Supporting Information for:

Simulation of Reversible Protein-Protein Binding and Calculation of Binding Free Energies using Perturbed Distance Restraints

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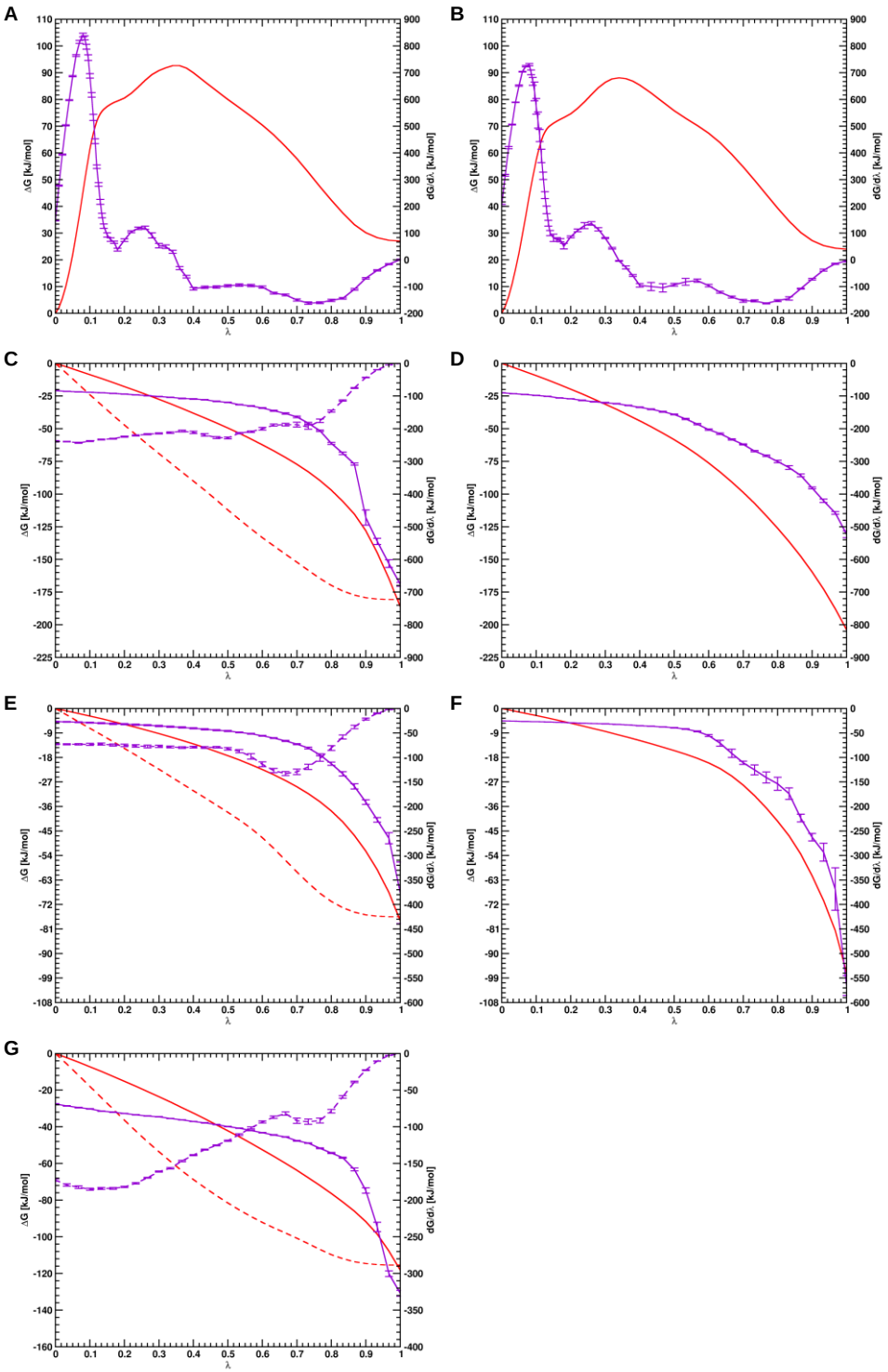

Figure S1. Derivatives of the free energy with respect to λ for simulations employing SCR (full violet lines) or HR (dashed violet lines) and free-energy differences calculated with BAR along λ for simulations employing SCR (full red lines) or HR (dashed red lines) for protein binding and unbinding (ΔG^{res}_{unbind}) in system RS of the wt UBM2 domain (A) and the P692A UBM2 domain (B), for the free-energy contribution of the ENs and specific intermolecular distance restraints in the bound state ($\Delta G^{b}_{en,dr}$) of the wt complex (C) and the complex involving P692A UBM2 (D), for the free-energy contributions of the ENs in the unbound state ($\Delta G^{u}_{en,1}, \Delta G^{u}_{en,2}$) of wt UBM2 (E), P692A UBM2 up to 100 ns total simulation time (F) and UBI (G).

