Supporting Information for:

Towards Automated Free Energy Calculation with Accelerated Enveloping Distribution Sampling (A-EDS)

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Table S1. Definition of the harmonic protein-ligand distance restraint for system TRP.

Protein atom	Ligand atom	Distance [nm]	Force constant [kJ·mol^{-1}·nm^{-2}]
ASP171 Cγ	Cε	0.42	250

Table S2. Definition of harmonic protein-ligand distance restraints for system PNMT.

Protein atom(s) (COM)	Ligand atoms (COM)	Distance [nm]	Force constant [kJ·mol^{-1}·nm^{-2}]
PHE161 Cδ1, Cδ2, Cε1, Cε2	Cα, Cβ, Cδ, Cε	0.38	250
TYR64 Cδ1, Cδ2, Cε1, Cε2	Cα, Cβ, Cδ, Cε	0.83	250
VAL32 Cβ	Cα, Cβ, Cδ, Cε	0.72	250

Table S3. Computed binding affinities for GRA2 ligands and comparison to experimental data and results obtained with different methods. Energy units are kJ·mol^{-1}.

Ligand	A-EDS (2σ)	A-EDS (3σ)	Experiment[1]	TI[1]	OSP[1]	A-EDS ($\Delta E_{max}^*=100$)[2]
1	-27.4	-28.0	-30.8	-31.9	-32.1	-30.6
2	-29.8	-37.8	-36.8	-34.4	-37.0	-39.4
3	-26.9	-33.3	-31.7	-27.2	-30.7	-34.2
4	-37.3	-20.5	-29.4	-28.5	-32.0	-26.8
5	-35.3	-37.3	-28.0	-34.8	-25.1	-25.7
6	-33.1	-43.6	n.a.	n.a.	-31.8	n.a.
7	-35.9	-26.0	n.a.	n.a.	-34.3	n.a.
8	-45.5	-48.9	n.a.	n.a.	-22.5	n.a.
9	-39.6	-24.1	n.a.	n.a.	-29.3	n.a.
10	-43.2	-41.3	n.a.	n.a.	-22.7	n.a.
11	-48.6	-25.9	n.a.	n.a.	-19.3	n.a.
12	-40.9	-31.6	n.a.	n.a.	-29.6	n.a.
13	-53.6	-52.8	n.a.	n.a.	-21.4	n.a.
14	-51.1	-35.4	n.a.	n.a.	-14.0	n.a.
15	-54.3	-31.7	n.a.	n.a.	-18.9	n.a.
16	-58.7	-41.6	n.a.	n.a.	-7.5	n.a.
RMSE to experiment	6.3	6.0		3.9	1.9	2.2

Table S4. Computed binding affinities for TRP ligands and comparison to experimental data and results obtained with different methods. Energy units are kJ·mol^{-1}.

Ligand	A-EDS (1σ)	A-EDS (2σ)	A-EDS (3σ)	Experiment[3]	TI[4]	OSP/TPF[4]
1	-29.1	-28.5	-28.6	-26.5	-23.1	-24.7
2	-19.5	-13.6	-27.8	-20.5	-32.4	-23.3
3	-24.4	-22.6	-18.4	-28.1	-21.0	-25.2
4	-31.2	-32.2	-36.3	-20.2	-28.3	-25.6
5	-16.5	-23.8	-20.9	-26.9	-22.7	-26.5
6	-28.0	-28.8	-22.9	-25.3	-23.0	-23.2
7	-21.8	-21.5	-16.2	-23.3	-20.0	-22.1
8	-4.3	-3.8	6.4	n.a.	14.5	20.0
RMSE to experiment	6.1	6.0	8.5		6.6	2.8

n.a.: not available

Table S5. Computed binding affinities for PNMT ligands and comparison to experimental data and results obtained with different methods. Energy units are kJ·mol^{-1}.

Ligand	A-EDS (2σ)	A-EDS (3σ)	Experiment[5-10]	TI[5]	EDS[11]	RE-EDS[12]
1	-21.2	-26.7	-30.1	-30.0	-23.6	-26.5
2	201.2	196.5	> -15.5[a,b]	48.9	n.a.	n.a.
3	-26.2	-28.6	-25.4[b]	-25.7	-23.8	-28.1
4	-35.2	-30.4	-26.6[a,b]	-31.1	-24.3	-29.4
5	-31.1	-37.2	-40.9	-39.7	-35.9	-36.8
6	-42.9	-31.7	-34.8	-40.3	-45.2	-32.4
7	-23.6	-41.2	-39.8	-41.5	-35.5	-39.3
8	-49.9	-36.3	-41.9[c]	-42.9	-43.0	-39.1
9	-35.6	-34.9	-33.9[b]	-29.0	-35.9	-34.8
10	-31.3	-30.2	-24.0[a,b]	-17.4	-29.9	-30.7
RMSE to experiment	8.8	3.8		3.7	5.2	4.9

