SUPPLEMENTAL MATERIAL

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	R_E (nm)	R_G (nm)
Conformation 1	5.39	2.00
Conformation 2	0.57	1.78
Conformation 3	6.73	2.68
Conformation 4	5.48	2.1

Table S1. End-to-end distance (R_E) and radius of gyration (R_G) values of four different Nup153[82] conformers obtained from simulated annealing and used as starting points for MD simulations at room temperature (see Materials and Methods for details).

Water model	A_{OW}^{*}	C_{OW}	A_{HW}	C_{HW}	$vdW\text{-}^{OW}_{min}$	$vdW^{\text{-}HW}_{min}$
TIP3P	$2.44 \cdot 10^{-6}$	$2.49 \cdot 10^{-3}$	-	-	-0.636386	-
TIPS3P	$2.44 \cdot 10^{-6}$	$2.49 \cdot 10^{-3}$	$1.29 \cdot 10^{-17}$	$3.15 \cdot 10^{-9}$	-0.636386	-0.192464
TIP4P	$2.51 \cdot 10^{-6}$	$2.55 \cdot 10^{-3}$	-	-	-0.64852	-
TIP5P	$2.28 \cdot 10^{-6}$	$2.47 \cdot 10^{-3}$	-	-	-0.66944	-
SPC	$2.64 \cdot 10^{-6}$	$2.62 \cdot 10^{-3}$	-	-	-0.650629	-
SPC/E	$2.64 \cdot 10^{-6}$	$2.62 \cdot 10^{-3}$	-	-	-0.650629	-
TIP4P-Ew	$2.74 \cdot 10^{-6}$	$2.73 \cdot 10^{-3}$			-0.680946	
TIP4P/2005	$3.06 \cdot 10^{-6}$	$3.08 \cdot 10^{-3}$			-0.7749	
TIP4P-D	$3.78 \cdot 10^{-6}$	$3.76 \cdot 10^{-3}$			-0.93565	

Table S2. Summary of the A and C parameters used to compute Lennard-Jones interactions of the oxygen (OW) and hydrogen (HW) atoms for the water models used in the simulations of Nup153[82] and additionally for the TIP4P-Ew, TIP4P/2005 water models. The units for the A and C parameters are kJ nm^{12}/mol and kJ nm^{6}/mol, respectively.

