Protocols Utilizing Constant pH Molecular Dynamics to Compute pH-Dependent Binding Free Energies

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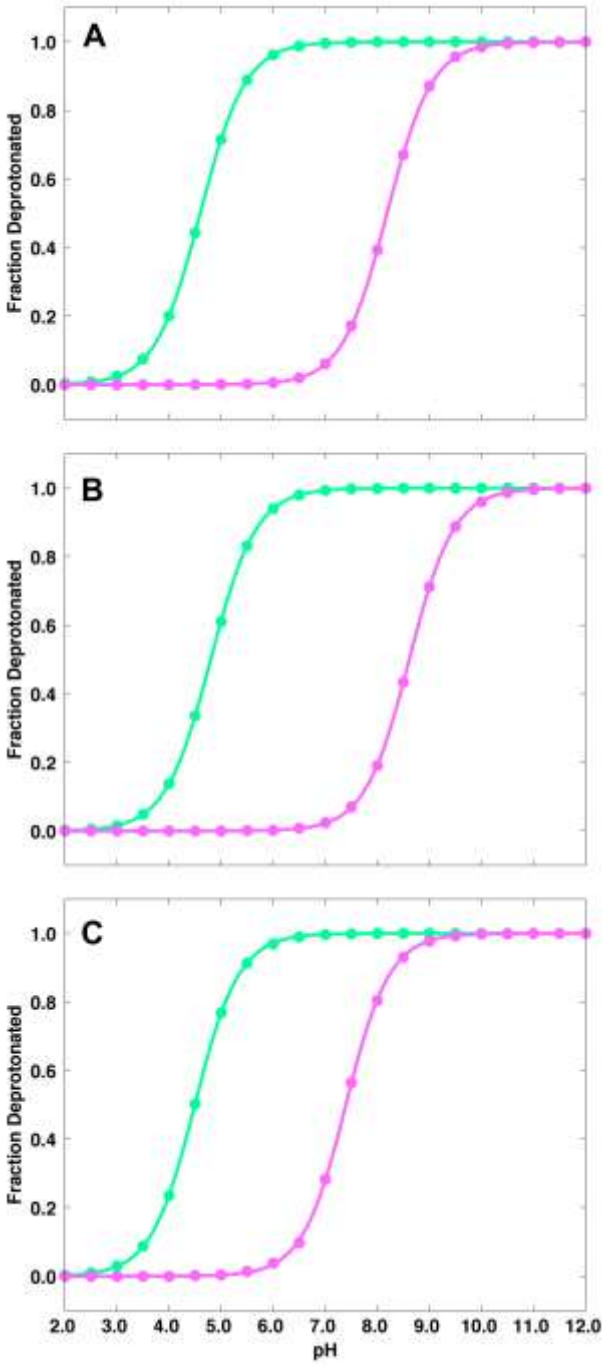

Figure S1. Titration curves from constant pH MD simulations of the guests free in solution (green) and in complex with CB[7] (purple). A. Thiabendazole. B. Fuberidazole. C. Carbendazim.

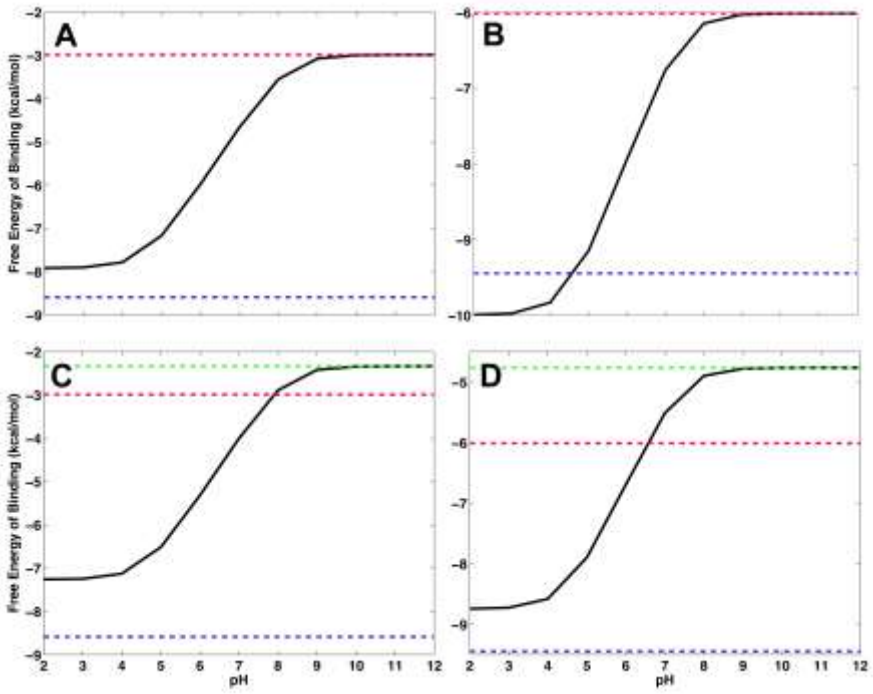

Figure S2. Binding free energies as functions of pH (black line). The top row is computed by the hybrid approach using the experimental reference binding energies ($\Delta G°_{ref,exp}$, red line) and the bottom row uses the full computational approach with the reference binding energies computed by thermodynamic integration ($\Delta G°_{ref,TI}$, green line). Experimentally derived binding free energies for the protonated guests are shown in blue. A, C. Thiabendazole. B, D. Carbendazim.