[a]experimental data refers to racemic mixture

[b]experimental data refers to binding to bovine PNMT

[c]experimental data refers to other enantiomer
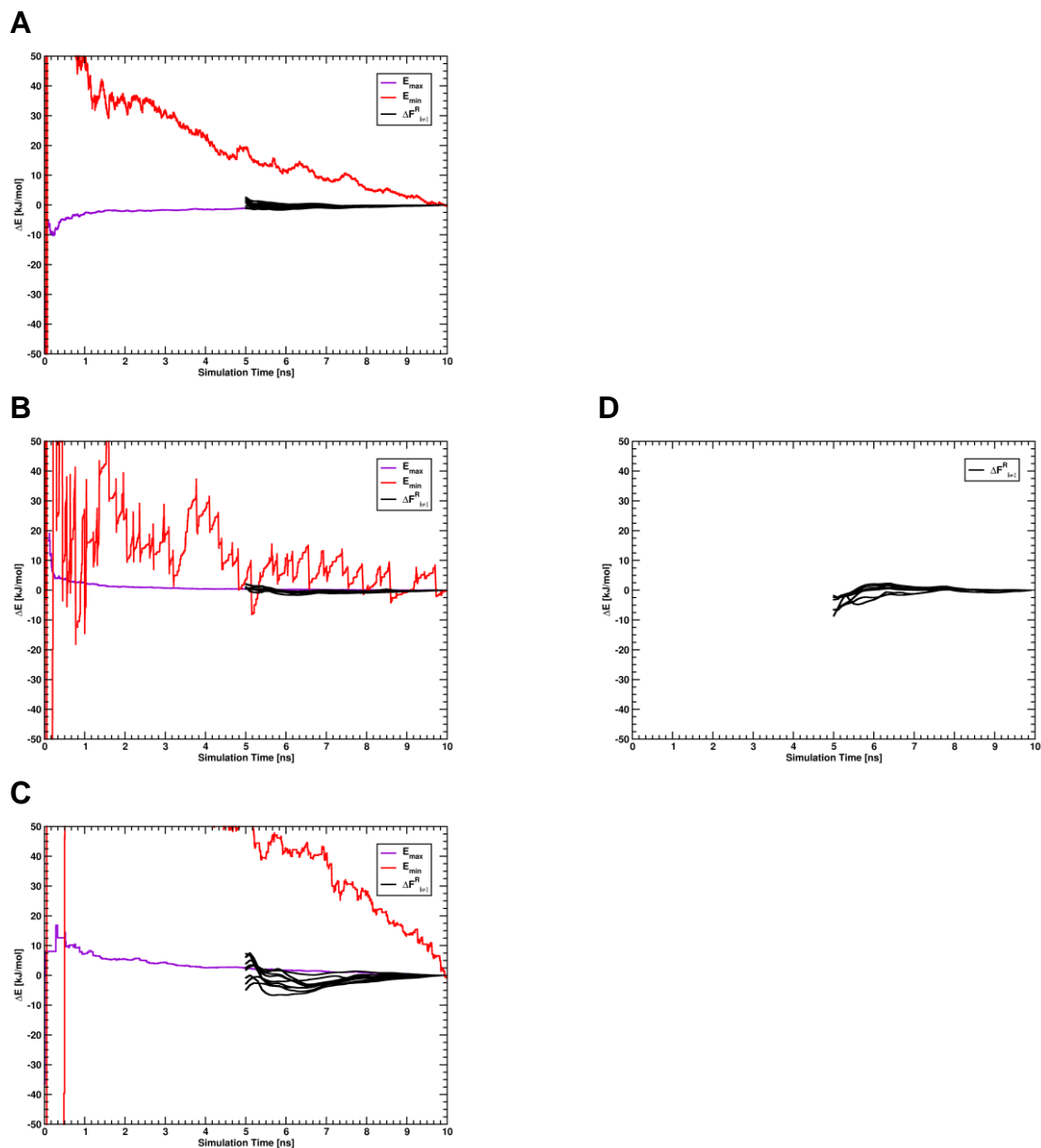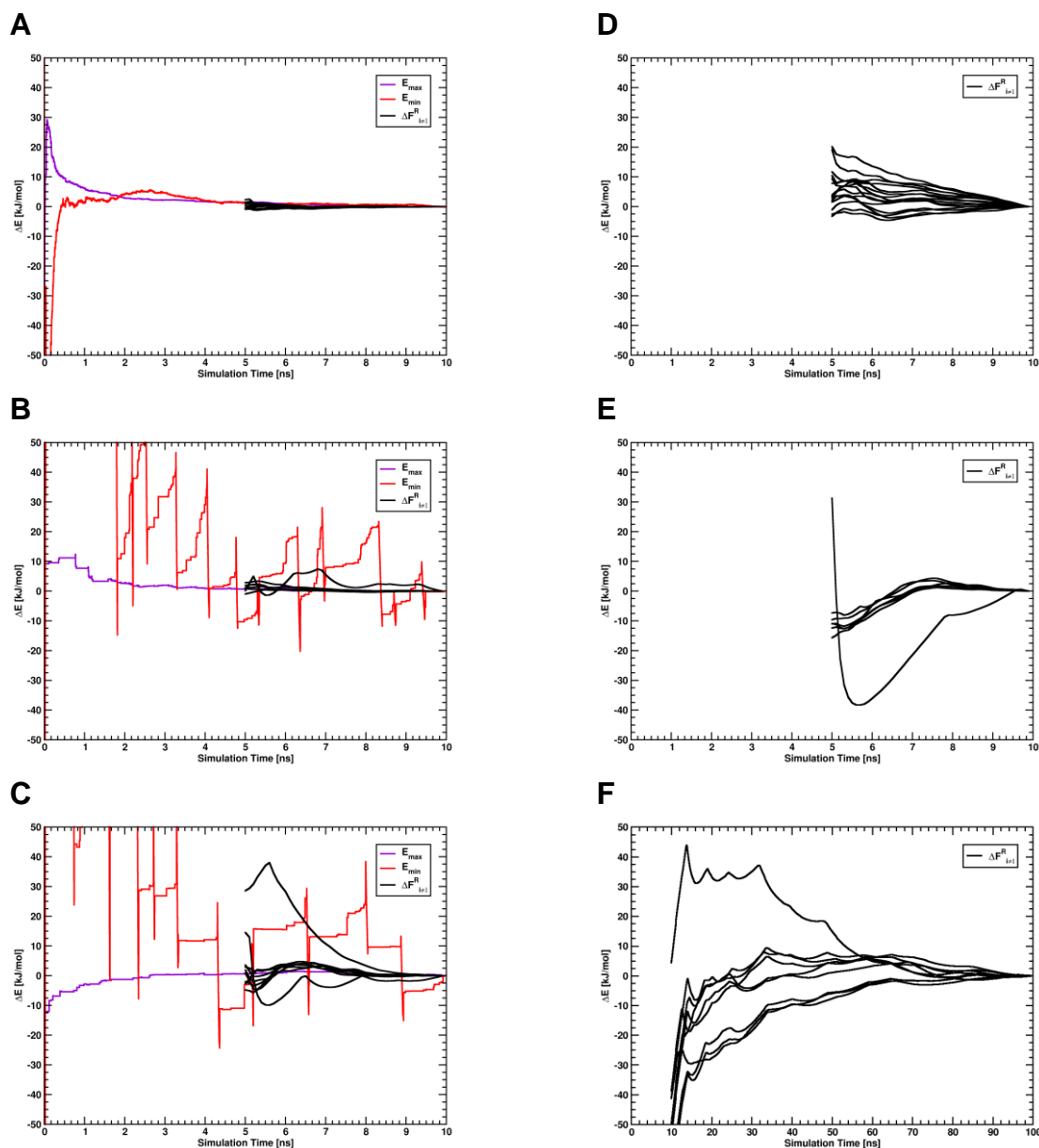
n.a.: not available