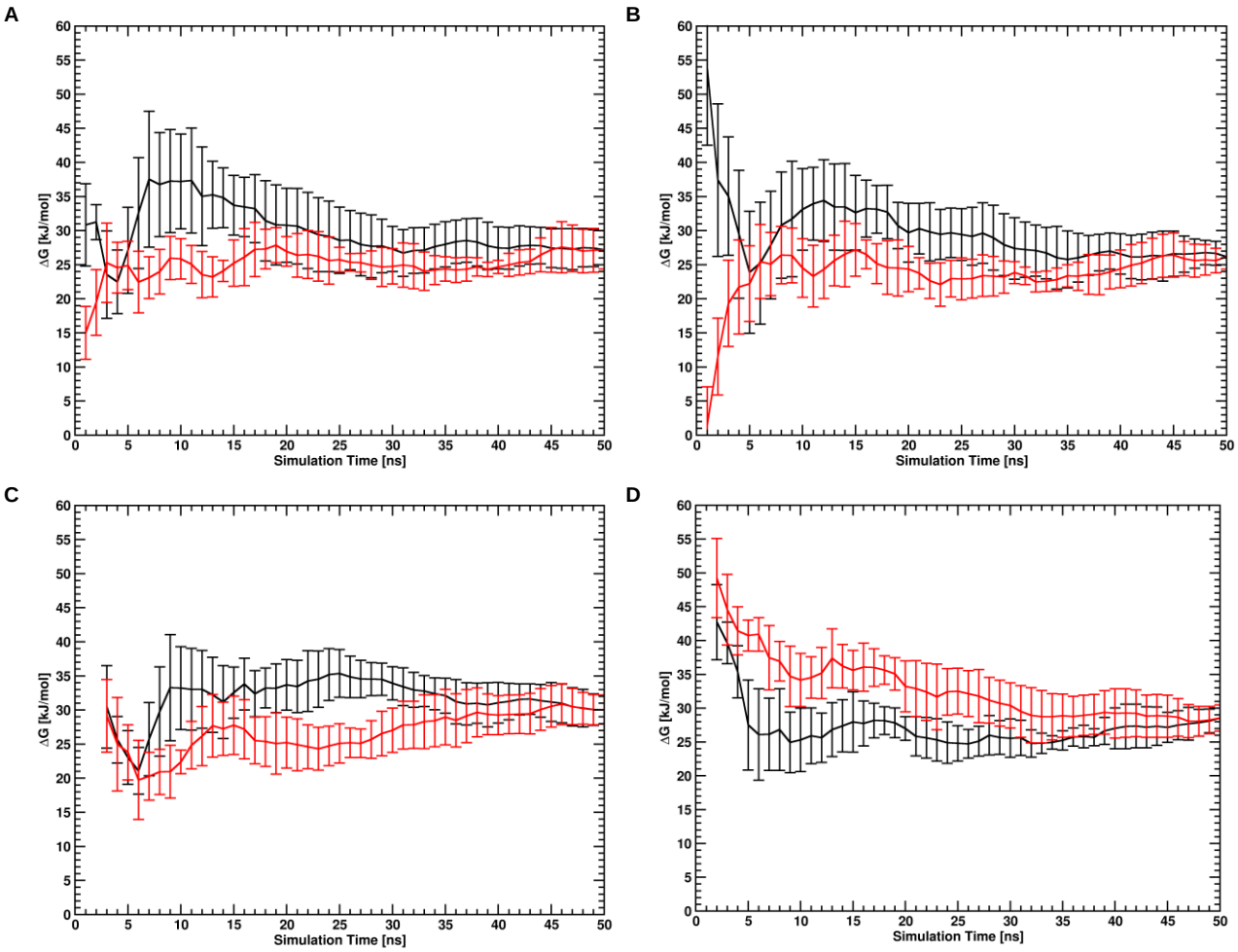

Figure S2. Forward (black lines) and reverse (red lines) cumulative averages along the trajectories of the free-energy differences calculated with BAR for protein binding and unbinding (ΔG^{res}_{unbind}) of the wt UBM2 domain simulated with system setup ZS (A) or ZL (C) and the P692A UBM2 domain simulated with system setup ZS (B) or ZL (D).

