Supporting Information

for

Thermodynamic Parameters for the Association of Fluorinated Benzenesulfonamides with Bovine Carbonic Anhydrase II

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Derivation of Eq. (4)

We start from the definition of the desired dissociation constant ($K_d^{ArSO_2NH^-}$):

$$K_d^{ArSO_2NH^-} = \frac{[ArSO_2NH^-][CA\text{-}Zn^{II}\text{-}OH_2^+]}{[CA\text{-}Zn^{II}\text{-}NHSO_2Ar][H_2O]}$$

We re-express $ArSO_2NH^-$ and CA-Zn^{II}-OH_2^+ in terms of the total concentrations of free arylsulfonamide and of free CA (CA-Zn^{II}-OH_2^+ and CA-Zn^{II}-OH), using $\theta_{ArSO_2NH^-}$ and $\theta_{CA\text{-}Zn^{2+}\text{-}OH_2^+}$ [defined in Eq. (3)] of the main text):

$$K_d^{ArSO_2NH^-} = \frac{\theta_{ArSO_2NH^-}\left([ArSO_2NH^-]+[ArSO_2NH_2]\right)\theta_{CA\text{-}Zn^{II}\text{-}OH_2^+}\left([CA\text{-}Zn^{II}\text{-}OH_2^+]+[CA\text{-}Zn^{II}\text{-}OH]\right)}{[CA\text{-}Zn^{II}\text{-}NHSO_2Ar][H_2O]}$$

Re-arranging the terms gives:

$$K_d^{ArSO_2NH^-} = \theta_{ArSO_2NH^-}\theta_{CA\text{-}Zn^{II}\text{-}OH_2^+}\frac{\left([ArSO_2NH^-]+[ArSO_2NH_2]\right)\left([CA\text{-}Zn^{II}\text{-}OH_2^+]+[CA\text{-}Zn^{II}\text{-}OH]\right)}{[CA\text{-}Zn^{II}\text{-}NHSO_2Ar][H_2O]}$$

The right-most term is just K_d^{obs} (the experimentally observed dissociation constant for the binding of arylsulfonamide to CA). Substituting for this term gives Eq. (4) of the main text:

$$\therefore K_d^{ArSO_2NH^-} = K_d^{obs}\theta_{ArSO_2NH^-}\theta_{CA\text{-}Zn^{II}\text{-}OH_2^+} \quad (4)$$

Derivation of Eq. (5)

The observed enthalpy of binding of arylsulfonamides to CA comprises the following terms: (i) the binding of the arylsulfonamide anion ($ArSO_2NH^-$) to the Zn^{II}-water form of CA (CA-Zn^{II}-OH_2^+), (i) the protonation of the fraction of CA that is present as the Zn^{II}-hydroxide bound form (CA-Zn^{II}-OH) with concomitant deprotonation of the buffer, and (iii) the deprotonation (ionization) of the fraction of free arylsulfonamide that is present in the neutral

form ($ArSO_2NH_2$) with concomitant protonation of the buffer. These processes can be expressed algebraically as follows (with the parameters defined in the main text):

$$\Delta H^\circ_{obs} = \Delta H^\circ_{ArSO_2NH^-} + \theta_{CA-Zn^{II}-OH}(\Delta H^\circ_{ion,buffer} - \Delta H^\circ_{ion,CA-Zn^{II}-OH_2^+}) + \theta_{ArSO_2NH_2}(\Delta H^\circ_{ion,ArSO_2NH_2} - \Delta H^\circ_{ion,buffer})$$

Rearranging to express the fractions of neutral arylsulfonamide and CA-Zn^{II}-OH in terms of the charged forms for both species, gives:

