental conditions (Table 6
623 and 7). For ubiquitin and villin HP36 the following steps were also used in the simula-
624 tion setup: (i) Secondary structure was assigned with DSSP (*Kabsch and Sander, 1983*)
625 in Martinize2. (ii) An elastic network model was applied with Martinize2. The elastic re-
626 straints consisted of a harmonic potential of $700 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ between backbone beads
627 within a 0.9 nm cut-off. For ubiquitin, we removed elastic restraints from the C-terminus
628 (residues 72–76) to allow for flexibility (*Lindorff-Larsen et al., 2005*). (iv) Dihedral and
629 angular potentials between side chains and backbone beads were added based on the
630 initial structures with the -scfix flag in Martinize2. For ubiquitin, villin HP36, α -synuclein,
631 and p15PAF we ran 10 replica simulations of 40 μ s per replica. For hTau40 and FUS_{LCD},
632 we ran 10 replica simulations of 13 μ s and 25 μ s per replica respectively.

633 We analyzed the population of the bound states in our simulations by calculating the
634 minimum distance between beads in the two protein copies over the trajectory with Gro-
635 macs mindist. The fraction bound was defined as the fraction of frames where the min-
636 imum distance was below 0.8 nm. For ubiquitin and villin HP36, we calculated the ex-
637 pected fraction of bound protein at the concentrations in our simulations based on the
638 respective K_d -values of 4.9 mM and 1.5 mM determined for self-association (*Liu et al.,*
639 *2012; Brewer et al., 2005*). The bound fraction was calculated as

$$\phi_b = \frac{4C_p + K_d - \sqrt{8K_d C_p + K_d^2}}{4C_p} \quad (1)$$

640 where ϕ_b is the bound fraction, C_p is the concentration of protein in the simulation box
641 (using the average box volume over all simulation trajectories), and K_d is the dissociation
642 constant.

643 **Amino acid side chain analogue simulations**

644 The Martini 3 parameters for amino acid side chain analogues were produced based
645 on the existing amino acid parameters by simply removing the backbone bead and any
646 potentials or exclusions involving the backbone bead. For simulations of Arg-Asp side
647 chain analogue self-association, the SC1 bead was also removed from Arg (leaving only
648 the SC2 bead of type SQ3p) in order to best emulate the guanidine-acetate system used
649 to measure the experimental affinity (*Springs and Haake, 1977*).

650 We ran MD simulations of two copies of Tyr and Phe side chain analogues, as well
651 as Tyr-Phe, Arg-Asp, and Lys-Asp side chain analogues using the Martini 3.0 force field
652 (*Souza et al., 2021*) either unmodified or with the well-depth, ϵ , in the Lennard-Jones po-
653 tential between all protein and water beads rescaled by a factor $\lambda_{pW}=1.10$ or ϵ in the
654 Lennard-Jones potential between all protein beads rescaled by a factor $\lambda_{pp}=0.88$. The
655 simulations were set up and run using the same protocol as for IDP simulations. The
656 two side chain analogues were placed in a cubic box with a side length of 5 nm. A NaCl
657 concentration of 150 mM was used for Phe-Phe, Tyr-Phe, Tyr-Tyr simulations. No NaCl
658 was added in the Lys-Asp and Arg-Asp systems. The simulations were run for 100 μ s each
659 at 300 K. The fraction bound was calculated from simulations and dissociation constants
660 (K_d) using the same approach as for protein self-association in solution described above.
661 Experimental association constants (K_a) of 0.4 M⁻¹ for Phe-Phe (benzene-benzene) and
662 0.6 M⁻¹ for Phe-Tyr (benzene-phenol) were obtained from *Christian and Tucker (1982)*.
663 Experimental K_a -values of 0.31 M⁻¹ for Lys-Asp (butylammonium-acetate) and 0.37 M⁻¹
664 for Arg-Asp (guanidine-acetate) were obtained from *Springs and Haake (1977)*. For equa-
665 tion 1, $K_d = 1/K_a$ was used. In order to calculate the free energy profiles along the COM
666 distance, we calculated the COM distance between side chain analogues using Gromacs
667 distance, calculated the probability density using the histogram function in NumPy (*Har-*
668 *ris et al., 2020*), and calculated the free energy (in units of $k_B T$) as: $\Delta G = -\ln(P(r_{COM}))$,
669 where $P(r_{COM})$ is the probability density along the COM distance.

