SUPPORTING INFORMATION

Water Networks Contribute to Enthalpy/Entropy Compensation in Protein-Ligand Binding

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Expression and Purification of Human Carbonic Anhydrase

We chose human carbonic anhydrase II (HCA, E.C. 4.2.1.1) as a model protein to study the enthalpy-entropy compensation (*H*/*S* compensation) in protein-ligand binding, because HCA: i) is well-characterized structurally with X-ray and neutron structures,[1-5] ii) is structurally rigid, and retains its secondary and tertiary structure upon binding of a ligand;[1, 5] iii) can be expressed in *E. coli* in the quantities necessary for calorimetry experiments and X-ray crystallography; iv) crystallizes readily, and the conditions for growing crystals, reproducibly, are known.[6]

We expressed the HCA protein according to procedures published previously by Fierke and coworkers,[7][8] and then purified the protein with methods described previously.[4]

Synthesis of the Partially Fluorinated Heteroarylsulfonamide Ligands

We synthesized the differently fluorinated benzoheteroarylsulfonamide ligands from commercially available starting materials, which we used without further purification (Sigma Aldrich). We characterized the final product of each synthesis with ^{1}H and ^{19}F NMR spectroscopy, and GC-MS (EI). We measured ^{1}H and ^{19}F spectra on a 300 or 400 MHz instrument, and report the chemical shifts (in ppm) relative to tetramethylsilane or residual un-deuterated solvent for ^{1}H NMR spectra, and relative to trichloro-fluoromethane for ^{19}F NMR spectra. Coupling constants (*J*) are given in Hz, and the apparent resonance multiplicities are abbreviated with (b)s ((broad) singlet), d (doublet), t (triplet), and m (multiplet). We measured the mass of each compound with an Agilent GC/MSD 5975A inert with a Triple-Axis Detector, controlled *via* Agilent GC ChemStation Software (version E.02.00.493), containing a HP 5MS column (5% diphenyl 95% dimethylpolysiloxane, 30 m x 0.25 mm).

We synthesized the non-fluorinated benzo-extended ligand benzo[*d*]thiazole-2-sulfonamide (**H_4BTA**)[5] and the fully-fluorinated ligand ,5,6,7-tetrafluorobenzo[*d*]thiazole-2-sulfonamide (**F_4BTA**)[4] according to previously published synthetic procedures. We stored all ligands under argon or nitrogen at room temperature.

General Synthetic Procedure for Partially Fluorinated Benzoheteroarylsulfonamide Ligands. Each of the fluorobenzo-extended ligands were synthesized according to the following general synthetic procedure (**GP**): The appropriately fluorinated aniline (25 mmol) was dissolved in DMF (30 mL) before potassium ethyl xanthate (2.2 equivalents) was added to the solution. The reaction mixture was heated to 120° C for 4 hours, and subsequently cooled to 25° C before being quenched with HCl (1M), and the product isolated by filtration.[9] The isolated thiazolethiones (10 mmol) were dissolved in NaOH (5% aq; 30 mL). Simultaneously, aliquots of NaOCl (5% aq; 20 mL) and of NH_4OH (10 mL) were added to the solution at 0° C dropwise over 15 minutes. After being stirred for 1.5 hours, the intermediate *S*-(fluorobenzo[*d*]thiazol-2-yl)thiohydroxylamines were filtered, dried *in vacuo*, and used without further purification in the next step.[10]

The thiohydroxylamines (10 mmol) were dissolved in DME (50 mL) and cooled to 0° C before *meta*-chloroperoxybenzoic acid (20 mmol, dissolved in 10 mL of DME) was added. The reaction was stirred overnight and allowed to warm to room temperature. EtOAc was added, and extracted with $NaHCO_3$ (5% aq.; 3 x 30 mL) yielded the desired crude partially fluorinated benzothiazolsulfonamides. The partially fluorinated ligands were recrystallized in EtOH.

7-fluorobenzo[d]thiazole-2-sulfonamide (7-F_1BTA). **7-F_1BTA** was synthesized, according to the **GP** outlined above, starting from 2,3-difluoroaniline.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (bs, 2H), 8.08 (dd, J = 0.8, 8.3, 1H), 7.73 (m, 1H), 7.56 (ddd, J = 0.8, 8.3, 9.7, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −110.9 (dd, J = 5.6, 9.3); GC-MS (EI, 70 eV): m/z 232.0 (M^+, 100%) @ t_R 21.0 min.

4-fluorobenzo[d]thiazole-2-sulfonamide (4-F_1BTA). **4-F_1BTA** was synthesized, according to the **GP** outlined above, starting from 2,6-difluoroaniline.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (bs, 2H), 8.10 (dd, J = 0.9, 8.1, 1H), 7.66 (m, 1H), 7.55 (ddd, J = 0.9, 8.1, 10.7, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −110.9 (dd, J = 5.9, 10.7); GC-MS (EI, 70 eV): m/z 232.0 (M^+, 100%) @ t_R 21.9 min.

5,6-difluorobenzo[d]thiazole-2-sulfonamide (5,6-F_2BTA). **5,6-F_2BTA** was synthesized, according to the **GP** outlined above, starting from 2,4,5-trifluoroaniline.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (bs, 2H), 8.39 (m, 2H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −133.5 (m), −138.4 (m); GC-MS (EI, 70 eV): m/z 250.0 (M^+, 100%) @ t_R 21.9 min.

4,7-difluorobenzo[d]thiazole-2-sulfonamide (4,7-F_2BTA). **4,7-F_2BTA** was synthesized, according to the **GP** outlined above, starting from 2,3,6-trifluoroaniline.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (bs, 2H), 7.65 (m, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −115.95 (m), −126.07 (m); GC-MS (EI, 70 eV): m/z 250.0 (M^+, 100%) @ t_R 21.4 min.

6,7-difluorobenzo[d]thiazole-2-sulfonamide (6,7-F_2BTA). **6,7-F_2BTA** was synthesized, according to the **GP** outlined above, starting from 2,3,4-trifluoroaniline.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (bs, 2H), 8.12 (ddd, J = 1.4, 4.3, 9.2, 1H), 7.83 (m, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −135.3 (dd, J = 8.0, 22.2), −139.0 (ddd, J = 3.6, 10.9, 21.5); GC-MS (EI, 70 eV): m/z 250.0 (M^+, 100%) @ t_R 20.6 min.

4,6-difluorobenzo[d]thiazole-2-sulfonamide (4,6-F_2BTA). **4,6-F_2BTA** was synthesized, according to the **GP** outlined above, starting from 2,4,6-trifluoroaniline.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 (bs, 2H), 8.03 (m, 1H), 7.67 (m, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −109.1 (m), −117.3 (dd, J = 6.8, 10.1); GC-MS (EI, 70 eV): m/z 250.0 (M^+, 100%) @ t_R 22.0 min.

5,6,7-trifluorobenzo[d]thiazole-2-sulfonamide (5,6,7-F_3BTA). **5,6,7-F_3BTA** was synthesized, according to the **GP** outlined above, starting from 2,3,4,5-tetrafluoroaniline.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (bs, 2H), 8.20 (s, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −130.9 (m), −131.3 (m), −162.1 (dt, J = 21.9, 5.0); GC-MS (EI, 70 eV): m/z 268.0 (M^+, 100%) @ t_R 20.1 min.

Physiochemical Characterization of the Fluorobenzothiazole Ligands

We summarize the physico-chemical data collected for each sulfonamide ligand in Table S1, and discuss the procedures in detail below.

Determination of pK_a. The pK_a of **H_4BTA** and **F_4BTA** (Figure S1) were determined previously.[5] We used the same procedure to determine the pK_a of the fluorobenzothiazole ligands: a solution of the ligand (20 mM in DMSO) was added to a buffered solution (10 mM, ranging from pH 1 to pH 13 in 0.5 increments of pH) in a cuvette, and a UV-VIS spectrum recorded.