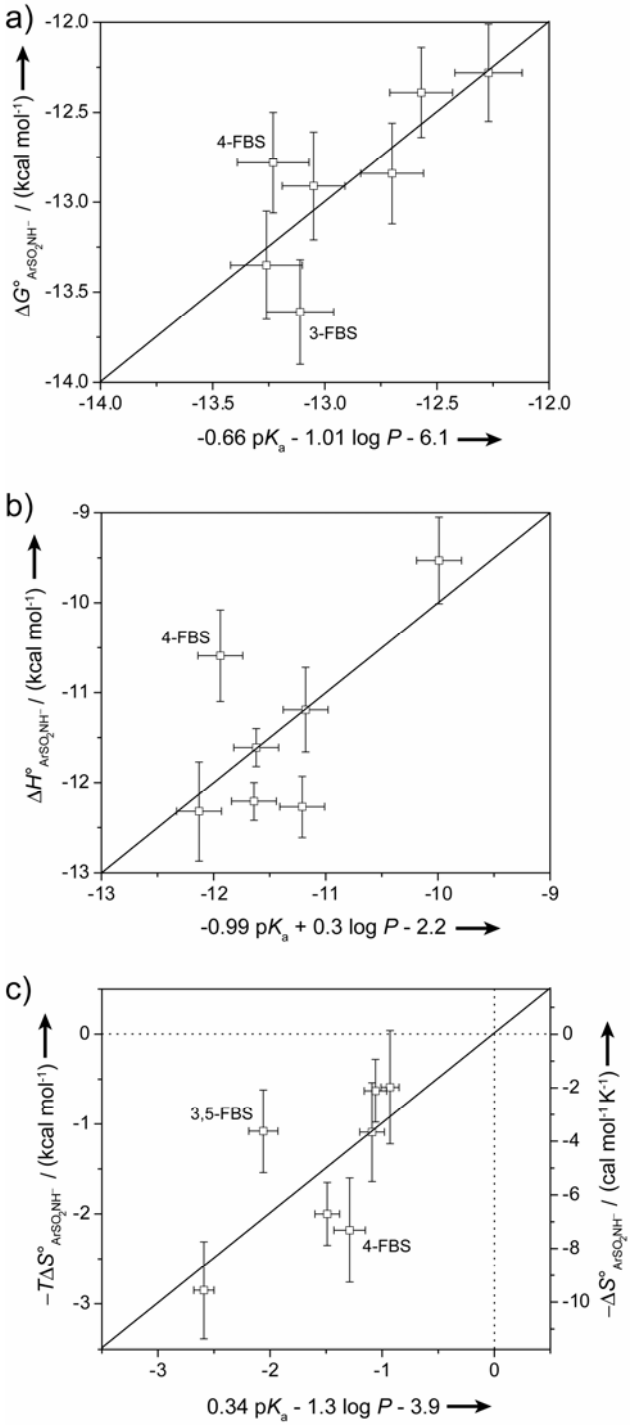
$$\Delta H^\circ_{obs} = \Delta H^\circ_{ArSO_2NH^-} + (1-\theta_{CA-Zn^{II}-OH_2^+})(\Delta H^\circ_{ion,buffer} - \Delta H^\circ_{ion,CA-Zn^{II}-OH_2^+}) + (1-\theta_{ArSO_2NH^-})(\Delta H^\circ_{ion,ArSO_2NH_2} - \Delta H^\circ_{ion,buffer})$$

Solving for $\Delta H^\circ_{ArSO_2NH^-}$ gives Eq. (5) of the main text:

$$\therefore \Delta H^\circ_{ArSO_2NH^-} = \Delta H^\circ_{obs} + (1-\theta_{CA-Zn^{II}-OH_2^+})(\Delta H^\circ_{ion,CA-Zn^{II}-OH_2^+} - \Delta H^\circ_{ion,buffer}) + (1-\theta_{ArSO_2NH^-})(\Delta H^\circ_{ion,buffer} - \Delta H^\circ_{ion,ArSO_2NH_2}) \quad (5)$$

Figure S.1. Quantitative Structure-Activity Relationships (QSARs) between a) $\Delta G^{\circ}_{ArSO_2NH^-}$, b) $\Delta H^{\circ}_{ArSO_2NH^-}$, and c) $-T\Delta S^{\circ}_{ArSO_2NH^-}$ and pK_a and log P (partition coefficient) for all of the fluorinated benzenesulfonamides (including 4-fluorobenzenesulfonamide, 4-FBS). The QSARs gave values of R^2 of a) 0.63, b) 0.46, and c) 0.48. The *y*-error bars are uncertainties described in Table 2 of the main text, and the *x*-error bars were obtained by propagating uncertainties in pK_a and log P. The horizontal and vertical dotted lines in (C) separate favorable ($-T\Delta S^{\circ} < 0$) from unfavorable ($-T\Delta S^{\circ} > 0$) entropy of binding.

Figure S.1 (Continued).