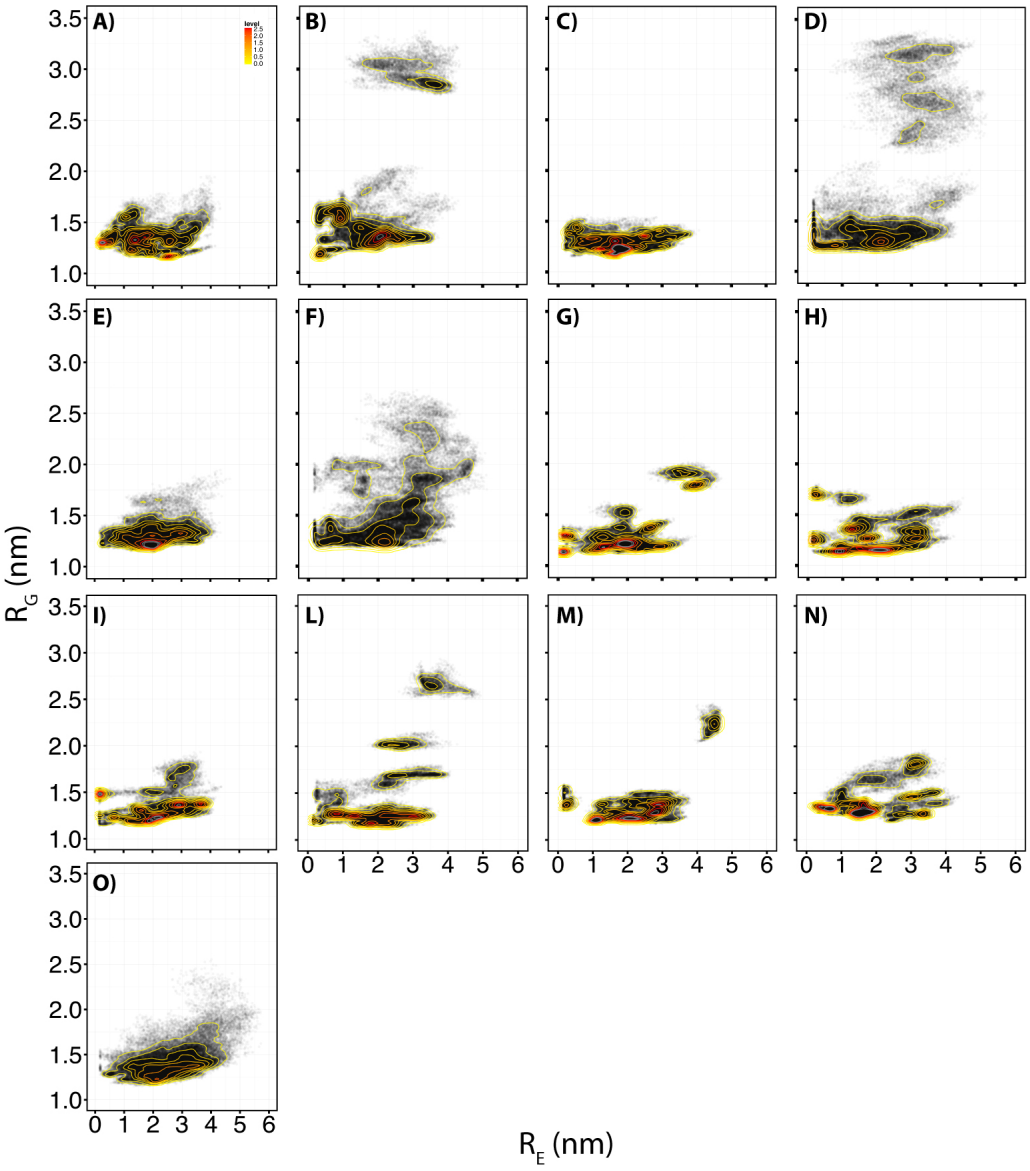

Figure S1. Radius of gyration (R_G) as a function of the end-to-end distance (R_E) for the last 50 ns of Nup153[82] conformational ensemble simulated using (A) AMBER99-sb*-ILDN with SPC, (B) AMBER99-sb*-ILDN with SPC/E, (C) AMBER99-sb*-ILDN with TIP4P, (D) CHARMM22* with SPC, (E) CHARMM22* with TIP3P, (F) CHARMM22* with SPC/E, (G) GROMOS 54a7 with SPC, (E) GROMOS 54a7 with SPC/E, (I) OPLS with TIP4P, (L) OPLS with SPC, (M) OPLS with SPC/E and (N) OPLS with tip5p (O) CHARMM22* with tips3p water models. Contour lines indicate the densities of the points and are colored accordingly as shown in the bar at the side of (A).