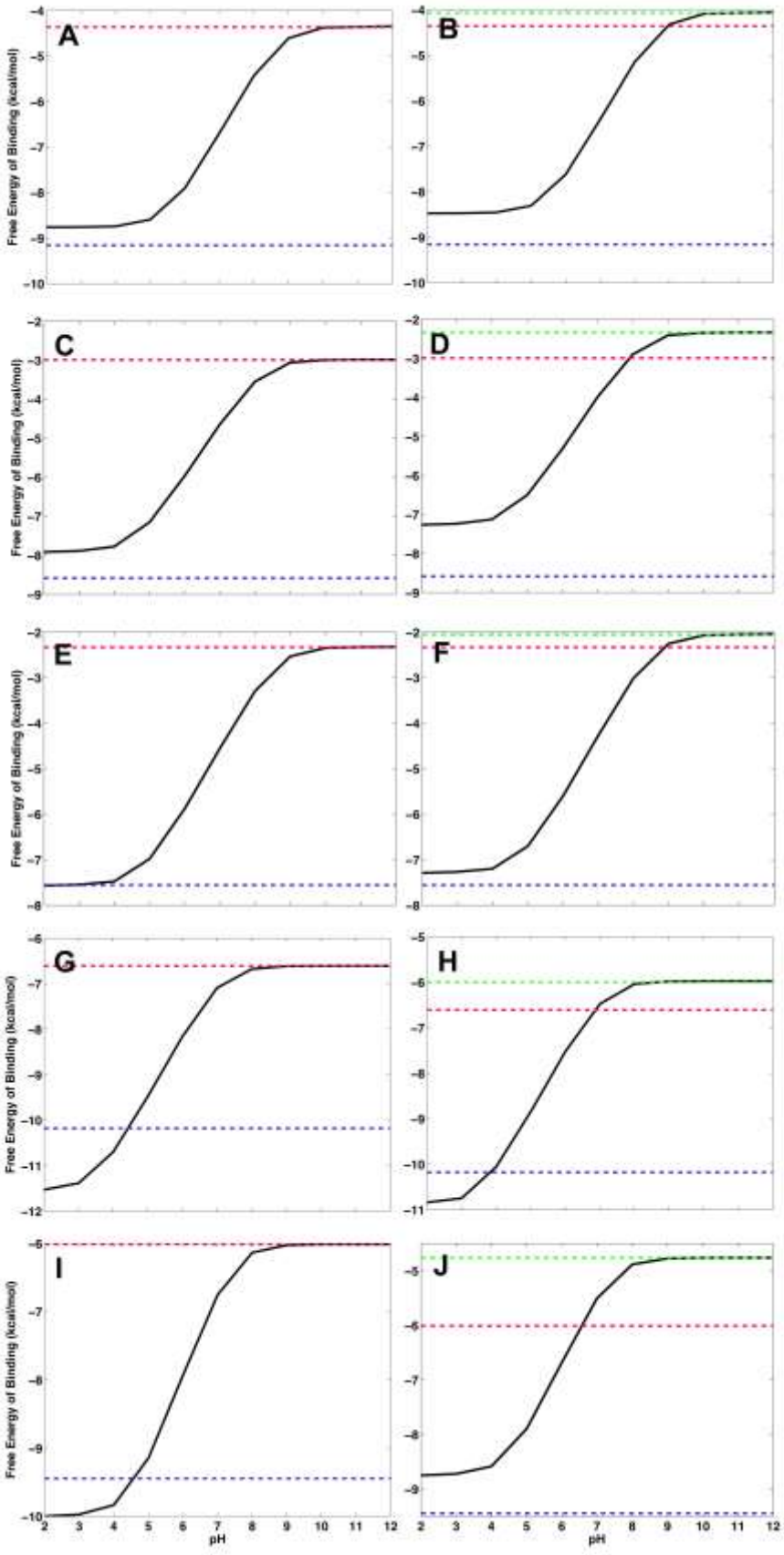
A
B
C
D
E
F
G
H
I
J
Free Energy of Binding (kcal/mol)
pH

Figure S3. Binding free energies as functions of pH (black line), computing using Eq. 8. The left row is computed by the hybrid approach using the experimental reference binding energies ($\Delta G°_{ref,exp}$, red line) and the right row uses the full computational approach with the reference binding energies computed by thermodynamic integration ($\Delta G°_{ref,TI}$, green line). Experimentally derived binding free energies for the protonated guests are shown in blue. A, B. Benzimidazole. C, D. Thiabendazole. E, F. Fuberidazole. G, H. Albendazole. I, J. Carbendazim.