670 We also ran MD simulations of the cyclohexane/water partitioning of the uncharged
671 amino acid side chain analogues for which experimental transfer free energies were
672 taken from *Radzicka and Wolfenden (1988)*; *Monticelli et al. (2008)* using unmodified
673 Martini 3.0 and Martini 3.0 with $\lambda_{pW}=1.10$. We prepared a simulation box with 716 copies
674 of Martini 3 cyclohexane (CHEX) and water (W) respectively. For each partitioning simu-
675 lation, we added a single copy of a side chain analog. Simulations were set up and run
676 using the same protocol as for IDP simulations. Each simulation was run for 100 μ s at
677 300 K.

678 We calculated the number of contacts between beads in the side chain analogue and
679 CHEX or W over the simulation using Gromacs mindist with a cut-off of 0.8 nm. For a
680 given frame, we considered the side chain analogue as partitioned to the phase with the
681 most contacts (frames with an equal number of CHEX and W contacts were discarded).
682 We then calculated the transfer free energy from cyclohexane to water as:

$$\Delta G_{\text{CHEX-W}} = RT \ln \left(\frac{(n_{\text{CHEX}}/\phi_{\text{CHEX}})}{(n_{\text{W}}/\phi_{\text{W}})} \right) \quad (2)$$

683 where R is the gas constant, T is the temperature, n_{CHEX} and n_{W} are the number of
684 simulation frames where the side chain analogue is in the cyclohexane or water phase
685 respectively, and ϕ_{CHEX} and ϕ_{W} are the respective volume fractions of the cyclohexane

686 and water phases in the simulations. To determine ϕ_{CHEX} and ϕ_{W} , we calculated the
687 average densities of cyclohexane and water along the z-coordinate in our partitioning
688 simulations of the Ser side chain analogue with unmodified Martini 3 and selected the
689 cut-off between the two phases as the crossover points of the respective densities. The
690 Pearson correlations with experimental transfer free energies were calculated using the
691 `pearsonr` function in SciPy stats and standard errors were determined with bootstrap-
692 ping using the `bootstrap` function in SciPy stats with 9999 resamples (*Virtanen et al.,*
693 *2020*).

694 **hnRNPA1 LCD variant simulations**

695 We ran MD simulations of a set of six variants of the hnRNPA1 LCD (-10R, -10R+10K, -
696 12F+12Y, -6R+6K, +7F-7Y, +7K+12D) for which the R_g has previously been determined by
697 SAXS experiments (*Bremer et al., 2022*). The variants contain substitutions to and from
698 charged and aromatic residues, but have the same sequence length as the wild-type pro-
699 tein, and were selected to have a relatively large deviation in R_g from the wild-type; pro-
700 tein sequences can be found in the supporting information of *Bremer et al. (2022)*. We
701 ran MD simulations with unmodified Martini 3.0 and Martini 3.0 with ϵ in the Lennard-
702 Jones potential between all protein beads rescaled by a factor $\lambda_{\text{PP}}=0.92$ or $\lambda_{\text{PP}}=0.88$. Sim-
703 ulations were set up using the same protocol as for the other IDPs described above. The
704 systems were equilibrated for 10 ns with a 2 fs time step using the Berendsen thermo-
705 stat and Berendsen barostat (*Berendsen et al., 1984*). Production simulations were run
706 for 100 μs with a 20 fs time step using the Velocity-Rescaling thermostat (*Bussi et al.,*
707 *2007*) and Parinello-Rahman barostat (*Parinello and Rahman, 1981*). Simulations were
708 run with 150 mM NaCl at 298 K and 1 bar.

709 **Peripheral membrane protein simulations**

710 We performed MD simulations of one negative control, three peripheral membrane pro-
711 teins, two multidomain proteins, and two intrinsically disordered regions with lipid bi-
712 layers of different compositions (Table 8). We ran simulations with the Martini 3 force
713 field (*Souza et al., 2021*), or with modified force fields in which ϵ in (i) the Lennard-Jones
714 potential between all protein beads were rescaled by a factor $\lambda_{\text{PP}}=0.88$ or (ii) with ϵ in
715 the Lennard-Jones potential between all protein and water beads rescaled by a factor
716 $\lambda_{\text{PW}}=1.10$.