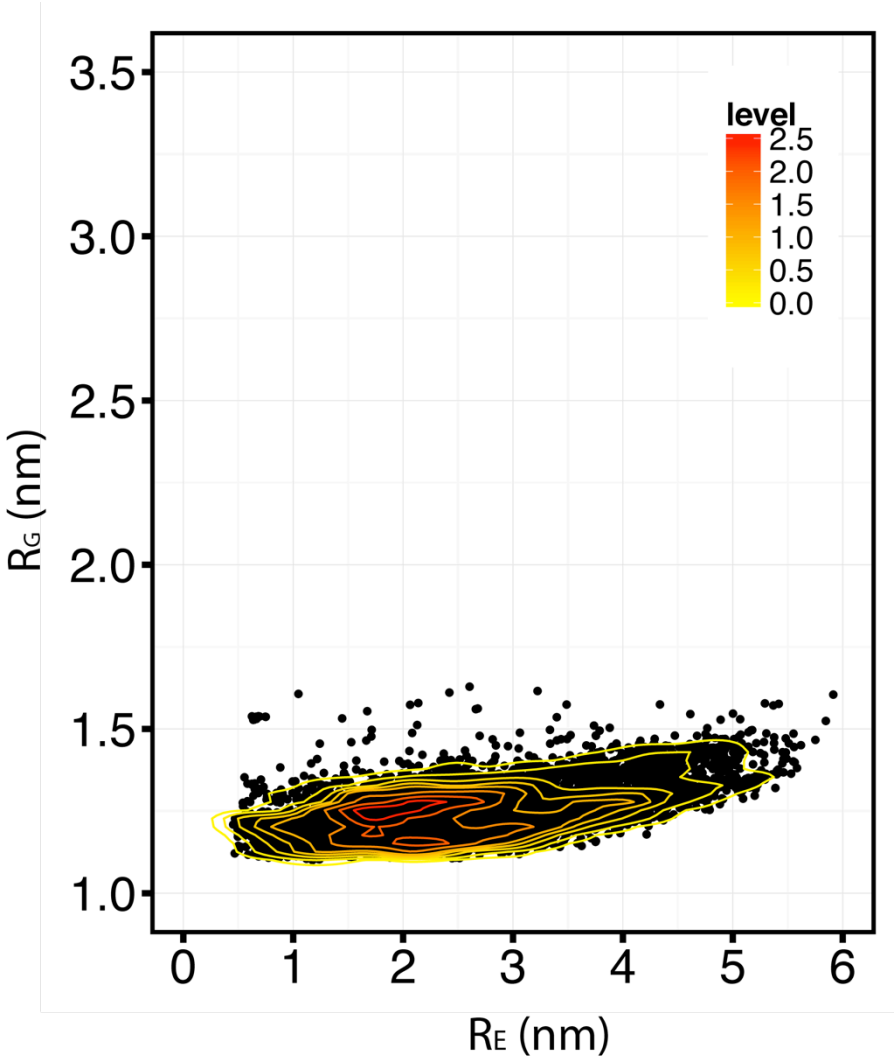

Figure S2 Radius of gyration (R_G) as a function of the end-to-end distance (R_E) for the conformational ensemble of Nup153[82] obtained from Replica Exchange Monte Carlo sampling, performed using OPLS for the protein and implicit solvation mimed by the ABSINTH solvation model. The reported ensemble has been collected at 300 K for comparison with MD simulations.