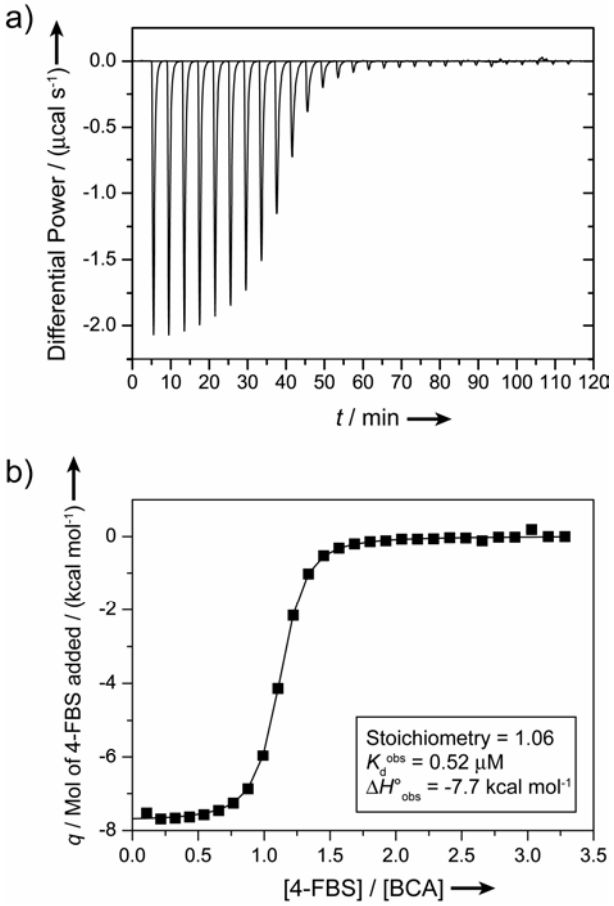
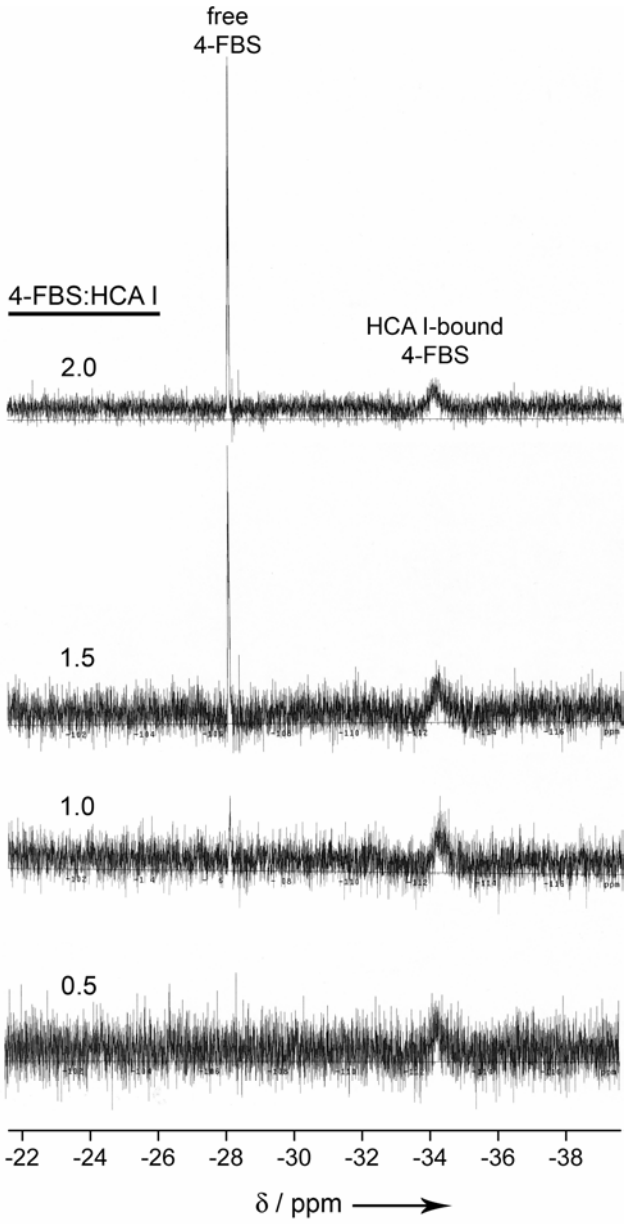


Figure S.2. An ITC experiment showing the titration of 4-fluorobenzenesulfonamide (4-FBS) into a solution of high concentration of bovine carbonic anhydrase II (BCA) at $T = 298$ K. The sample cell contained 68.2 µM BCA in 20 mM sodium phosphate buffer pH 7.5 and 5% DMSO (v/v) (to solubilize the ligand). The injection syringe contained 1.00 mM 4-fluorobenzene-sulfonamide (4-FBS) in the same buffer. One injection of 2.0 µL preceded 28 injections of 10.0 µL. The interval between injections was 4 min. a) Data after baseline correction. b) Data after peak integration, blank subtraction, and normalization to moles of injectant. The solid line shows a sigmoid fit to a single-site binding model (with the first datum omitted) with the optimized fitting parameters shown in the box. The only inflection point present in the thermogram is near unity (at the optimized stoichiometry of binding of ligand to protein).