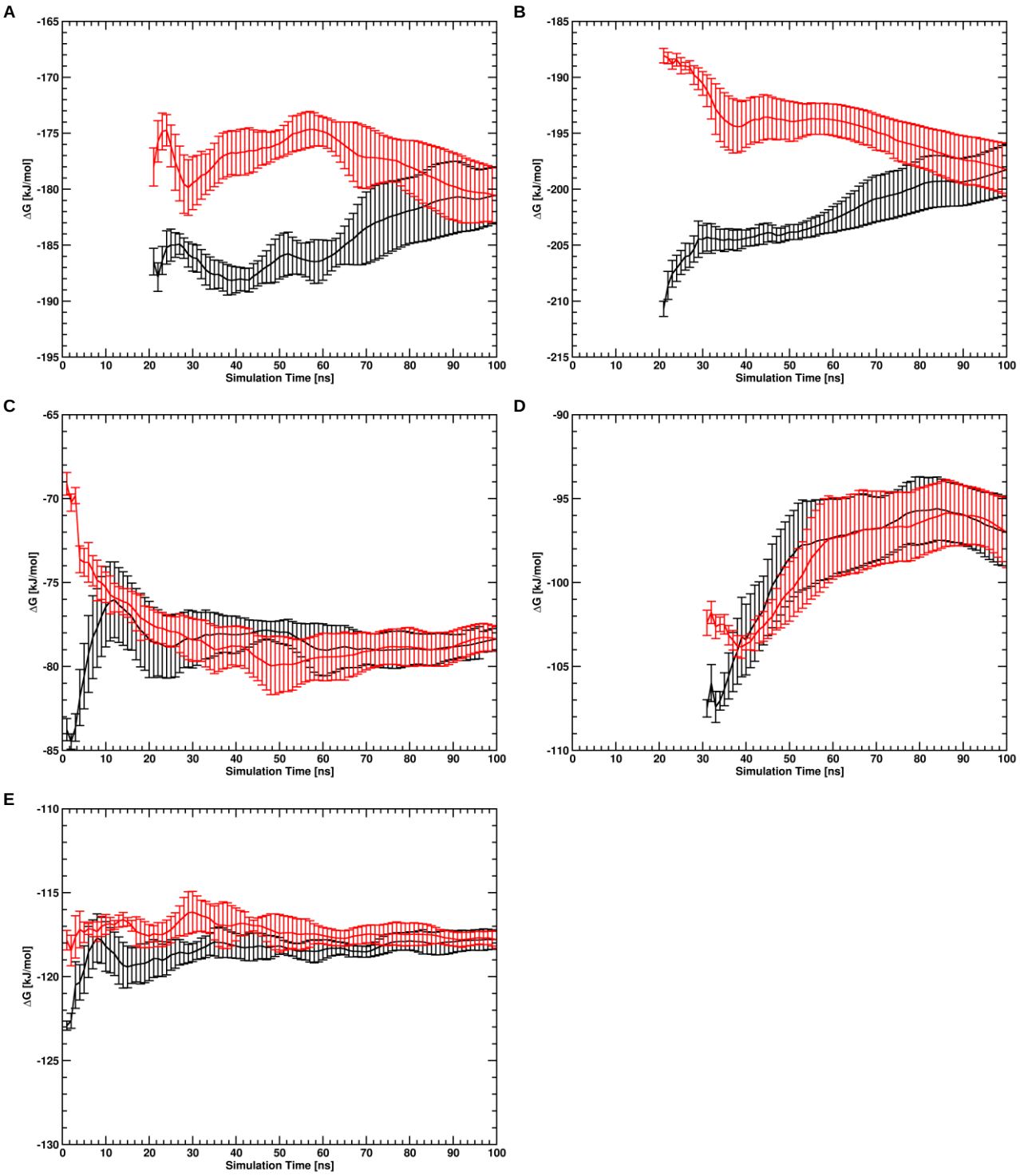

Figure S3. Forward (black lines) and reverse (red lines) cumulative averages along the trajectories continued up to 100 ns of the free-energy differences calculated with BAR for the free-energy contribution of the ENs and specific intermolecular distance restraints in the bound state ($\Delta G^{b}_{en,dr}$) of the wt complex (A) and the complex involving P692A UBM2 (B), for the free-energy contributions of the ENs in the unbound state ($\Delta G^{u}_{en,1}, \Delta G^{u}_{en,2}$) of wt UBM2 (C), P692A UBM2 (D) and UBI (E). The simulations of all were performed using SCR potential energy functions.