Figure S1. Convergence of the A-EDS acceleration parameters E_{max} and E_{min} and the free-energy offset parameters $\Delta F_{i\neq1}^{R}$ relative to their final values for the A-EDS parameter search simulation with an acceleration σ-level of 1σ, for the unbound ligands in water of system GRA2 (A), TRP (B) and PNMT (C), and for the ligands bound to the protein of system TRP (D). The convergence of the free-energy offset parameters $\Delta F_{i\neq1}^{R}$ is shown as forward cumulative average over the free-energy offset trajectory from which the first 5 ns were discarded as non-equilibrated region. Note that in panels A, B and C the A-EDS parameter E_{min} (red solid line) falls off the plot in the first half of the trajectory.

Figure S2. Convergence of the A-EDS acceleration parameters E_{max} and E_{min} and the free-energy offset parameters $\Delta F_{i\neq1}^R$ relative to their final values for the A-EDS parameter search simulation with an acceleration σ-level of 3σ, for the unbound ligands in water of system GRA2 (A), TRP (B) and PNMT (C), and for the ligands bound to the protein of system GRA2 (D), TRP (E) and PNMT (F). The convergence of the free-energy offset parameters $\Delta F_{i\neq1}^R$ is shown as forward cumulative average over the free-energy offset trajectory from which the first 5 or 10 ns were discarded as non-equilibrated region. Note that in panels A, B and C the A-EDS parameter E_{min} (red solid line) falls off the plot in the first half of the trajectory.