717 Initial structures of proteins were obtained either from the RCSB database (*Rose et al.,*
718 *2012*) or from the AlphaFold protein structure database (*Varadi et al., 2022*). For Com-
719 plexin CTM, we used ColabFold v1.5.2 (*Mirdita et al., 2022*) to model 16-residues long (AT-
720 GAFETVKGFFPFGK) disordered region. The N-terminal IDR of TRPV4 (residues 2–134) was
721 taken from the full-length AlphaFold structure of TRPV4 (A0A1D5PXA5). Initial structure
722 of the FERM domains in Talin (PDB:3IVF) had missing residues (134–172), which we mod-
723 elled using MODELLER (*Webb and Sali, 2016*) via the Chimera interface (*Pettersen et al.,*
724 *2004*). CG structures of proteins were generated using Martinize2, with DSSP (*Kabsch*
725 *and Sander, 1983*) flag to assign secondary structures. An elastic network was applied
726 with a harmonic potential of a force constant $700 \text{ kJ mol}^{-1} \text{ nm}^{-2}$ between all backbone
727 beads within a cut-off of 0.8 nm. We removed elastic network potentials between dif-
728 ferent domains and in linkers and in IDRs of multidomain proteins. Secondary structure
729 and elastic network was not assigned to the two IDRs.

Protein	PDB ID	N_R	Bilayer composition
Lysozyme	1AKI	129	DOPC
Phospholipase2	1POA	118	DOPC
Arf1-GTP bound	2KSQ	181	DOPC
Lact-C2	3BN6	158	DOPC
PTEN (1-351)	AF-F6KD01-F1	351	DOPC:DOPS (8:2)
Talin's FERM	3IVF	368	POPC:PIP ₂ (10% PIP ₂ in upper leaflet)
Complexin CTM	Modelled with ColabFold	16	POPC:POPS (7:3)
TRPV4 IDR	AF-A0A1D5PXA5-F1	133	POPC:DOPS:PIP ₂ (7:2:1)

Table 8. Membrane-protein systems. Structure (PDB ID), number of amino acid residues (N_R) in protein, and lipid composition in the membrane bilayer used in the simulations. Structures starting with AF prefix are AlphaFold-predicted structures (*Varadi et al., 2022*).

730 All the lipid bilayers, with initial lateral dimension of 20 nm × 20 nm, were gener-
731 ated using CHARMM-GUI Martini maker (*Qi et al., 2015*), except in the systems where
732 phosphoinositol-(4,5)-phosphate (PIP2) lipids were needed, which instead were gener-
733 ated using the Insane python script (*Wassenaar et al., 2015*). We used the parameter for
734 SAP2_45 lipids (*Borges-Araújo et al., 2021*) to model PIP2 in the bilayer. The bilayers gener-
735 ated from CHARMM-GUI were then minimized and equilibrated following the 6-step
736 equilibration protocol. To compute protein-membrane interactions, systems were gener-
737 ated as previously described (*Srinivasan et al., 2021*), with a minimum distance of 3 nm
738 between any bead of protein and any beads of lipid. Systems were first energy minimized
739 using steepest descent algorithm after which a short MD run of 200 ps was performed
740 with the protein backbone beads restrained. Production simulations (four replicas for
741 each system) were run for 3 μs with a time step of 20 fs using velocity-rescale thermostat
742 (*Bussi et al., 2007*) and Parrinello-Rahman barostat (*Parrinello and Rahman, 1981*).

743 We performed MD simulation of the two IDRs (Complexin CTM and TRPV4 IDR) in solu-
744 tion with unmodified Martini 3 and both of the modified versions of Martini 3. For these
745 simulations, we took the CG structure and placed it in a cubic box using Gromacs edit-
746 conf, and solvated and ionized with a concentration of 150 mM of NaCl. Then the system
747 was minimized for 10000 steps with steepest descent algorithm and a short equilibra-
748 tion run was performed with Berendsen thermostat and Berendsen barostat (*Berend-
749 sen et al., 1984*) with a time step of 2 fs. Production simulations were run for 10 μs with
750 a 20 fs time-step using Parrinello-Rahman barostat (*Parrinello and Rahman, 1981*) and
751 velocity-rescaling thermostat (*Bussi et al., 2007*). All the simulations were performed with
752 GROMACS 2021.5 (*Abraham et al., 2015*). Initial 100 ns of production run were discarded
753 from all the trajectories for further analysis.