Determination of ΔH^o_{ion}. We could not measure the ΔH^o_{ion} of the fluorobenzo-extended ligands directly with calorimetry due to their low solubility. To approximate values of ΔH^o_{ion}, we correlated the chemical shift of the proton in the sulfonamide group, measured with ^{1}H NMR spectroscopy, with the chemical shift of the proton in the sulfonamide group of known heteroarylsulfonamide ligands, which was described previously.[4]

Partitioning experiments. We measured the equilibrium constant of partitioning, between sodium phosphate buffer (10 mM, pH = 7.6) and octanol, for each of the fluorobenzothiazole ligands using the shake-flask method described previously.[5]

Solution calorimetry. We followed the procedure reported previously,[5] and measured the heat of dissolution for solid samples of each ligand (5–10 mg) with a TAMIII calorimeter (TA Instruments)

Table S1. Physical properties of **H_4BTA**, the partially fluorinated heteroarylsulfonamides used in this study, **F_4BTA**, and the Zn(II)-bound-water form of HCA II (HCA-Zn(II)-OH_2^+).

System ligand	SASA [Å^2]	pK_a NMR [c]	$\Delta H°_{ion}$ [kcal mol^{-1}] [a]	log$P$ [b]	$\Delta G°_{WO}$ [kcal mol^{-1}]	$\Delta G°_{WO}$ per Å^2 [cal mol^{-1} Å^{-2}]	μ [D] [e]
H_4BTA	449	8.2	7.4	2.28	1.45 ± 0.03	4.00	7.73
4-F_1BTA	457	8.2.	7.5	2.63	1.66 ± 0.02	4.50	7.15
7-F_1BTA	456	8.1	7.3	2.18	1.80 ± 0.1	4.87	7.39
6,7-F_2BTA	468	8.0	7.3	2.68	1.91 ± 0.02	5.07	6.67
4,7-F_2BTA	468	7.9	7.2	1.91	2.17 ± 0.1	5.78	7.20
5,6-F_2BTA	467	8.3	7.5	1.89	1.80 ± 0.1	4.81	5.64
4,6-F_2BTA	467	8.2	7.4	2.07	1.73 ± 0.1	4.60	6.39
5,6,7-F_3BTA	476	8.0	7.2	1.86	2.21 ± 0.3	5.78	□□1
F_4BTA	483	7.9	7.2	2.71	2.67 ± 0.08	7.05	5.33
HCA-Zn(II)-OH_2^+ [c]	---	6.9	6.9	---	---	---	---

a) NMR experiments were performed in DMSO-d_6 at 25° C.
b) Calculated *via* log$P$ = log$D$ + log(1 + 10^{pKa-pH}).
c) Values estimated according to the literature.[1]

Isothermal Titration Calorimetry

We prepared 20 mM stock-solutions of each sulfonamide in d^6-DMSO using a 25 mL volumetric flask. We measured the concentrations of the stock solutions of each ligand by ^{1}H NMR. These solutions were diluted to 100 μM in sodium phosphate buffer (10 mM, pH = 7.6; same buffer from the last dialysis of each protein purification) with micropipettes (Eppendorf), mixed, and immediately used for the ITC experiments. We conducted all ITC experiments on an Auto VP-ITC instrument (Microcal) at 298.15 K. The titration of the sulfonamide ligand (100 μM) into a solution of HCA II (5 μM) comprised 20 injections (one initial injection of 1 μL followed by nineteen injections of 7.8 μL per injection, with a 300-second interval between injections, stirring of 300 rpm, and a reference power of 14 μcal/sec). We deleted the initial data point from the 1 μL injection from the integrated data to minimize the effect of diffusive mixing that occurs during equilibration of the instrument. We carefully prepared a stock solution of the methazolamide solution using a 50 mL volumetric flask and determined the concentration by NMR spectroscopy. We cross-checked and corrected the actual concentration of each partially fluorinated sulfonamide solution, which was used during the ITC experiment, by running an ITC experiment of a stock solution of an internal standard (methazolamide) after each ITC experiment. The uncertainty of the target concentration was less than 5%.

We measured the differential power between the sample cell and the reference cell and used Origin 7.0, and a macro (VPViewer 2000) provided by MicroCal to analyze the ITC data. We estimated the free energy of binding (ΔG^{o}_{bind}) and entropy of binding ($-T\Delta S^{o}_{bind}$) with a nonlinear, single-binding site model. Values of K_a, ΔG^{o}_{bind}, ΔH^{o}_{bind}, and $-T\Delta S^{o}_{bind}$ are the average

of at least seven experiments and the uncertainties are one standard deviation from the mean (see Table S3).

pK_a-correction of observed ITC data. We corrected the K_a of each fluorobenzothiazole ligand—to reflect the binding of the arylsulfonamide anion ($ArSO_2NH^-$) to the zinc-bound water form of HCA (HCA-Zn^{II}-OH_2^+)—using a previously reported method,[11] which is explained in detail by Snyder *et al*.[5] Table S2 lists the observed, and the pK_a-corrected values of ΔG^o_{bind}, ΔH^o_{bind}, and $-T\Delta S^o_{bind}$ for the sulfonamide ligands investigated in this study. Table S3 lists all observed thermodynamic parameters for the individual titrations. Figure S1 plots $\Delta G°_{bind}$, $\Delta H°_{bind}$, and $T\Delta S°_{bind}$ as function of the difference in solvent-accessible surface area ($\Delta SASA_{bound} = SASA_{ligand} + SASA_{protein} - SASA_{complex}$) for each ligand. Figure S2 also plots the free energy (ΔG^o_{OW}), the enthalpy (ΔH^o_{OW}) and entropy ($-T\Delta S^o_{OW}$) of partitioning from octanol to buffer of **H_4BTA**, **7-F_1BTA**, **4,6-F_2BTA** and **F_4BTA**. Figure S2 plots representative thermograms of each partially fluorinated ligand.

Table S2. Average observed and pK_a-corrected values for the free energy of binding ($\Delta G°_{bind}$), enthalpy of binding ($\Delta H°_{bind}$), and entropy of binding ($-T\Delta S°_{bind}$) of the anionic form of the fluorobenzothiazolesulfonamide ligands (**y-F$_x$BTA**)$^-$ to HCA.

Ligand	N[a]	SASA (Å^2)	$\Delta G°_{bind}$ (kcal mol^{-1})		$\Delta H°_{bind}$ (kcal mol^{-1})		$-T\Delta S°_{bind}$ (kcal mol^{-1})	
			observed	corrected[b]	observed	corrected[b]	observed	corrected[b]
H_4BTA	9	449	−11.4±0.3	−13.5±0.3	−18.6±0.6	−18.9±0.5	7.1±0.8	5.5±0.7
7-F_1BTA	7	457	−12.8±0.1	−14.7±0.1	−18.8±0.6	−18.6±0.2	6.1±0.1	3.9±0.3
4-F_1BTA	7	456	−11.3±0.4	−13.1±0.4	−13.7±0.3	−13.4±0.3	2.8±0.4	0.3±0.4
6,7-F_2BTA	7	468	−12.7±0.2	−14.5±0.2	−18.1±0.2	−17.9±0.3	5.4±0.3	2.8±0.3
4,7-F_2BTA	8	468	−11.6±0.1	−13.3±0.1	−18.3±0.4	−17.5±0.4	6.7±0.4	4.2±0.4
5,6-F_2BTA	7	467	−11.6±0.4	−13.6±0.4	−18.1±1.2	−18.8±0.7	6.5±0.9	5.1±0.6
4,6-F_2BTA	8	467	−11.5±0.2	−13.7±0.2	−13.9±0.1	−13.9±0.4	2.2±0.1	~ □±0.5
5,6,7-F_3BTA	12	476	−12.0±0.2	−13.7±0.2	−18.1±0.7	−17.4±0.7	6.1±0.7	3.7±0.7
F_4BTA	9	483	−11.3±0.2	−13.0±0.2	−17.3±0.6	−16.3±0.6	6.0±0.5	3.4±0.5

a) Number of experiments used for analyses.
b) Corrected for the pK_a-values determined as described above.