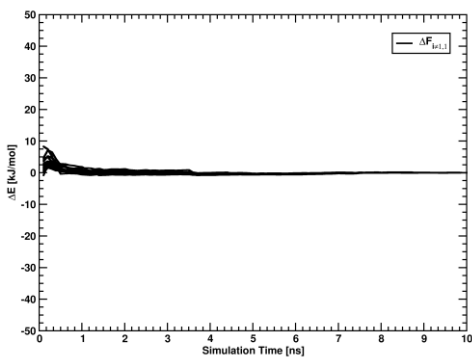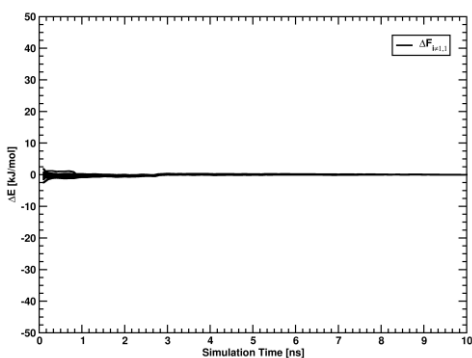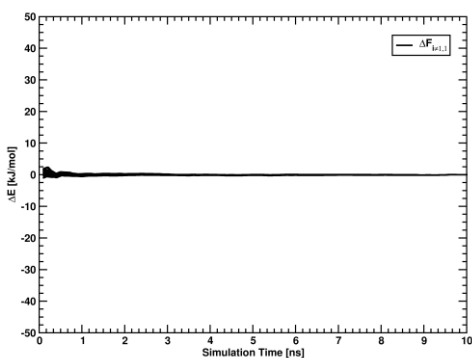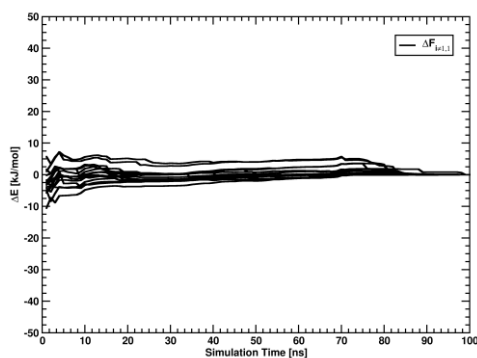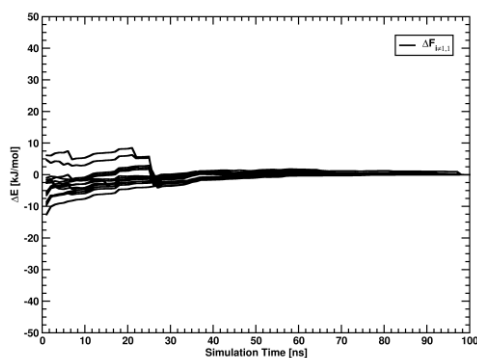
Figure S3. Convergence of the calculated relative free-energy differences $\Delta F_{i\neq 1,1}$ between the ligand end-states relative to their final values from the equilibrium A-EDS simulations of the unbound reference-state ligand in water for acceleration σ-levels of 1σ (A), 2σ (B) and 3σ (C), and for the reference-state ligand bound to the protein for acceleration σ-levels of 2σ (D) and 3σ (E) of system GRA2.
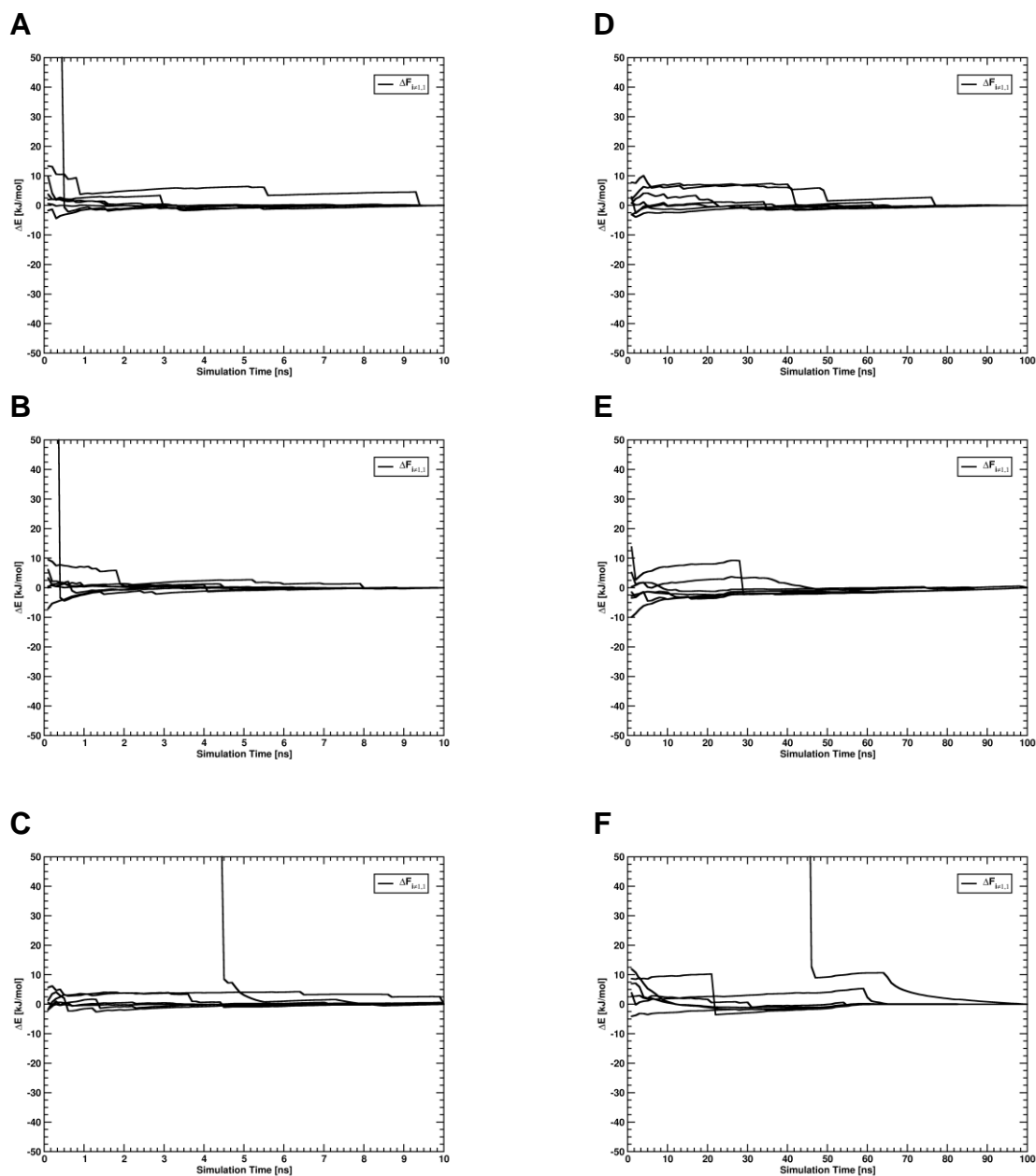
Figure S4. Convergence of the calculated relative free-energy differences $\Delta F_{i \neq 1,1}$ between the ligand end-states relative to their final values from the equilibrium A-EDS simulations of the unbound reference-state ligand in water for acceleration σ-levels of 1σ (A), 2σ (B) and 3σ (C), and for the reference-state ligand bound to the protein for acceleration σ-levels of 1σ (D), 2σ (E) and 3σ (F) of system TRP. Note that in panels C and F one relative free-energy difference $\Delta F_{i \neq 1,1}$ falls off the plot in the first half of the trajectory.