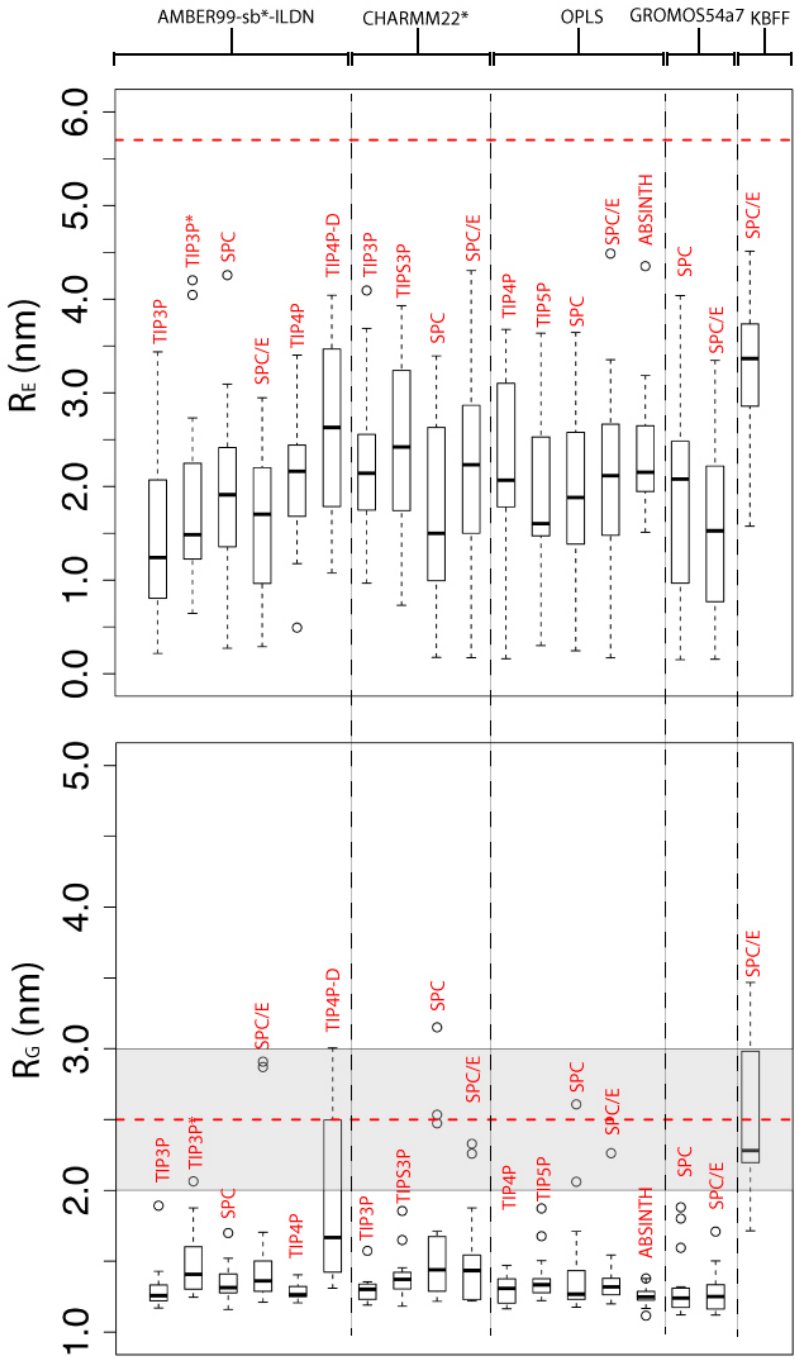

Figure S3.

End-to-end distances (R_E) and radius of gyration (R_G) distributions sampled using molecular dynamics simulations obtained only considering the last simulated frame for each replica. The different force fields and water models are reported on the top of the graphs and boxes respectively. The dashed red lines correspond to the experimental average of R_E and R_G obtained by smFRET and SAXS (Figure 1, E-F), and the gray shade reports the error in R_G from SAXS.