Table S3. Observed Thermodynamic Parameters for the individual titrations.

H_4BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
1.03	1.28E+08	-18.34	7.81E-09	-11.06	7.27
1.02	1.95E+08	-18.94	5.13E-09	-11.31	7.68
0.99	2.18E+08	-18.84	4.59E-09	-11.37	7.45
0.98	1.17E+08	-19.09	8.55E-09	-11.00	8.07
0.98	1.63E+08	-19.07	6.13E-09	-11.20	7.86
1.10	2.83E+08	-17.42	3.53E-09	-11.53	5.90
1.12	5.00E+08	-17.72	2.00E-09	-11.87	5.84
0.98	4.73E+08	-18.68	2.11E-09	-11.83	6.85
0.98	4.55E+08	-19.01	2.20E-09	-11.81	7.21

7-F_1BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
0.98	2.24E+09	-18.78	4.46E-10	-12.76	6.02
0.98	1.83E+09	-18.90	5.46E-10	-12.64	6.26
0.98	2.21E+09	-18.77	4.52E-10	-12.74	6.02
1.01	3.49E+09	-18.43	2.87E-10	-13.01	5.81
1.00	3.00E+09	-18.58	3.33E-10	-12.93	6.02
1.01	2.06E+09	-18.64	4.84E-10	-12.70	6.02
1.00	2.27E+09	-18.82	4.41E-10	-12.76	6.29

4-F_1BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
1.00	7.66E+08	-13.97	1.31E-09	-12.10	1.84
1.02	1.11E+08	-13.36	9.01E-09	-10.97	2.74
1.01	8.72E+07	-13.54	1.15E-08	-10.83	3.13
1.04	3.04E+08	-13.72	3.29E-09	-11.57	2.89
1.02	2.55E+08	-13.63	3.92E-09	-11.46	2.89
1.02	1.31E+08	-13.39	7.63E-09	-11.07	3.04
1.02	1.47E+08	-14.11	6.80E-09	-11.14	2.95

5,6-F_2BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal	K_d (M)	$\Delta G°_{obs}$ (kcal	$-T\Delta S°_{obs}$ (kcal
1.00	3.38E+08	-18.09	2.96E-09	-11.64	6.44
0.97	2.46E+08	-18.31	4.07E-09	-11.44	6.85
1.01	4.64E+08	-17.84	2.16E-09	-11.82	6.02
1.00	2.87E+08	-17.82	3.48E-09	-11.54	6.29
0.95	5.46E+08	-19.46	1.83E-09	-11.92	7.54
0.95	4.65E+08	-19.34	2.15E-09	-11.82	7.51
1.01	2.38E+08	-17.14	4.20E-09	-12.79	4.35

4,7-F_2BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
0.99	2.64E+08	-18.37	3.79E-09	-11.49	6.88
0.99	4.20E+08	-18.15	2.38E-09	-11.76	6.38
0.98	3.73E+08	-18.19	2.68E-09	-11.69	6.50
0.98	4.33E+08	-17.97	2.31E-09	-11.78	6.20
0.97	2.57E+08	-18.26	3.89E-09	-11.47	6.79
0.97	3.09E+08	-19.09	3.24E-09	-11.58	7.51
0.97	2.50E+08	-17.89	4.00E-09	-11.45	6.44
0.99	3.17E+08	-18.52	3.15E-09	-11.59	6.91

6,7-F_2BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
1.00	2.62E+09	-18.20	3.82E-10	-12.85	5.45
1.01	3.61E+09	-18.10	2.77E-10	-13.04	5.54
1.01	2.92E+09	-18.19	3.42E-10	-12.91	5.04
1.00	9.12E+09	-18.25	1.10E-10	-13.58	5.03
0.97	9.96E+09	-18.31	1.00E-10	-13.64	5.93
1.01	9.46E+09	-18.84	1.06E-10	-13.61	5.19
1.00	2.87E+09	-18.22	3.48E-10	-12.90	5.39

4,6-F_2BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
1.00	3.81E+08	-14.00	2.62E-09	-11.71	2.30
1.00	3.39E+08	-13.86	2.95E-09	-11.64	2.23
0.99	3.77E+08	-13.86	2.65E-09	-11.69	2.16
1.06	2.18E+08	-13.75	4.59E-09	-11.37	1.74
1.04	2.29E+08	-12.95	4.37E-09	-11.40	0.91
1.01	1.86E+08	-13.73	5.38E-09	-11.28	2.45
1.01	1.26E+08	-14.13	7.94E-09	-11.05	2.15
1.01	3.96E+08	-13.54	2.53E-09	-11.73	1.85

5,6,7-F_3BTA

N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
1.02	4.58E+08	-17.88	2.18E-09	-11.82	6.08
0.99	5.58E+08	-17.75	1.79E-09	-11.93	5.81
1.02	1.13E+09	-17.31	8.85E-10	-12.35	4.95
1.02	8.74E+08	-17.86	1.14E-09	-12.19	5.66
0.94	2.49E+08	-18.03	4.02E-09	-11.45	6.62
0.99	4.29E+08	-18.03	2.33E-09	-11.77	6.26
1.03	7.77E+08	-17.91	1.29E-09	-12.13	5.78
0.99	5.09E+08	-17.80	1.96E-09	-11.88	5.93
1.07	6.42E+08	-17.27	1.56E-09	-12.02	5.24
1.06	7.86E+08	-18.85	1.27E-09	-12.14	6.71
1.01	5.61E+08	-19.47	1.78E-09	-11.94	7.54
0.99	6.90E+08	-18.97	1.45E-09	-12.06	6.91

F_4BTA N	K_a (M^{-1})	$\Delta H°_{obs}$ (kcal mol^{-1})	K_d (M)	$\Delta G°_{obs}$ (kcal mol^{-1})	$-T\Delta S°_{obs}$ (kcal mol^{-1})
0.95	1.68E+08	-16.65	5.59E-09	-11.22	5.42
0.95	1.46E+08	-17.01	6.85E-10	-11.14	5.87
0.92	1.50E+08	-17.35	6.67E-09	-11.15	6.20
0.92	1.54E+08	-17.05	6.49E-09	-11.17	5.87
0.92	1.16E+08	-17.26	8.62E-09	-11.00	6.26
0.92	2.73E+08	-17.08	3.66E-09	-11.51	5.57
0.99	2.15E+08	-17.12	4.65E-09	-11.37	5.75
0.98	2.62E+08	-18.52	3.82E-09	-11.48	7.03
0.99	2.96E+08	-17.79	3.38E-09	-11.56	6.23

Figure S1. (A) Thermodynamics of binding for the anion of each partially fluorinated arylsulfonamide ligand to HCA as a function of the difference in solvent-accessible surface area between the bound and unbound states of the ligand as determined from the crystal structures ($\Delta SASA_{unbound} = \Delta SASA_{ligand} + \Delta SASA_{protein} - \Delta SASA_{complex}$). Each datum is the average of at least seven independent measurements, and the error bars represent one standard deviation from the mean. (B) Thermodynamics of partitioning of **H_4BTA**, **7-F_1BTA**, **4,6-F_2BTA** and **F_4BTA** from water to octanol. The equilibrium constant for partitioning was measured by a shake-flask method, and corrected for the ionization of the sulfonamide.