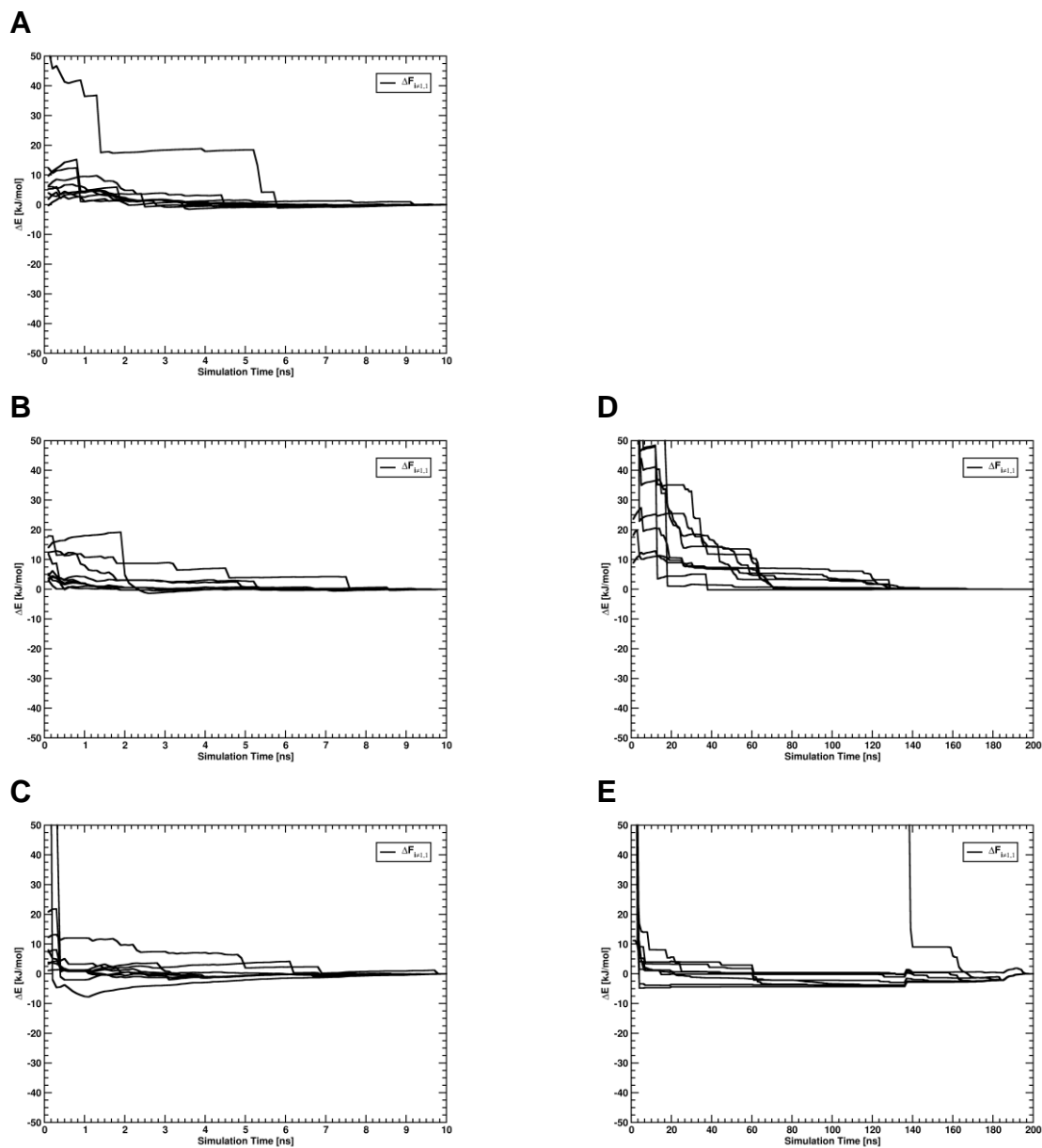
Figure S5. Convergence of the calculated relative free-energy differences $\Delta F_{i\neq1,1}$ between the ligand end-states to relative their final values from the equilibrium A-EDS simulations of the unbound reference-state ligand in water for acceleration σ-levels of 1σ (A), 2σ (B) and 3σ (C), and for the reference-state ligand bound to the protein for acceleration σ-levels of 2σ (D) and 3σ (E) of system PNMT. Note that in panels A, C, D and E relative free-energy differences $\Delta F_{i\neq1,1}$ falls off the plot.
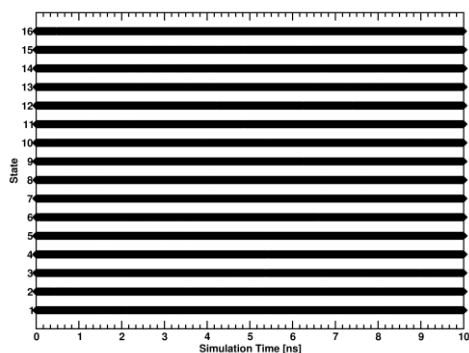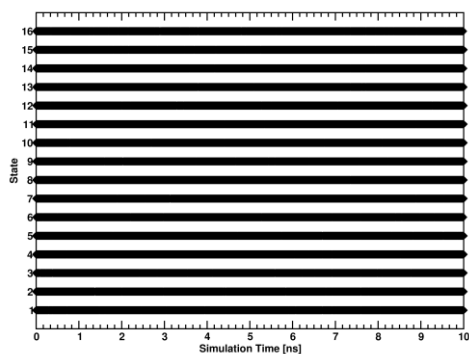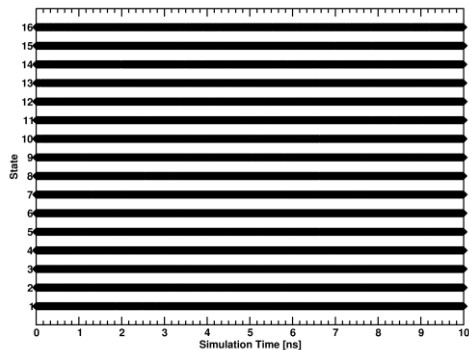
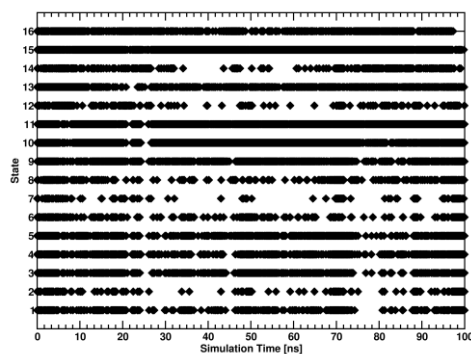**Figure S6.** Ligand end-state timeseries in the equilibrium A-EDS simulations of the unbound reference-state ligand in water for acceleration σ-levels of 1σ (A), 2σ (B) and 3σ (C), and for the reference-state ligand bound to the protein for acceleration σ-levels of 2σ (D) and 3σ (E) of system GRA2. At every timepoint, the state that is currently sampled is marked. Regular exchanges lead to continuous bars, due to the size of the marker.