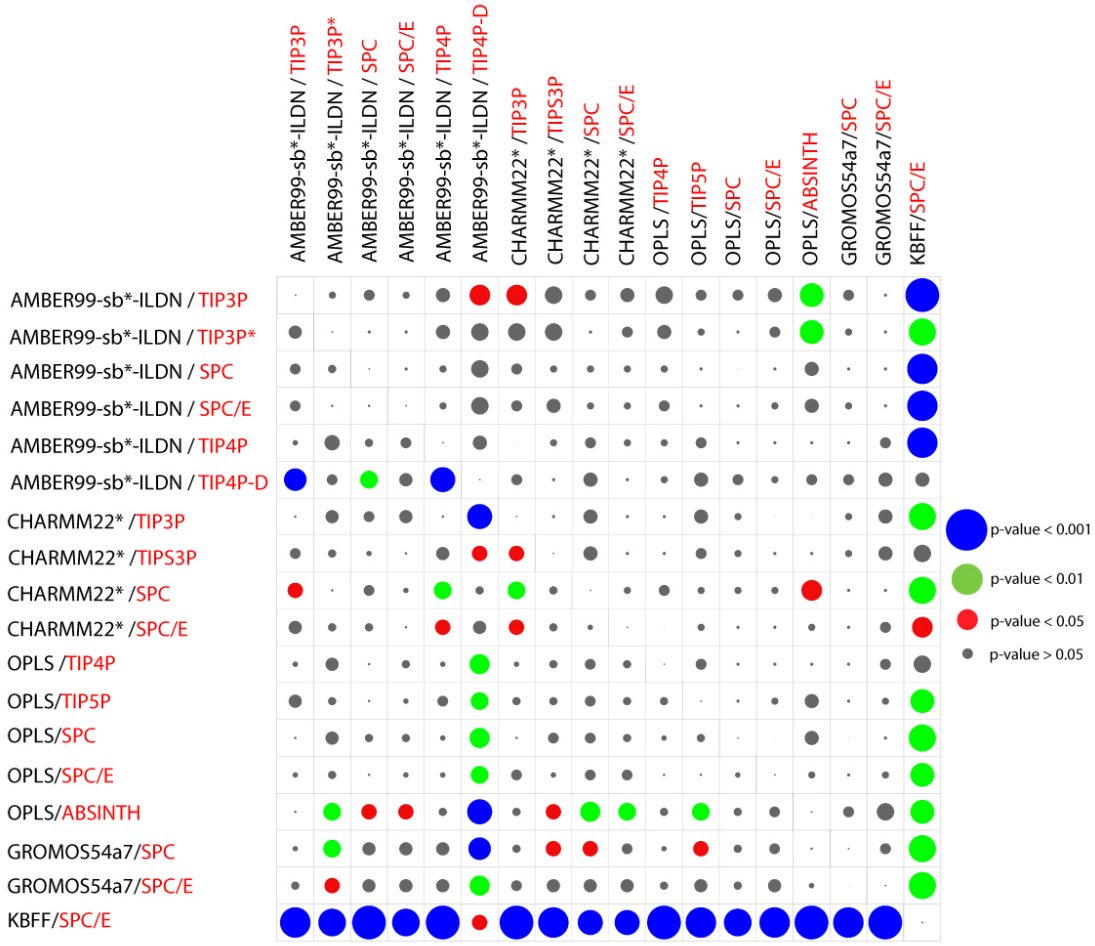

Figure S4. Statistical significance for the differences of R_E (above diagonal) and R_G distributions (below diagonal) shown in Figure S3 estimated using the Kolmogorov-Smirnov test of significance after having detected the lack of normality using a Shapiro-Wilk test on each collected distribution.

Circle diameters are proportional to –ln(p-value) whereas the color indicates the range of significance as blue for $p<0.001$, green for $p<0.01$, red for $p<0.05$, and grey for non-significant differences. In the case of the sampling achieved using Replica Exchange Monte Carlo simulations 15 sampled points were randomly chosen from the distribution.