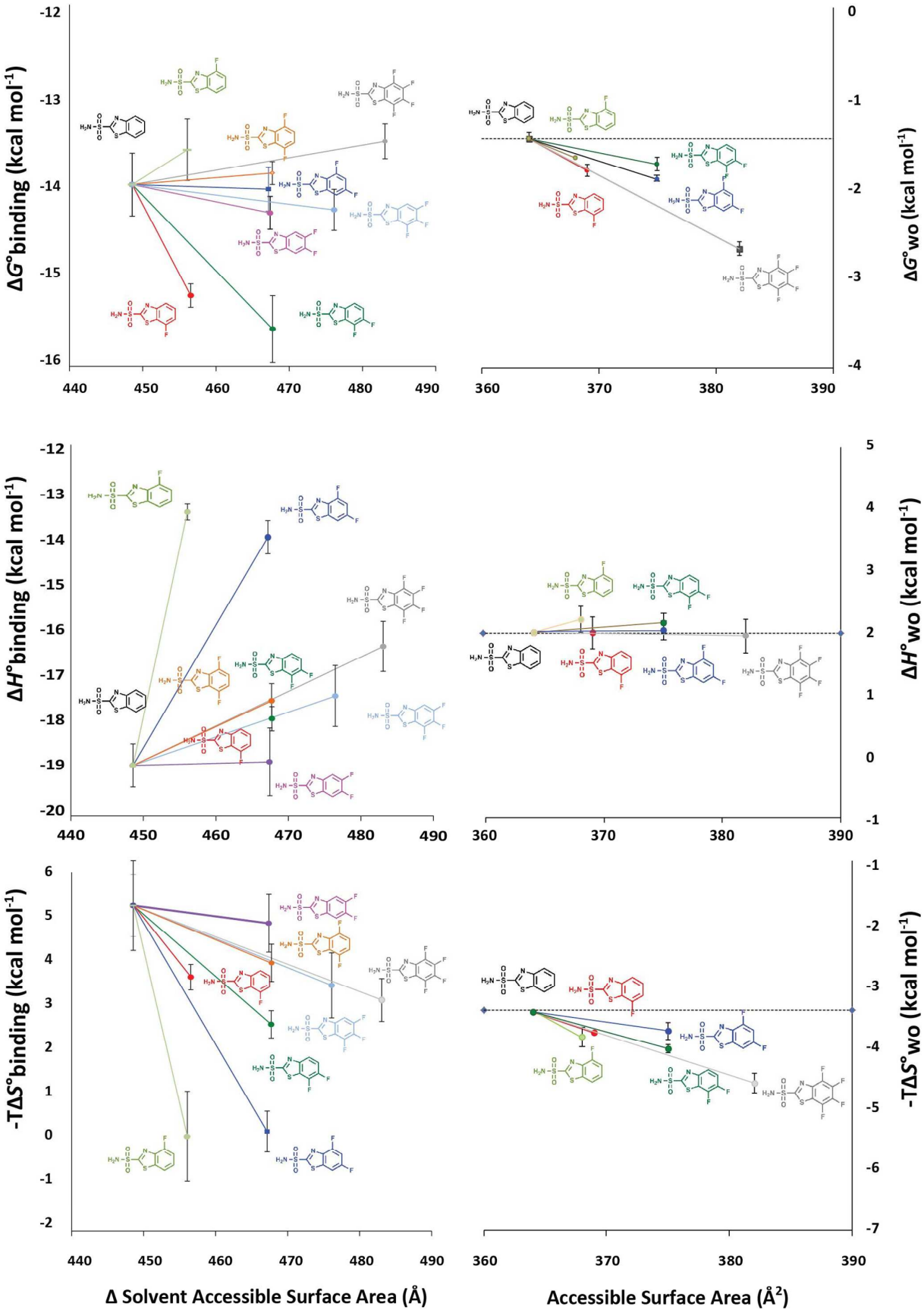
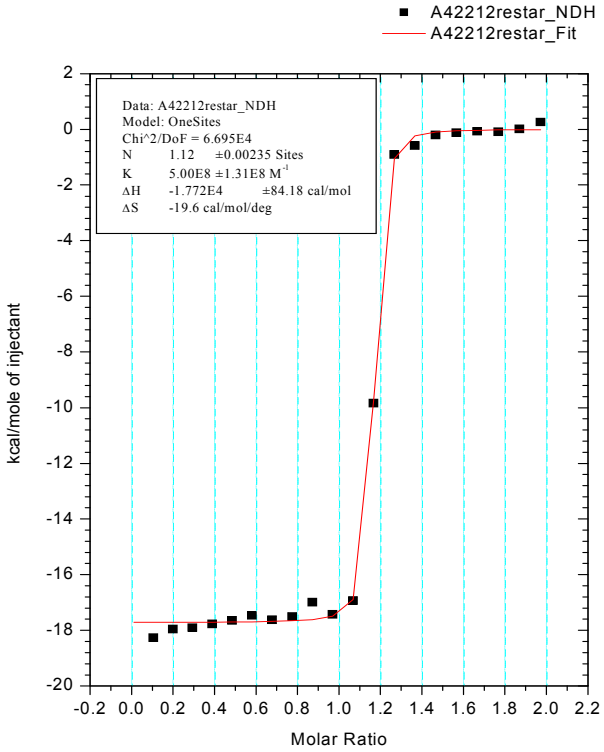
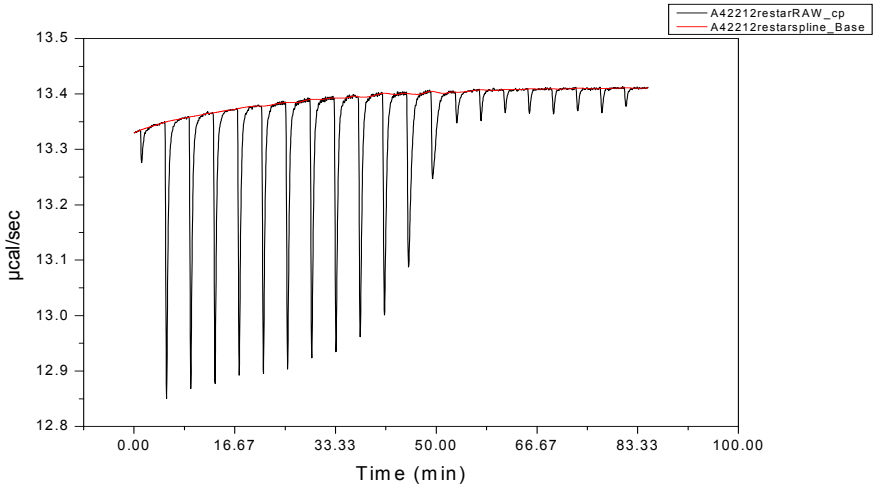

ΔG°binding (kcal mol-1)
ΔG°wo (kcal mol-1)
ΔH°binding (kcal mol-1)
ΔH°wo (kcal mol-1)
-TΔS°binding (kcal mol-1)
-TΔS°wo (kcal mol-1)
Δ Solvent Accessible Surface Area (Å)
Accessible Surface Area (Å2)

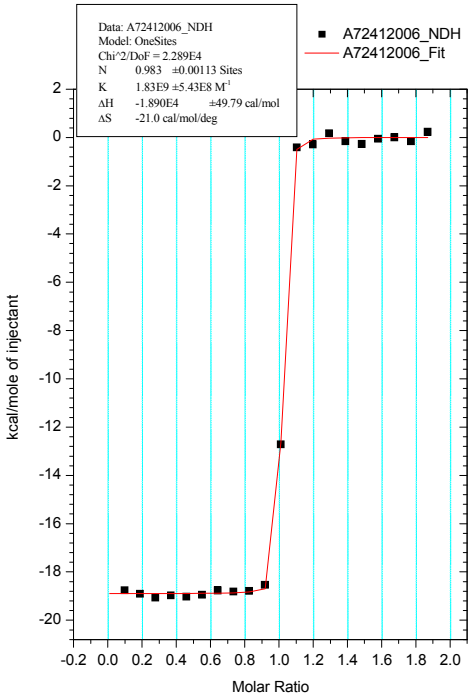
Figure S2. (A) Representative thermograms of each of the partially fluorinated ligand to HCA studied in this work.