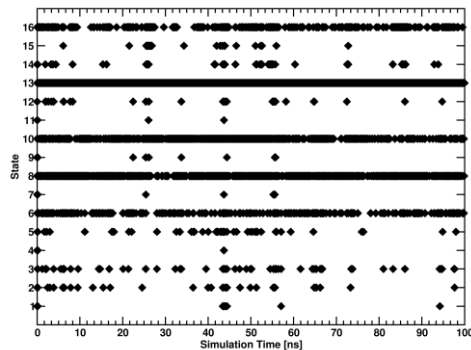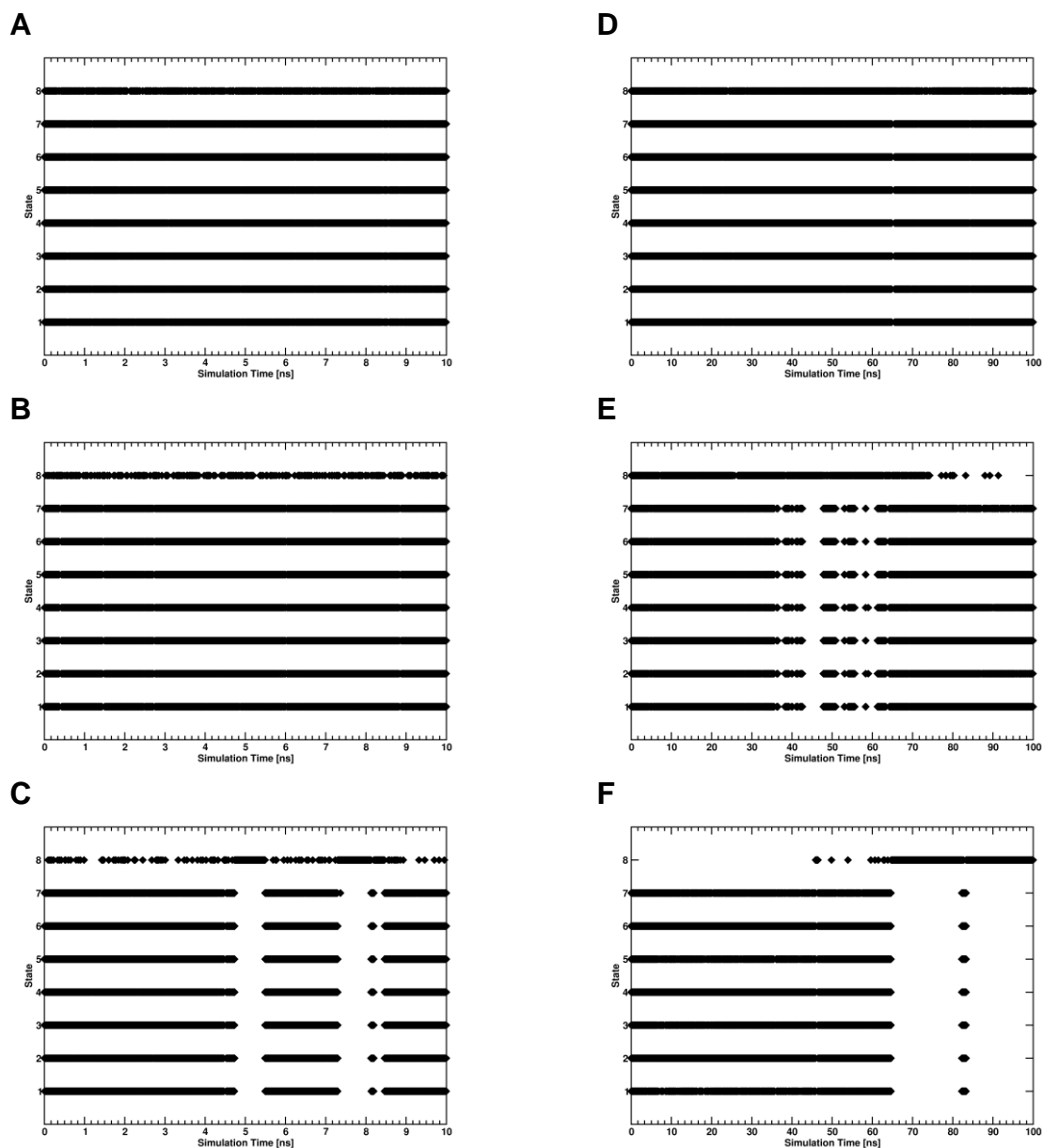
Figure S7. Ligand end-state timeseries in the equilibrium A-EDS simulations of the unbound reference-state ligand in water for acceleration σ-levels of 1σ (A), 2σ (B) and 3σ (C), and for the reference-state ligand bound to the protein for acceleration σ-levels of 1σ (D), 2σ (E) and 3σ (F) of system TRP. At every timepoint, the state that is currently sampled is marked. Regular exchanges lead to continuous bars, due to the size of the marker.
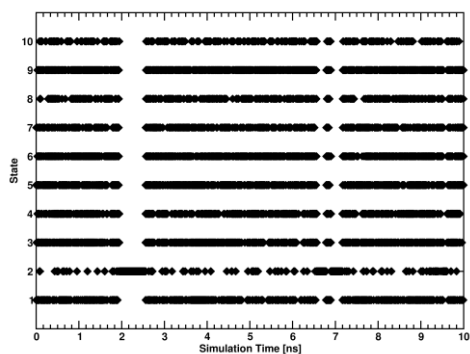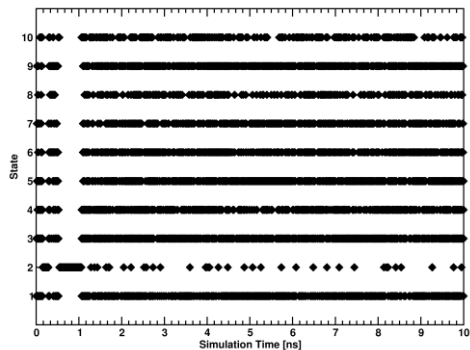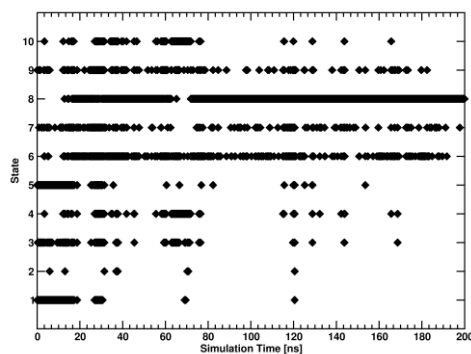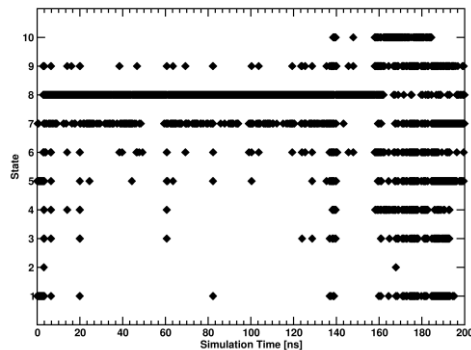
Figure S8. Ligand end-state timeseries in the equilibrium A-EDS simulations of the unbound reference-state ligand in water for acceleration σ-levels of 1σ (A), 2σ (B) and 3σ (C), and for the reference-state ligand bound to the protein for acceleration σ-levels of 2σ (D) and 3σ (E) of system PNMT. At every timepoint, the state that is currently sampled is marked. Regular exchanges lead to continuous bars, due to the size of the marker.
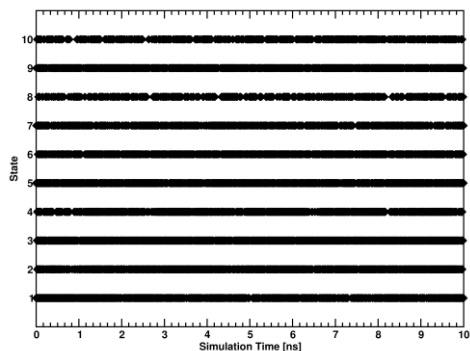
Table S6. Number of transitions between end-states during the first 10 ns of the A-EDS parameter search simulations with the ligand reference state bound to the protein.