754 Simulations of transmembrane protein self-association

755 We performed simulation of the transmembrane domains of two protein dimers from
756 the RTK family to calculate the free energy of association, ΔG , using the Martini 3.0 force
757 field (*Souza et al., 2021*) either unmodified or with the well-depth, ϵ , in the Lennard-Jones
758 potential between all protein and water beads rescaled by a factor $\lambda_{PW}=1.10$ or ϵ in the
759 Lennard-Jones potential between all protein beads rescaled by a factor $\lambda_{PP}=0.88$. Sim-
760 ulations were performed with Gromacs 2021.5. PDB 2K1L (*Bocharov et al., 2008*) was

Protein	PDB ID	Bilayer	T (K)	c_s (M)	Self-association ref.
EphA1	2K1L	DLPC	303	0.15	<i>Artemenko et al. (2008)</i>
ErbB1	2M0B	DLPC	303	0.5	<i>Chen et al. (2009)</i>

Table 9. Transmembrane proteins with available self-association data. Structure (PDB ID), lipid composition in the membrane bilayer used in simulations, temperature (T), salt concentration (c_s), and the reference for the self-association data.

761 used as the starting structure for EphA1. We used Charmm-GUI (*Qi et al., 2015*) to em-
762 bed the EphA1 dimer in a bilayer of 400 DLPC lipids and 0.5 M NaCl corresponding to the
763 conditions in the reference experiment (*Artemenko et al., 2008*), as in (*Javanainen et al.,*
764 *2017*). The system was equilibrated using the standard six-step protocol in Charmm-GUI.
765 For ErbB1, the starting structure of the system, based on PDB 2M0B (*Bocharov et al.,*
766 *2016*), was taken from *Souza et al. (2021)*. The system has 400 DLPC lipids and 0.15 M
767 NaCl corresponding to the conditions in the reference experiment (*Chen et al., 2009*).
768 The system was equilibrated for 50 ns in the NPT ensemble with position restraints of
769 1000 kJ/(mol nm²) on both chain of the dimer.

770 For both systems, pulling simulations were run for 100 ns at a rate of 0.05 nm/ns. The
771 2D COM distance between the protein subunits, r_{COM} , was used as the reaction coordi-
772 nate. Frames ranging from 0.6 nm to 3.4 nm with a spacing of 0.2 nm were extracted
773 from the pulling simulation trajectories as umbrella sampling windows, to be consistent
774 with previous work (*Souza et al., 2021*). A spring constant of 400 kJ/(mol nm²) was ap-
775 plied as the umbrella potential in production runs. The temperature was maintained at
776 303 K separately for peptides, lipids, and solvents and semi-isotropic pressure coupling
777 was applied at 1 bar. The production run was performed for 10 μ s in each window using
778 a time-step of 20 fs and the Gromacs wham tool (*Hub et al., 2010*) was used to obtain
779 the PMF. The error in PMF plots represents the standard deviation of 4 profiles calcu-
780 lated from 2 μ s blocks, with the first 2 μ s were discarded. All PMFs plateaued before
781 $r_{COM}=3.4$ nm and were aligned to zero at this value. ΔG values were estimated from the
782 minimum of zero aligned PMFs for comparison with experimental values.

783 SAXS calculations

784 We extracted 20,000 evenly distributed frames from each back-mapped trajectory to cal-
785 culate SAXS profiles using Pepsi-SAXS (*Grudin et al., 2017*). To avoid overfitting the
786 parameters for the contrast of the hydration layer ($\delta\rho$) and the displaced solvent (r_0) by
787 fitting them individually to each structure, we used the fixed values for these parameters
788 determined in *Pesce and Lindorff-Larsen (2021)*. We globally fitted the scale and constant
789 background with least-squares regression weighted by the experimental errors using
790 Scikit-learn (*Pedregosa et al., 2011*). To assess the agreement between the experimental
791 SAXS profiles and those calculated from simulations, we calculated the χ_r^2 between the
792 ensemble-averaged calculated SAXS intensities (I_{calc}) and the experimental SAXS inten-
793 sity (I_{exp}):

$$\chi_r^2 = \frac{1}{m} \sum_q^m \left(\frac{I_q^{calc} - I_q^{exp}}{\sigma_q^{exp}} \right)^2 \quad (3)$$