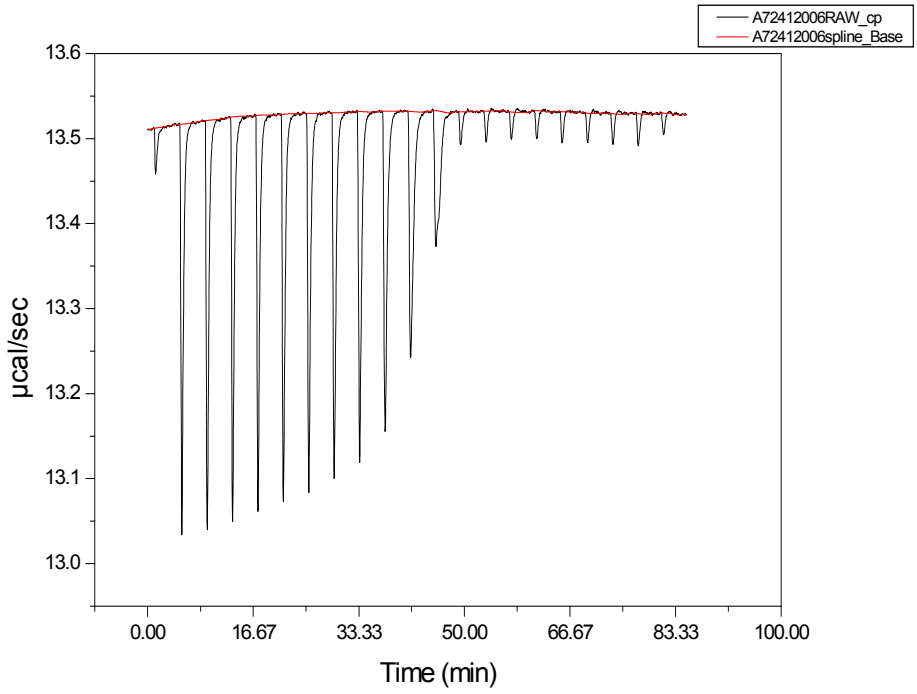
BTA:



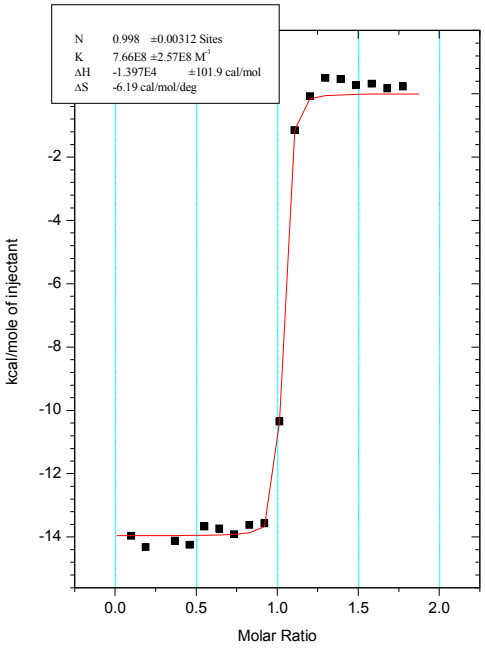


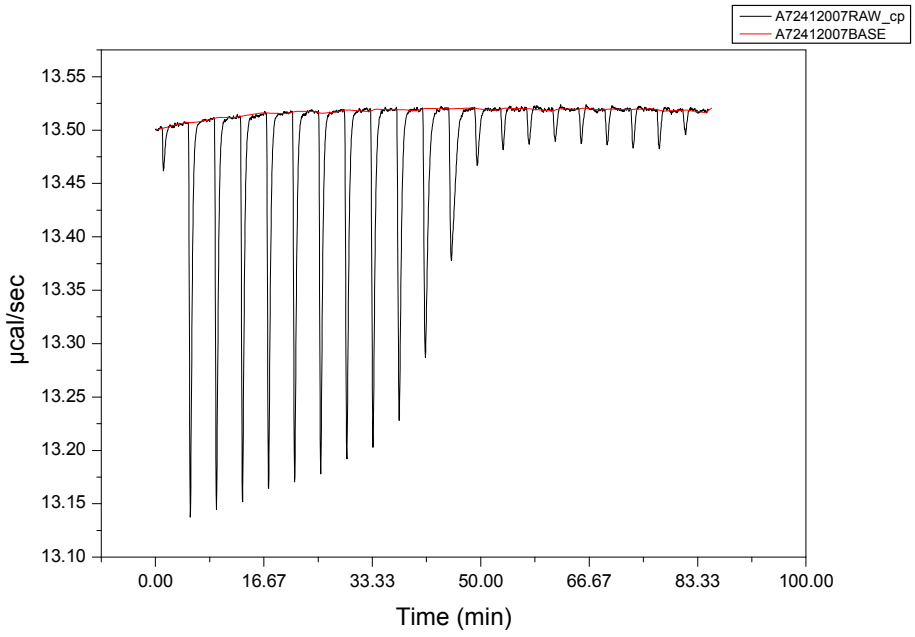
7-F_1BTA


Data: A72412006_NDH
Model: OneSites
Chi^2/DoF = 2.289E4
N 0.983 ±0.00113 Sites
K 1.83E9 ±5.43E8 M-1
ΔH -1.890E4 ±49.79 cal/mol
ΔS -21.0 cal/mol/deg
A72412006_NDH
A72412006_Fit
kcal/mole of injectant
Molar Ratio

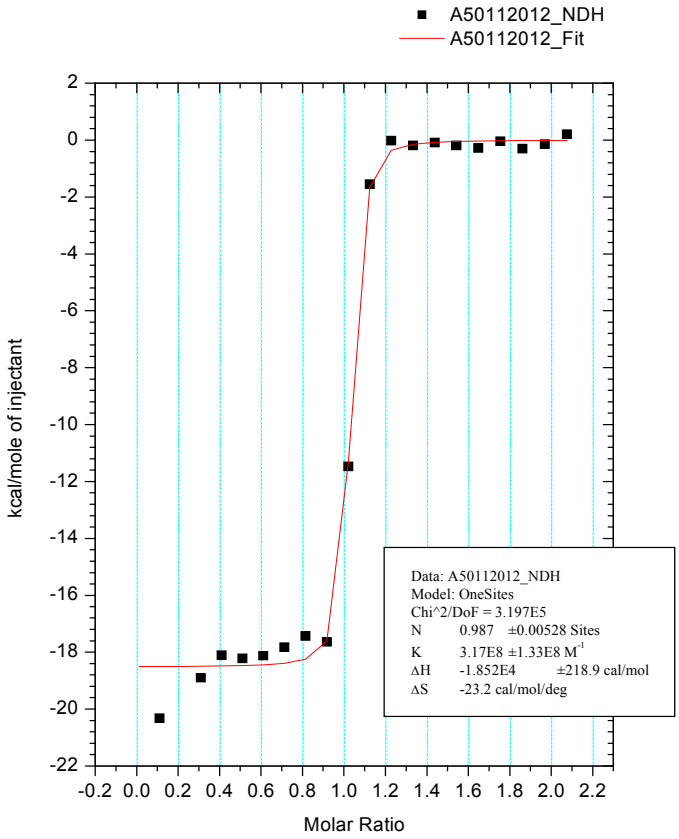

A72412006RAW_cp
A72412006spline_Base
µcal/sec
Time (min)