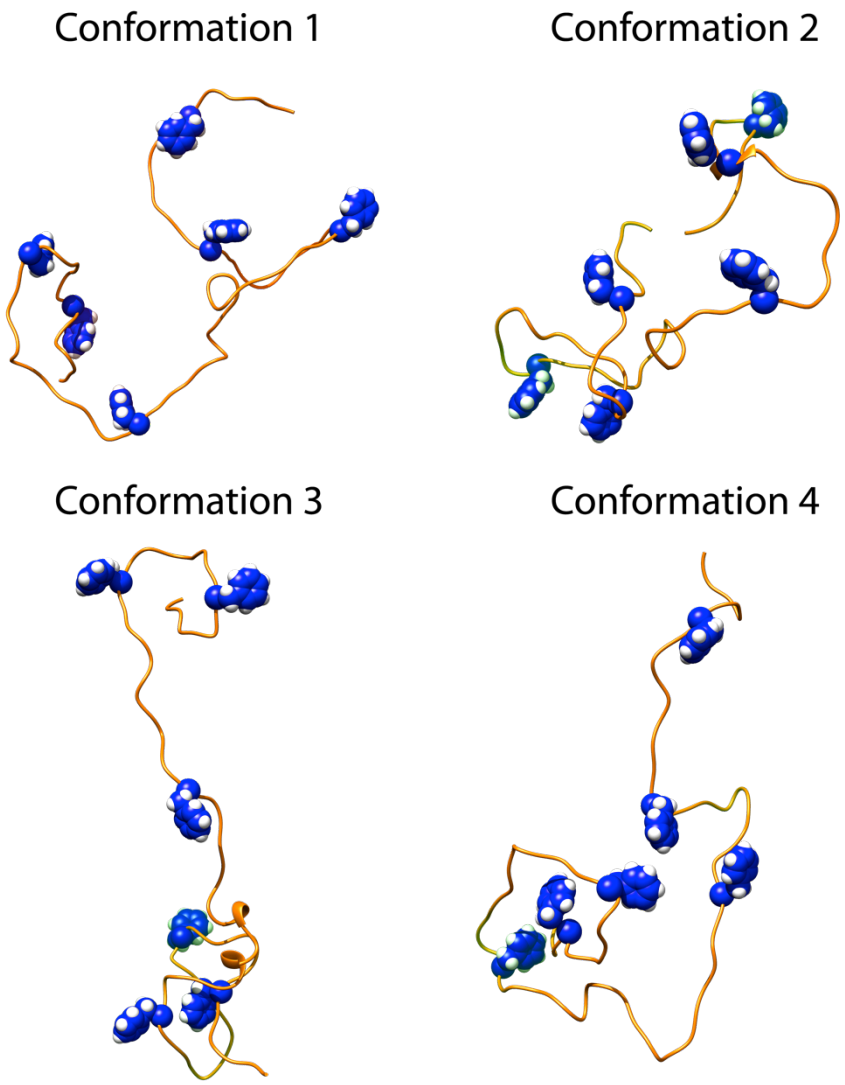

Figure S5. Nup153[82] conformations derived from simulated annealing (see Materials and Methods for details) and used as starting points for MD simulations at room temperature. The R_E and R_G for each of the reported structures are reported in Table S1.