Acceleration Level	System GRA2	System TRP	System PNMT
1σ	-	25655	11030
2σ	21331	25210	9979
3σ	17434	20719	4821

Table S7. A-EDS free-energy offset parameter search results for the three different acceleration σ-levels for system GRA2. Energy units are kJ·mol^{-1}.

	Unbound Ligand			Two Bound Ligands		
	1σ	2σ	3σ	1σ	2σ	3σ
ΔF_2^R	-93.1	-100.0	-208.6	-	-189.6	-208.9
ΔF_3^R	38.9	31.9	57.0	-	81.3	74.3
ΔF_4^R	27.0	19.4	30.0	-	20.0	29.6
ΔF_5^R	1.4	-23.2	-74.0	-	-55.6	-72.5
ΔF_6^R	-57.6	-71.1	-157.8	-	-113.9	-142.6
ΔF_7^R	-70.3	-84.5	-185.6	-	-155.3	-179.6
ΔF_8^R	-68.2	-99.2	-232.4	-	-200.5	-235.9
ΔF_9^R	93.9	79.1	142.4	-	161.4	160.6
ΔF_{10}^R	-6.1	-36.9	-107.4	-	-75.7	-101.9
ΔF_{11}^R	20.1	-11.1	-56.6	-	-39.4	-52.9
ΔF_{12}^R	-7.3	-27.7	-78.2	-	-26.7	-63.1
ΔF_{13}^R	-79.1	-115.3	-270.0	-	-231.0	-271.1
ΔF_{14}^R	-53.4	-89.6	-219.2	-	-179.8	-219.3
ΔF_{15}^R	40.3	3.8	-32.0	-	-4.7	-26.5
ΔF_{16}^R	-35.8	-77.1	-199.0	-	-152.3	-194.8