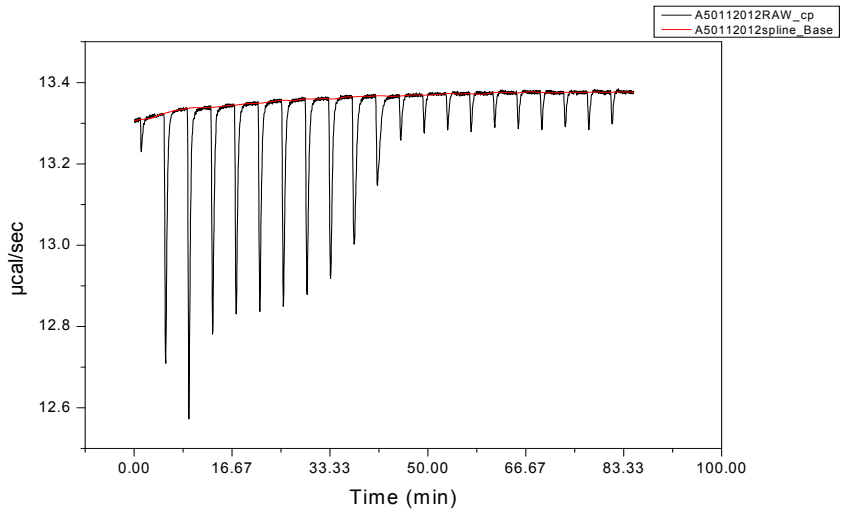
4-F_1BTA


N 0.998 ±0.00312 Sites
K 7.66E8 ±2.57E8 M-1
ΔH -1.397E4 ±101.9 cal/mol
ΔS -6.19 cal/mol/deg
kcal/mole of injectant
Molar Ratio

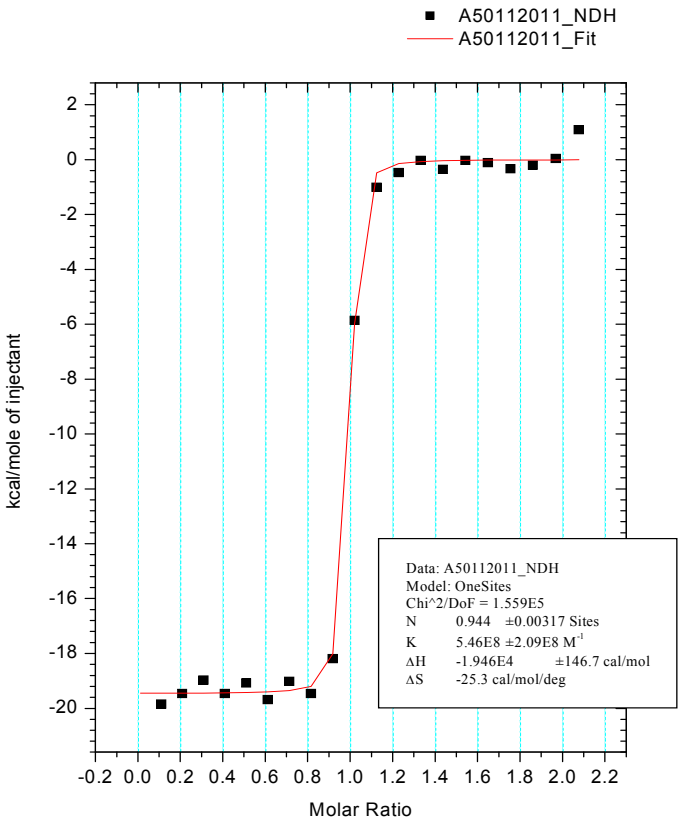

A72412007RAW_cp
A72412007BASE
µcal/sec
Time (min)

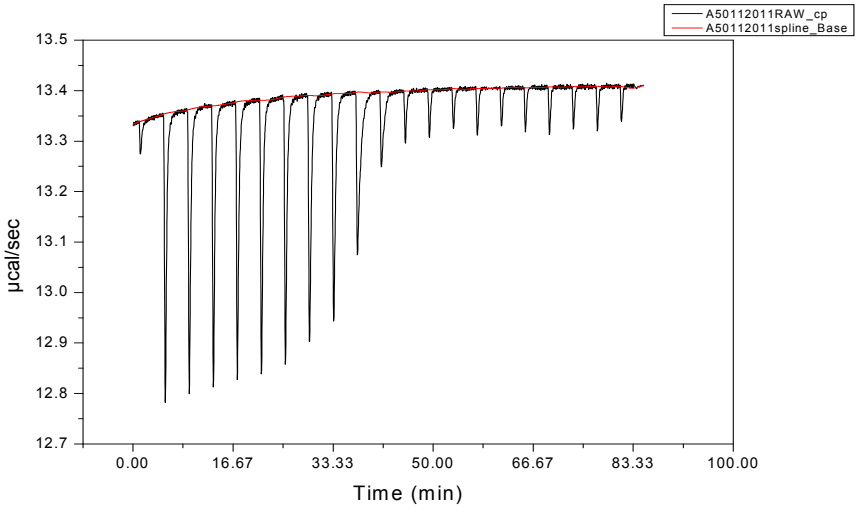
4,7-F_2BTA


A50112012_NDH
A50112012_Fit
kcal/mole of injectant
Molar Ratio
Data: A50112012_NDH
Model: OneSites
Chi^2/DoF = 3.197E5
N 0.987 ±0.00528 Sites
K 3.17E8 ±1.33E8 M-1
ΔH -1.852E4 ±218.9 cal/mol
ΔS -23.2 cal/mol/deg

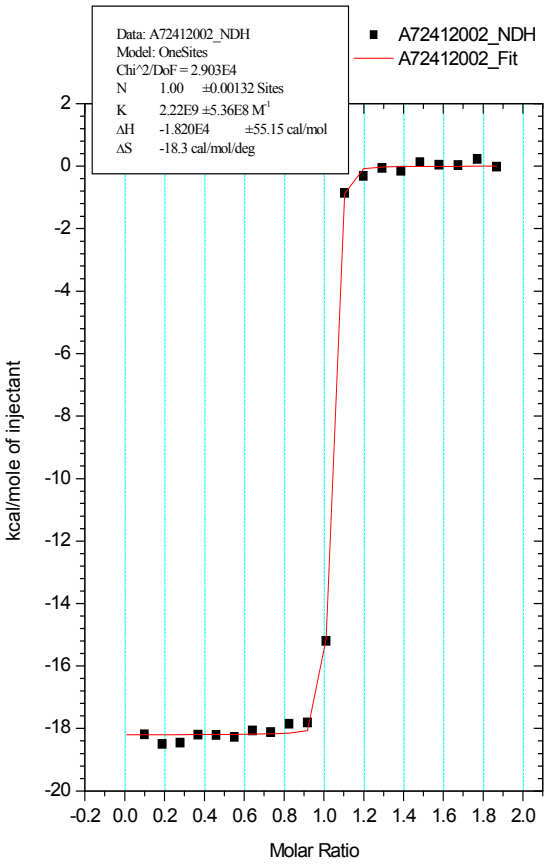

A50112012RAW_cp
A50112012spline_Base
µcal/sec
Time (min)