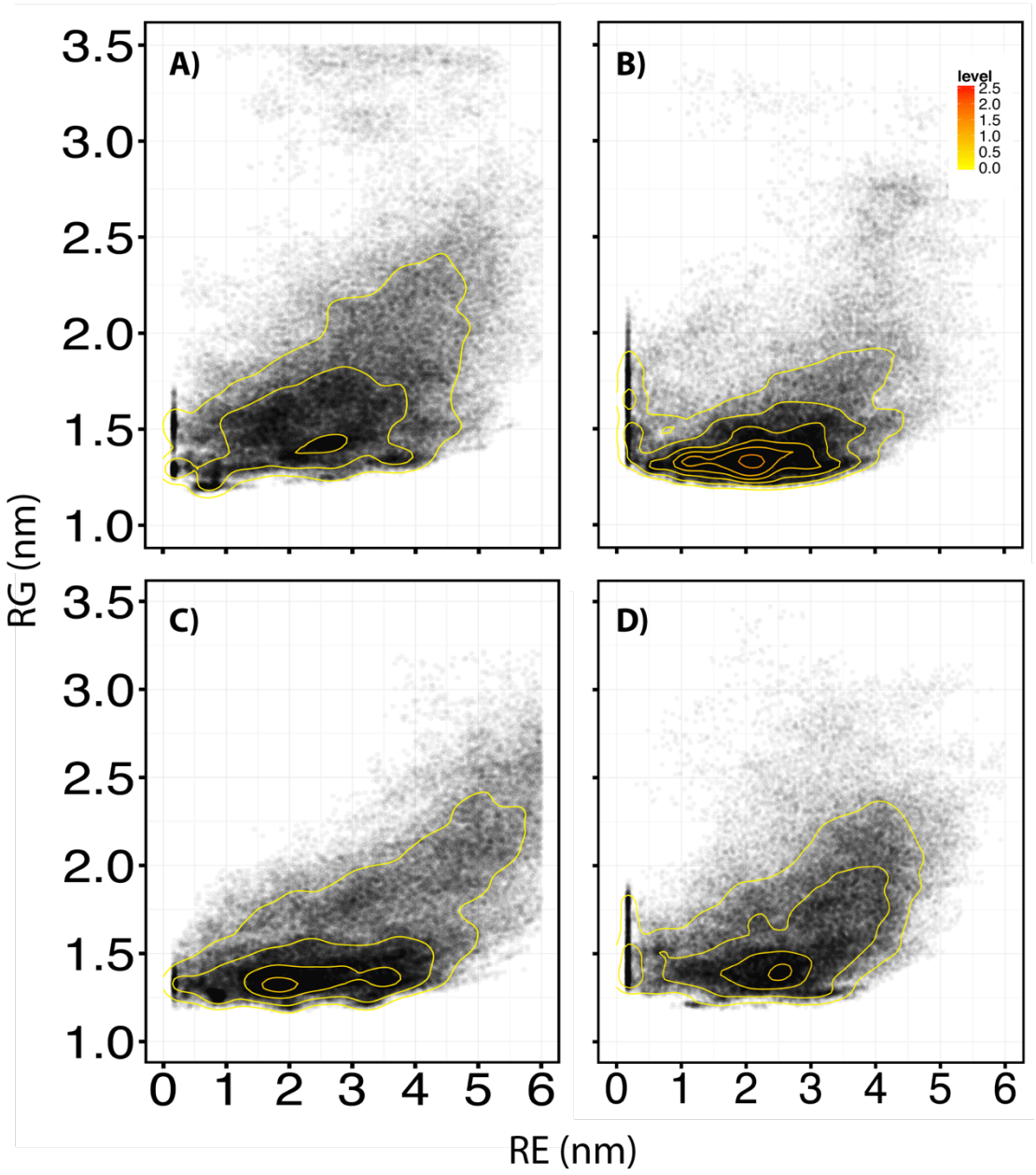

Figure S6 Conformational dynamics of Nup153[82] starting from four different conformations. Radius of gyration (R_G) as a function of the end-to-end distance (R_E) of Nup153[82] conformational ensemble simulated using CHARMM22* with TIPS3P. The four different starting conformations retrieved through simulated annealing are showed in Figure S6 and their dimensions is reported in Table S1. The conformational dynamics of conformers 1-4 is reported in panels A-D respectively. Contour lines indicate the density of the points and are colored as shown in the bar at the side of panel B.

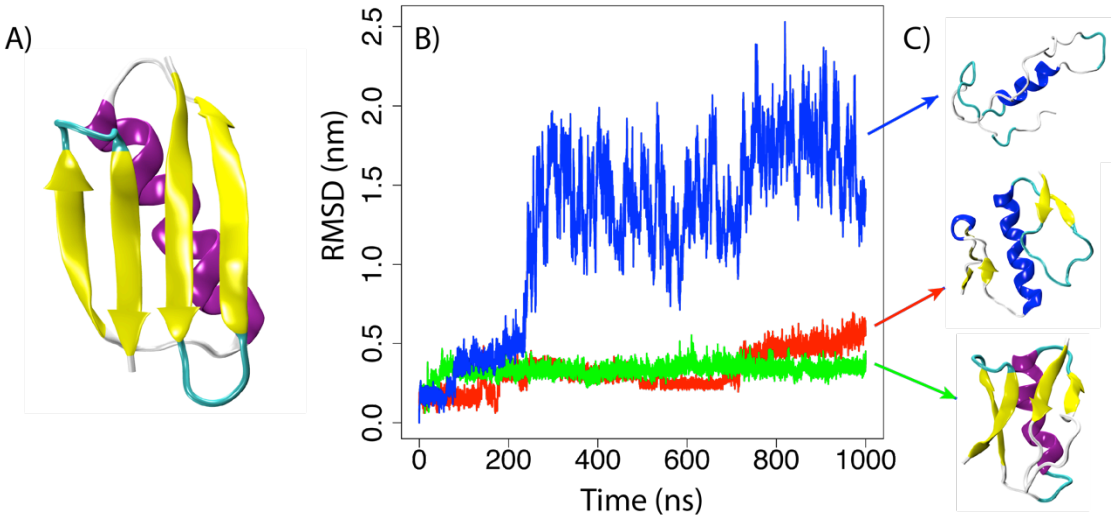

Figure S7. KBFF renders a folded protein unstable.

MD simulations of the B1 immunoglobulin domain of the streptococcal protein G (GB1) performed using the KBFF force field. (A) The native fold of the domain, colored by secondary structure elements, is shaped by a single α-helix (magenta), and a four strands β-sheet (yellow). (B) RMSD of the GB1 domain as retrieved from 1 μs-long MD simulations. The three independent simulations performed are reported in blue, red and green. (C) Conformations of GB1 after 1 μs simulation. α-helices are colored in magenta, 3_{10} helices in blue, β-strands in yellow, turns in cyan and coil in white.