Guest	$\Delta G°_{ref,exp}$	$\Delta G°^{+}_{exp}$	$\Delta G°^{+}_{hybrid}$	$\Delta G°_{ref,exp}$ (pH = 7)	$\Delta G°_{hybrid}$ (pH = 7)
BZ	-4.4	-9.2	-8.8	-7.1	-6.7
TBZ	-3.0	-8.6	-7.9	-5.2	-4.6
FBZ	-2.3	-7.6	-7.6	-4.5	-4.6
ABZ	-6.6	-10.2	-11.4	-6.7	-7.1
CBZ	-6.0	-9.5	-10.0	-6.4	-6.8

Table S1. Binding free energies of the guests upon complex formation with CB[7], computed using the hybrid approach with Eq. 8. All energies are reported in kcal/mol. $\Delta G°_{ref,exp}$ is the experimental[1] binding free energy for the reference deprotonated guest; $\Delta G°^{+}_{exp}$ is the binding free energy for the protonated guest derived from the $\Delta G°_{ref,exp}$; and $\Delta G°^{+}_{hybrid}$ is the free energy obtained by using $pK_a^{C,calc}$ with $\Delta G°_{ref,exp}$ in Eq. 8.

Guest	$\Delta G°_{ref,exp}$	$\Delta G°_{ref,TI}$	$\Delta G°^{+}_{exp}$	$\Delta G°^{+}_{TI}$	$\Delta G°_{ref,exp}$ (pH = 7)	$\Delta G°_{ref,TI}$ (pH = 7)
BZ	-4.4	-4.1 ± 2.0	-9.2	-8.5	-7.1	-6.4
TBZ	-3.0	-2.3 ± 2.6	-8.6	-7.3	-5.2	-4.0
FBZ	-2.3	-2.1 ± 2.6	-7.6	-7.3	-4.5	-4.3
ABZ	-6.6	-6.0 ± 3.0	-10.2	-10.8	-6.7	-6.5
CBZ	-6.0	-4.8 ± 2.7	-9.5	-8.7	-6.4	-5.5

Table S2. Binding free energies of the guests, computed using full computational approach (CpHMD/TI) and compared to experiment.[1] All energies are reported in kcal/mol. $\Delta G°_{ref,exp}$ is the experimental[1] binding free energy for the reference deprotonated guest; $\Delta G°_{ref,TI}$ is the absolute binding free energy obtained from TI computations for the reference state; $\Delta G°^{+}_{exp}$ is the binding free energy for the protonated guest derived from the $\Delta G°_{ref,exp}$; and $\Delta G°^{+}_{TI}$ is the binding free energy obtained by using $pK_a^{C,calc}$ with $\Delta G°_{ref,TI}$ in Eq. 8.

System	Segment	ΔG_{elec}	ΔG_{vdw}	ΔG_1	$\Delta G_3°$	$\Delta G°_{ref,TI}$	$\Delta G°_{ref,exp}$
CB[7]: BZN	ΔG_2	6.14 ± 0.60	16.22 ± 1.10	6.28 ± 0.74	-14.28	-4.07 ± 2.05	-4.36
	ΔG_4	10.13 ± 0.85	0.16 ± 1.16				
CB[7]: TBN	ΔG_2	-36.45 ± 0.82	16.10 ± 1.51	4.48 ± 0.62	-14.36	-2.33 ± 2.58	-2.99
	ΔG_4	-33.68 ± 0.97	1.12 ± 1.55				
CB[7]: FBN	ΔG_2	-10.64 ± 0.76	15.40 ± 1.50	4.34 ± 0.59	-14.36	-2.05 ± 2.52	-2.33
	ΔG_4	-7.88 ± 0.92	0.59 ± 1.54				
CB[7]: ABN	ΔG_2	249.13 ± 0.94	17.65 ± 1.84	3.61 ± 0.57	-14.85	-6.00 ± 3.01	-6.61
	ΔG_4	248.75 ± 1.05	0.79 ± 1.83				
CB[7]: CBN	ΔG_2	257.78 ± 0.89	16.63 ± 1.54	4.42 ± 0.62	-14.69	-4.76 ± 2.69	-6.01
	ΔG_4	258.17± 1.11	1.21 ± 1.56				

Table S3. Free energy for each segment in the thermodynamic cycle (Scheme 2) for absolute binding free energy computations for the guests. All energies are reported in kcal/mol. ΔG_1 is the free energy for gradually turning on restraints on; ΔG_2 is the free energy for decoupling the guest while bound to the host in the presence of the restraints; $\Delta G_3°$ is the free energy for turning off the restraint and correcting for the standard state; and ΔG_4 is the solvation free energy for the decoupled guest. Statistical errors are reported as standard deviations. Following Scheme 2, $\Delta G°_{ref,TI}$ is computed as - ΔG_1 - ΔG_2 - $\Delta G_3°$ + ΔG_4.