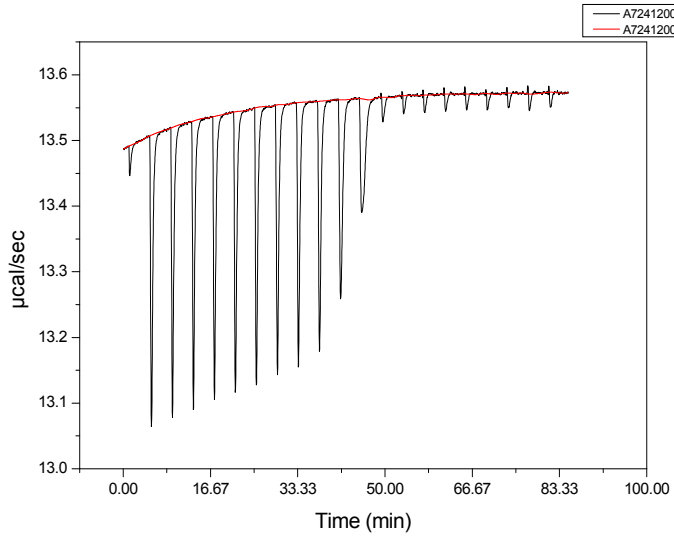
5,6-F_2BTA


A50112011_NDH
A50112011_Fit
kcal/mole of injectant
Molar Ratio
Data: A50112011_NDH
Model: OneSites
Chi^2/DoF = 1.559E5
N 0.944 ±0.00317 Sites
K 5.46E8 ±2.09E8 M-1
ΔH -1.946E4 ±146.7 cal/mol
ΔS -25.3 cal/mol/deg

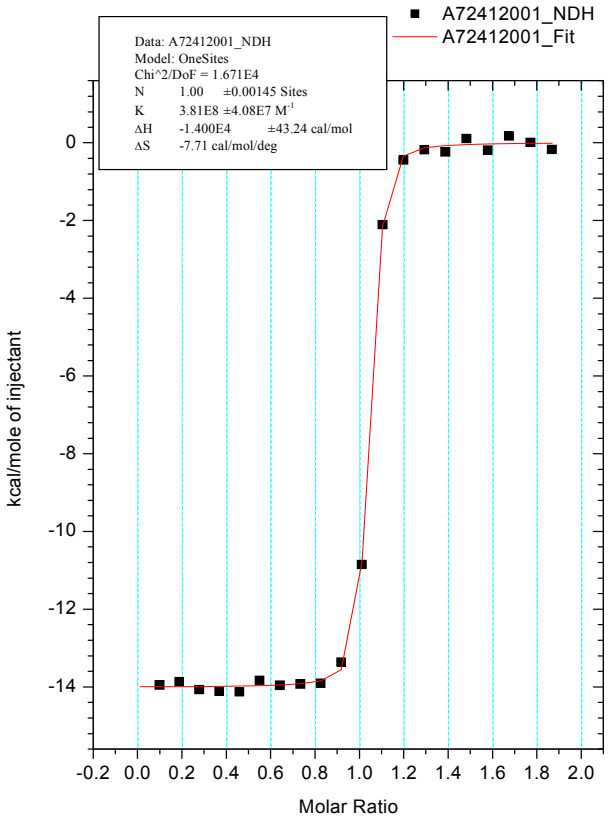

A50112011RAW_cp
A50112011spline_Base
µcal/sec
Time (min)

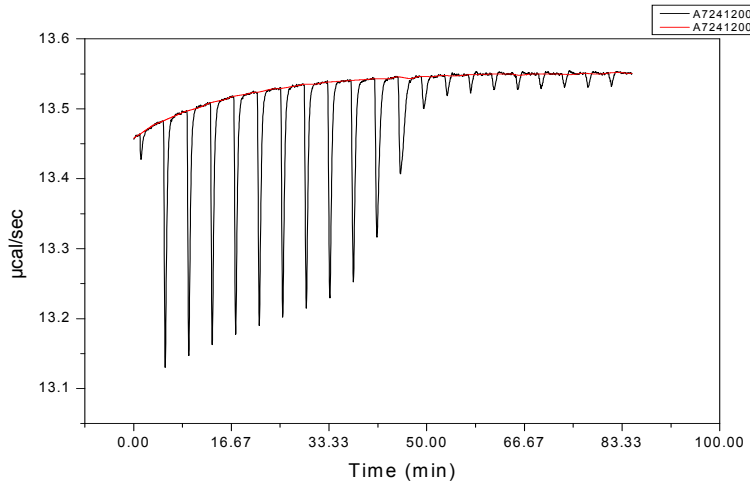
6,7-F_2BTA


Data: A72412002_NDH
Model: OneSites
Chi^2/DoF = 2.903E4
N 1.00 ±0.00132 Sites
K 2.22E9 ±5.36E8 M-1
ΔH -1.820E4 ±55.15 cal/mol
ΔS -18.3 cal/mol/deg
A72412002_NDH
A72412002_Fit
kcal/mole of injectant
Molar Ratio

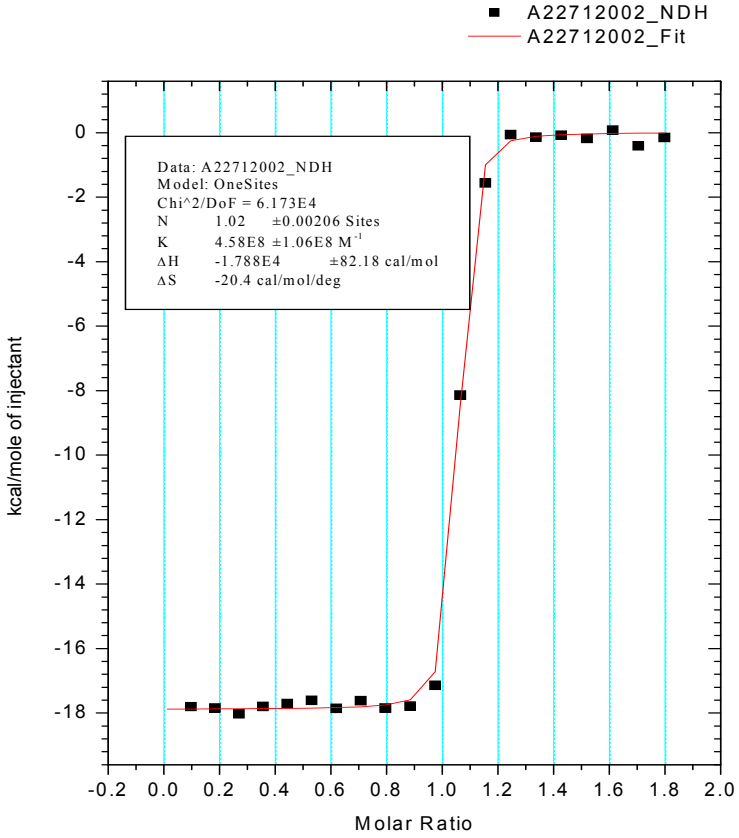

A72412002RAW_cp
A72412002spline_Base
µcal/sec
Time (min)

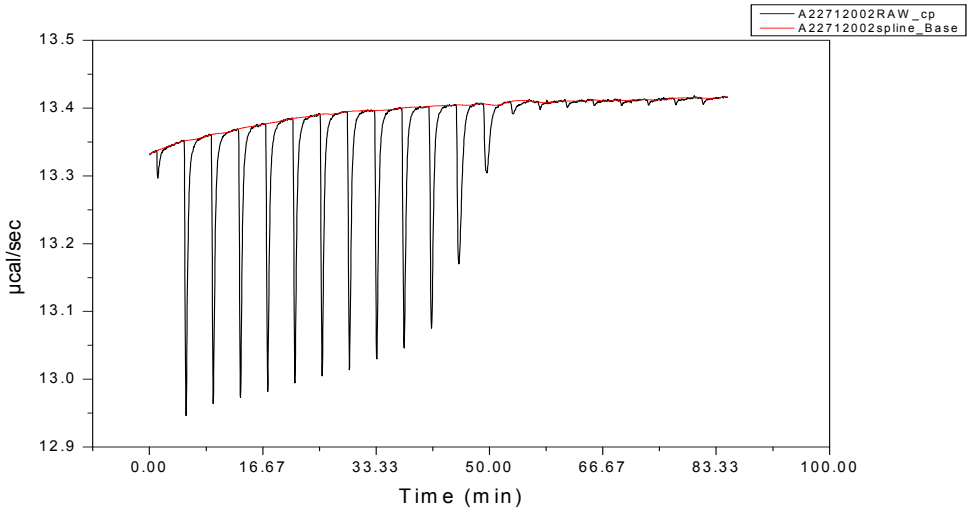
4,6-F_2BTA


A72412001_NDH
A72412001_Fit
Data: A72412001_NDH
Model: OneSites
Chi^2/DoF = 1.671E4
N 1.00 ±0.00145 Sites
K 3.81E8 ±4.08E7 M-1
ΔH -1.400E4 ±43.24 cal/mol
ΔS -7.71 cal/mol/deg
kcal/mole of injectant
Molar Ratio