Table S8. A-EDS free-energy offset parameter search results for the three different acceleration σ-levels for system TRP. Energy units are kJ·mol^{-1}.

	Unbound Ligand			Bound Ligand		
	1σ	2σ	3σ	1σ	2σ	3σ
ΔF_2^R	-42.8	-49.1	-54.1	-32.0	-40.0	-12.8
ΔF_3^R	-88.8	-90.4	-91.9	-90.4	-94.2	-77.9
ΔF_4^R	165.4	159.4	155.5	146.4	139.0	178.0
ΔF_5^R	-105.0	-105.8	-107.1	-98.9	-102.0	-87.2
ΔF_6^R	31.9	29.2	26.4	24.7	18.7	54.0
ΔF_7^R	17.1	11.2	6.2	11.5	5.7	34.7
ΔF_8^R	-158.0	-180.5	-198.5	-137.4	-152.8	-161.4

Table S9. A-EDS free-energy offset parameter search results for the three different acceleration σ-levels for system PNMT. Energy units are kJ·mol^{-1}.

	Unbound Ligand			Bound Ligand		
	1σ	2σ	3σ	1σ	2σ	3σ
ΔF_2^R	-50.5	-168.8	-189.8	-	-97.6	-107.2
ΔF_3^R	117.6	81.5	77.0	-	73.1	72.6
ΔF_4^R	178.7	120.7	113.0	-	108.6	117.3
ΔF_5^R	142.6	99.5	94.9	-	79.9	90.9
ΔF_6^R	206.4	131.1	121.5	-	114.7	119.2
ΔF_7^R	-228.0	-297.6	-303.7	-	-297.0	-300.0
ΔF_8^R	-186.9	-285.5	-297.8	-	-291.0	-288.8
ΔF_9^R	207.2	143.5	135.0	-	126.2	132.3
ΔF_{10}^R	55.9	-16.8	-17.4	-	-29.3	-5.8

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