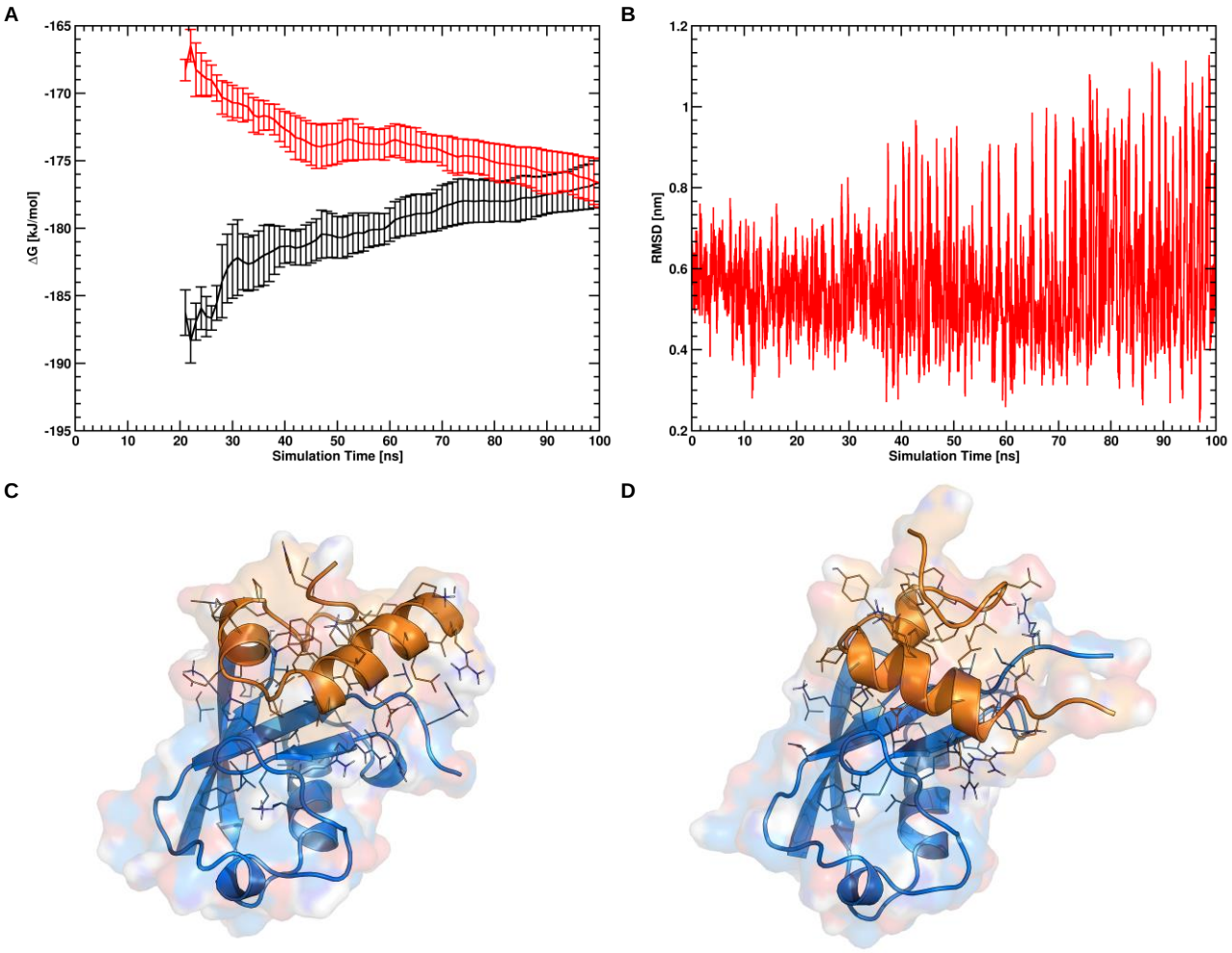

Figure S4. (A) forward (black lines) and reverse (red lines) cumulative averages along the trajectories continued up to 100 ns of the free-energy differences calculated with BAR and HR for the free-energy contribution of the ENs and specific intermolecular distance restraints in the bound state ($\Delta G^{b}_{en,dr}$) of the wt complex. (B) RMSD of the Cα atoms of the wt UBM2 domain in the unrestrained state of the same simulation to the Cα atoms of the wt UBM2 domain in the bound structure that was used to define the ENs and specific intermolecular distance restraints of the bound state. (C) experimentally determined complex structure of UBI (blue) and wt UBM2 (orange) as given in the PDB entry 2KTF. (D) non-canonically bound UBM2 in the unrestrained end-state of the simulation used to calculate the free-energy contribution of the specific intermolecular distance restraints and ENs in the bound state ($\Delta G^{b}_{en,dr}$).