A72412001RAW_cp
A72412001spline_Base
µcal/sec
Time (min)

4,5,6-F_3BTA


A22712002_NDH
A22712002_Fit
Data: A22712002_NDH
Model: OneSites
Chi^2/DoF = 6.173E4
N 1.02 ±0.00206 Sites
K 4.58E8 ±1.06E8 M-1
ΔH -1.788E4 ±82.18 cal/mol
ΔS -20.4 cal/mol/deg
kcal/mole of injectant
Molar Ratio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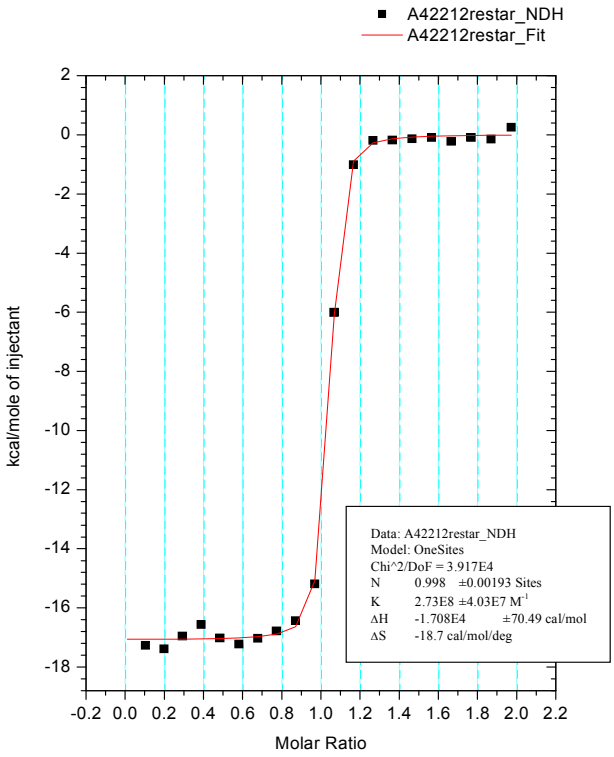
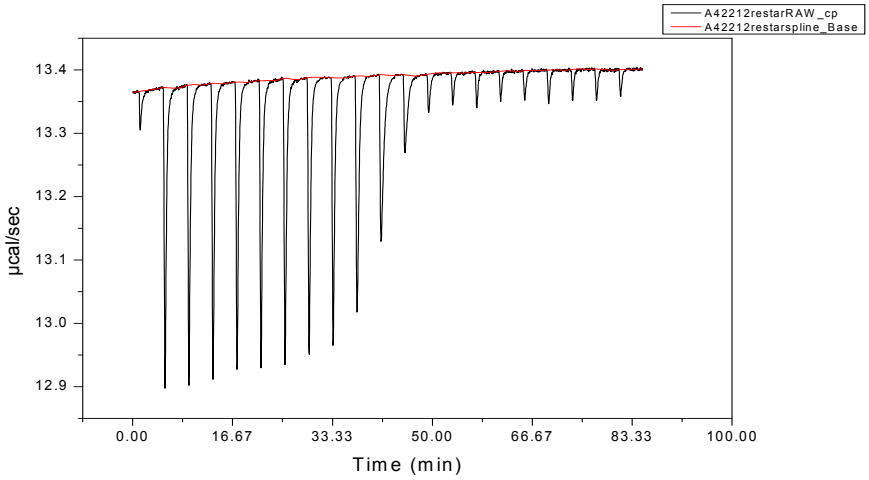
µcal/sec
Time (min)

F_4BTA:


A42212restar_NDH
A42212restar_Fit
kcal/mole of injectant
Molar Ratio
Data: A42212restar_NDH
Model: OneSites
Chi^2/DoF = 3.917E4
N 0.998 ±0.00193 Sites
K 2.73E8 ±4.03E7 M-1
ΔH -1.708E4 ±70.49 cal/mol
ΔS -18.7 cal/mol/deg


A42212restarRAW_cp
A42212restarspline_Base
µcal/sec
Time (min)

Biostructural analyses

Protein Crystallization*:* Monoclinic crystals of HCA were prepared with the hanging drop diffusion method published by McKenna and coworkers,[6] and the crystals of HCA were left undisturbed (at 4 ^{o}C) until needed for soaking experiments.

Ligand Soaking Experiments*.* We soaked the crystals of HCA in saturated solutions of the benzo- and fluorobenzo-extended ligands using the procedure described previously.[5]

X-ray Crystallography*.* X-ray diffraction data of each crystal was collected under a stream liquid nitrogen (i.e., cryo-cooled) at Brookhaven National Laboratory on the ADSC Quantum Q315 CCD detector at the National Synchrotron Light Source (beamline X-25) in collaboration with the Mail-Program, Brookhaven National Laboratory.[16] Reflections were indexed and integrated using HKL2000, and scaled using SCALEPACK.[17]

Solution of Crystal Structures*.* Diffraction data were analyzed using the CCP4i suite of crystallography software[18] using previously published procedures. Table S3 summarizes the crystallographic details for each protein-ligand structure.

Table S3. Crystallography data for the ligand-HCA complexes

	7-F_1BTA	4-F_1BTA	4,6-F_2BTA	4,7-F_2BTA	5,6-F_2BTA	6,7-F_2BTA
Data collection and processing						
No. crystals analyzed	1	1	1	1	1	1
Wavelength	1.100 Å	1.100 Å	1.100 Å	1.100 Å	1.100 Å	1.100 Å
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Unit cell parameters						
a	42.319	42.324	42.445	42.222	42.430	42.361
b	41.331	41.409	41.605	41.301	41.512	41.444
c	72.276	72.761	73.016	72.726	72.885	73.092
α	90.00	90.00	90.00	90.00	90.00	90.00
β	104.55	104.66	104.83	104.62	104.72	104.76
γ	90.00	90.00	90.00	90.00	90.00	90.00
Diffraction data						
High resolution bin	1.230 – 1.199	1.230 – 1.199	1.230 – 1.199	1.438 – 1.420	1.180 – 1.150	1.392 – 1.428
# of reflections	68571	71500	72345	35635	80635	44817
Refinement						
Resolution range	40.00 – 1.20	35.69 – 1.20	29.21 – 1.20	40.89 – 1.40	35.77 – 1.15	40.19 – 1.39
Completeness	95.01	98.31	98.46	78.49	97.32	95.67
R(obs)	0.17305	0.17311	0.16951	0.13135	0.16093	0.15375
R(work)	0.17187	0.17234	0.16857	0.12868	0.15992	0.15183
R(free)	0.19564	0.18754	0.18745	0.18159	0.17997	0.18979
B(avg)	11.539	12.028	10.092	15.148	13.879	16025
Bond lengths	0.038	0.032	0.031	0.022	0.031	0.025
Bond angles	3.723	2.533	2.590	2.069	2.680	2.273
Protein residues	261	261	261	261	261	261
Zinc ions	1	1	1	1	1	1
Water molecules	349	341	293	283	417	248
Inhibitor atoms	14	14	15	15	15	15

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[7] The pACA plasmid containing the HCA II gene was a kind gift of the Carol Fierke laboratory (University of Michigan, Department of Chemistry).

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