794 where σ^{exp} is the error of the experimental SAXS intensity and m is the number of mea-

795 sured SAXS intensities. We used the Bayesian Indirect Fourier Transform algorithm (BIFT)
796 to rescale the errors of the experimental SAXS intensities, in order to obtain a more con-
797 sistent error estimate across the different proteins (*Hansen, 2000; Larsen and Pedersen,*
798 *2021*).

799 PRE calculations

800 We used the DEER-PREDict software (*Tesei et al., 2021a*) to calculate intrachain PREs from
801 the back-mapped trajectories of α -synuclein, FUS_{LCD}, hnRNPA2_{LCD}, OPN and hTau40 (Ta-
802 ble 4), and interchain PREs from the back-mapped trajectories of two copies of FUS_{LCD}.
803 DEER-PREDICT uses a rotamer library approach to model the MTSL spin-label (*Polyhach*
804 *et al., 2011*) and a model-free formalism to calculate the spectral density (*Iwahara et al.,*
805 *2004*). We assumed an effective correlation time of the spin label, τ_l , of 100 ps, a molecu-
806 lar correlation time, τ_c , of 4 ns (*Gillespie and Shortle, 1997*), a transverse relaxation rate
807 for the diamagnetic protein of 10 s^{-1} and a total INEPT time of the HSQC measurement
808 of 10 ms (*Battiste and Wagner, 2000*). For the simulations of two copies of FUS_{LCD}, τ_c
809 was not fixed to 4 ns. We instead scanned values of τ_c from 1 to 20 ns in steps of 1 ns
810 and selected the τ_c that minimized the χ_r^2 to the experimental PRE data for each force
811 field. The optimal values were 1 ns, 8 ns, and 9 ns for unmodified Martini 3, $\lambda_{PW}=1.10$
812 and $\lambda_{PP}=0.88$ respectively. The agreement between calculated and experimental PREs
813 was assessed by calculating the χ_r^2 over all spin-label positions,

$$\chi_r^2 = \frac{1}{N_{labels} N_{res}} \sum_j^{N_{labels}} \sum_i^{N_{res}} \left(\frac{Y_{ij}^{exp} - Y_{ij}^{calc}}{\sigma_{ij}^{exp}} \right)^2 \quad (4)$$

814 where N_{labels} and N_{res} are the number of spin-labels and residues, Y_{ij}^{exp} and Y_{ij}^{calc} are
815 the experimental and calculated PRE rates for label j and residue i , and σ_{ij}^{exp} is the exper-
816 imental error of the PRE rate for label j and residue i . For the simulations of two copies
817 of the FUS_{LCD}, the χ_r^2 was calculated as an average over the 10 replica simulations.

818 Radii of gyration

819 We calculated the R_g from CG simulation trajectories using Gromacs gyrate (*Abraham*
820 *et al., 2015*) and calculated the error of the average R_g using block-error analysis (*Fly-*
821 *vbjerg and Petersen, 1989*) (<https://github.com/fpesceKU/BLOCKING>). Experimental R_g -
822 values and corresponding error bars were calculated from SAXS profiles by Guinier analy-
823 sis using ATSAS AUTORG with default settings (*Petoukhov et al., 2007*), except in the case
824 of the hnRNPA1_{LCD} variants, for which we used the R_g -values reported in *Bremer et al.*
825 (*2022*), which were determined from SAXS data using an empirical molecular form factor
826 approach. Pearson correlation coefficients were calculated using the pearsonr function
827 in SciPy stats and standard errors were determined with bootstrapping using the boot-
828 strap function in SciPy stats with 9999 resamples (*Virtanen et al., 2020*).