Figure S.3. ^{19}F NMR spectra of 4-fluorobenzenesulfonamide (4-FBS) in the presence of human carbonic anhydrase I (HCA I, 0.75 mM). The number of equivalents of 4-FBS to HCA I is indicated. Chemical shifts (δ in ppm) are reported relative to trifluoroacetic acid as "external" standard (in a sealed capillary). All samples were in 20 mM $Na_2D_2PO_4$ ("pH" 7.5) at T = 298 K. As with the binding of 4-FBS to BCA II (Figure 7 of the main text), a peak corresponding to free, unbound 4-FBS begins to appear when the stoichiometry of ligand to protein is greater than unity. This result differs from that of Dugad and Gerig where free ligand did not appear until the stoichiometry of ligand to protein was greater than two (ref [19] of the main text). The slight peak corresponding to free 4-FBS at a stoichiometry of ligand to protein of unity is due to the less than 100% activity of HCA I, presumably due to UV-active contaminants in the commercial source (see Figure S.4 and main text).

Figure S.3 (Continued).



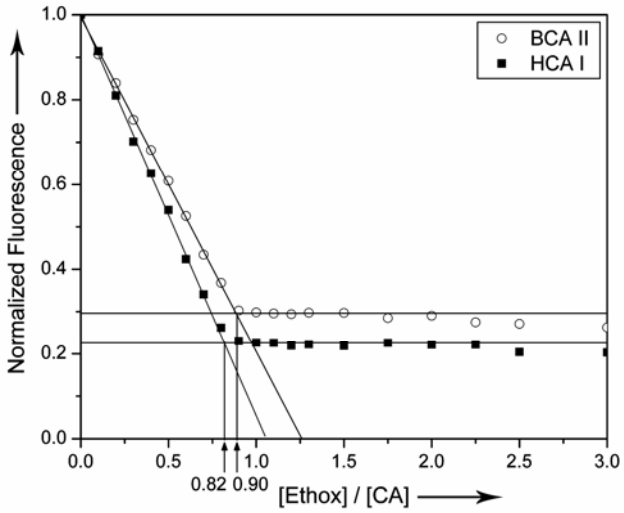


Figure S.4. Activity of carbonic anhydrase isozymes used in ^{19}F NMR titration experiments. Samples of CA that had been prepared for ^{19}F NMR experiments (see Experimental Section of the main text) were diluted to 1 μM (a concentration that is ≥500-fold larger than the K_d of ethoxzolamide for these proteins) in 20 mM sodium phosphate pH 7.5 buffer (ref [49] of the main text). The fluorescence of the sample was monitored (excitation wavelength = 290 nm, emission wavelength = 340 nm; both slit widths = 5 nm for BCA II, and excitation slit width = 5 nm and emission slit width = 7 nm for HCA I) as the sample was titrated with a stock solution of ethoxzolamide (a sulfonamide that has been shown to bind 1:1 with CA) (refs [43] and [49] of the main text). The data were corrected for background fluorescence of ethoxzolamide, dilution, and the inner filter effect due to absorption of excitation light by ethoxzolamide (using a titration of soybean trypsin inhibitor as a control because it has been shown not to bind sulfonamides; ref [21] of the main text). The break points in fluorescence reveal that the activities of both proteins are near unity (that the values are slightly lower than unity suggests either impurities in the commercial source, or loss in activity of the proteins that may have resulted from the preparation of the samples for NMR).

Table S.1. Data collection and refinement statistics for HCA II-ligand complexes.

HCA II Complex	4-FBS	2,6-FBS	3,5-FBS
Number of measured reflections	103,880	75,607	46,233
Number of unique reflections	19,051	17,251	15,077
Maximum resolution (Å)	1.93	2.00	2.09
R_{merge}[a]	0.122	0.125	0.090
Completeness of data (%)	98.6	97.1	95.1
Reflections used in refinement (>2s)	17,338	15,383	13,211
Reflections in R_{free} test set	884	788	702
R_{cryst}[b]	0.219	0.221	0.211
R_{free}[b]	0.296	0.287	0.299
Number of nonhydrogen atoms[c]	2,071	2,072	2,072
Number of solvent molecules[c]	60	89	56
Root-mean-square deviations from:			
Ideal bond lengths (Å)	0.012	0.012	0.011
Ideal bond angles (˚)	1.6	1.6	1.6
Ideal dihedral angles (˚)	25.3	25.2	25.4
Ideal improper dihedral angles (˚)	1.4	1.4	1.4
RCSB accession codes	1IF4	1IF5	1IF6

[a] $R_{merge} = \Sigma|I_h - \langle I_h\rangle|/\Sigma I_h$; I_h = intensity measured for reflection h, $\langle I_h\rangle$ = average intensity for reflection h calculated from replicate data. [b] $R = \Sigma\ ||F_o| - |F_c||/\Sigma|F_o|$, where R_{cryst} and R_{free} are calculated using the working and test reflection sets, respectively. [c] Per asymmetric unit.