Table S1. Number and spacing of the λ points used for the simulation of reversible protein binding.

λ Point No.	λ Value	λ Point No.	λ Value
1	0.000	28	0.240
2	0.010	29	0.260
3	0.020	30	0.280
4	0.030	31	0.300
5	0.040	32	0.320
6	0.050	33	0.340
7	0.060	34	0.360
8	0.070	35	0.380
9	0.080	36	0.400
10	0.085	37	0.433
11	0.090	38	0.467
12	0.095	39	0.500
13	0.100	40	0.533
14	0.105	41	0.567
15	0.110	42	0.600
16	0.115	43	0.633
17	0.120	44	0.667
18	0.125	45	0.700
19	0.130	46	0.733
20	0.135	47	0.767
21	0.140	48	0.800
22	0.150	49	0.833
23	0.160	50	0.867
24	0.170	51	0.900
25	0.180	52	0.933
26	0.200	53	0.967
27	0.220	54	1.000

Table S2. Parameters for intermolecular distance restraints between UBI and UBM2 for the bound and the unbound state of the simulation of reversible protein-protein binding. Residue numbering corresponds to the residue numbering in the experimental structure of the complex of wt UBM2 and UBI (PDB-ID 2KTF).

Restraint Type	UBI Residue	UBM2 Residue	Bound Distance [nm]	Bound Force Constant [kJ·mol^{-1}·nm^{-2}]	Unbound Distance [nm]	Unbound Force Constant [kJ·mol^{-1}·nm^{-2}]
Cα-Cα	73	702	0.84	250*	3.34	0
Cα-Cα	72	699	0.81	250*	3.31	0
Cα-Cα	42	698	1.32	250*	3.82	0
Cα-Cα	70	696	1.02	250*	3.52	0
Cα-Cα	44	695	0.96	250*	3.46	0
Cα-Cα	48	694	0.99	250*	3.49	0
Cα-Cα	47	692	0.62	250*	3.12	0
Cα-Cα	68	690	0.94	250*	3.44	0
Cα-Cα	6	687	0.72	250*	3.22	0
Cα-Cα	8	688	0.71	250*	3.21	0
Cα-Cα	9	683	0.50	250*	3.00	0
Cα-Cα	10	684	0.80	250*	3.30	0
Radial Cα COM-COM (systems RS)	1-76	-3, -2, -1, 679-707	1.89	0	4.39	3000
X-Component Cα COM-COM (systems ZS & ZL)	1-76	-3, -2, -1, 679-707	0.00	3000	0.00	3000
Y-Component Cα COM-COM (systems ZS & ZL)	1-76	-3, -2, -1, 679-707	0.00	3000	0.00	3000
Z-Component Cα COM-COM (systems ZS & ZL)	1-76	-3, -2, -1, 679-707	1.89	0	4.39	3000

*effective force constant in the bound state – the λ-dependence of the force constant is given in equation (3) with hidden restraints parameters $n = 0$, $m = 2$ and k_A= 62.5 kJ·mol^{-1}·nm^{-2}.

Table S3. Different structural properties and number of NOE distance upper bound violations of the generated ensembles or experimentally determined structures of unbound UBI and wt UBM2.

		# NOE violations > 0.0 nm / > 0.3 nm	% DSSP α-helix / β-sheet
wt UBM2	straight MD	85 / 16	47.2 / 0.0
	unbound, unrestrained	116 / 23	31.7 / 0.0
	unbound, restrained	64 / 19	56.0 / 0.0
	unbound, binding (restrained)	68 / 21	56.0 / 0.0
	experimental bundle (PDB-ID 2L0G)	0 / 0	53.9 / 0.0
UBI	straight MD	207 / 12	13.1 / 29.2
	unbound, unrestrained	273 / 24	12.4 / 27.2
	unbound, restrained	266 / 23	15.6 / 28.1
	unbound, binding (restrained)	246 / 16	15.8 / 28.9
	experimental bundle (PDB-ID 1D3Z)	11 / 0	15.6 / 30.3

Table S4. Structural properties of the generated ensembles of unbound P692A UBM2.

	% DSSP α-helix / β-sheet
straight MD	37.2 / 0.0 12.3 / 0.0*
unbound, unrestrained	12.2 / 0.9
unbound, restrained	55.8 / 0.0
unbound, binding (restrained)	55.9 / 0.0

*values from a second, independent simulation.