829 Principal component analysis

830 We used PCA based on the pairwise distances between backbone beads to compare our
831 unmodified, $\lambda_{PW}=1.10$, $\lambda_{PP}=0.88$, and $\lambda_{PW-BB}=1.22$ Martini 3 simulations of IDPs and mul-
832 tidomain proteins. PCA was performed with PyEMMA (*Scherer et al., 2015*). For each
833 protein, all four ensembles were pooled for PCA in order to project into the same two
834 principal components. For all IDPs except PRN_{NT} and CoR_{NID}, and for the multidomain

835 proteins Gal-3, MyBP-C_{MTHB-C2}, Ubq₂ and Ubq₃, the pairwise distances between all back-
836 bone beads were used as features for PCA. For the remaining proteins, the pairwise dis-
837 tances between every 10th backbone bead were used as features for PCA.

838 **Comparison with atomistic ensembles**

839 We compared our unmodified Martini 3 and $\lambda_{pp}=0.88$ Martini 3 simulations of α -synuclein
840 with a 20 μ s simulation with the Amber03ws force field and a 73 μ s simulation with the
841 Amber99SB-disp force field from *Robustelli et al. (2018)*. Because of problems caused by
842 interactions between periodic images of the protein in the originally published Amber99SB-
843 disp simulation, we used the corrected version of the simulation also used in *Ahmed*
844 *et al. (2021)*. We also compared our unmodified Martini 3 and $\lambda_{pp}=0.88$ Martini 3 simula-
845 tions of hnRNPA1 with an atomistic ensemble from *Ritsch et al. (2022)* (Protein Ensemble
846 Database PED00212). We used the dimensionality reduction ensemble similarity (DRES)
847 approach in Encore (*Lindorff-Larsen and Ferkinghoff-Borg, 2009; Tiberti et al., 2015*) im-
848 plemented in MDAnalysis (*Michaud-Agrawal et al., 2011*) to quantify the ensemble sim-
849 ilarity based on Ca RMSD. We used the all-atom back-mapped versions of our Martini
850 simulations (described above). For hnRNPA1, we used every 10th frame from our Martini
851 simulations for a total of 4001 frames per simulation and all structures from the atom-
852 istic ensemble. Different constructs of hnRNPA1 were used for our simulations and the
853 *Ritsch et al. (2022)* ensemble, so the Encore DRES calculations were only performed for
854 residues 2-258, which are identical in both constructs (*Martin et al., 2021; Ritsch et al.,*
855 *2022*). For α -synuclein, we used every 10th frame from each simulation for a total of 4001
856 frames per Martini simulation, 2998 frames from the Amber03ws simulation, and 2998
857 frames from the Amber99sb-disp simulation.

858 **Data availability**

859 The data generated for this paper is available via [https://github.com/KULL-Centre/_2023_](https://github.com/KULL-Centre/_2023_Thomassen_Martini)
860 [Thomassen_Martini](https://github.com/KULL-Centre/_2023_Thomassen_Martini). Simulation data and starting structures for simulations are available
861 at <https://doi.org/10.5281/zenodo.8010043>. Data for protein membrane simulations are
862 available at <https://zenodo.org/record/8154919>. Force field files for Martini 3 with inter-
863 actions between protein beads rescaled by $\lambda_{pp}=0.88$ are available at [https://github.com/](https://github.com/KULL-Centre/_2023_Thomassen_Martini/tree/main/force_field)
864 [KULL-Centre/_2023_Thomassen_Martini/tree/main/force_field](https://github.com/KULL-Centre/_2023_Thomassen_Martini/tree/main/force_field)

865 **Code availability**

866 Code and scripts used for this paper is available via [https://github.com/KULL-Centre/_2023_](https://github.com/KULL-Centre/_2023_Thomassen_Martini)
867 [Thomassen_Martini](https://github.com/KULL-Centre/_2023_Thomassen_Martini).

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879 Author contributions

880 F.E.T., S.V. and K.L.-L. conceived the overall study. F.E.T and T.S. performed and anal-
881 ysed simulations of proteins in water under the supervision of K.L.-L., and A.K. and S.S.
882 performed and analysed simulations of proteins interacting with membranes under the
883 supervision of S.V.. F.E.T. wrote the first draft of the manuscript with input from K.L.-L.
884 All authors contributed to the writing of the manuscript.

885 Competing interests

886 K.L.-L. holds stock options in and is a consultant for Peptone Ltd. All other authors declare
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