													Notes
ITC experiment													
temperature		25	C										
estimated Ka		1.00E+06	1/M										
sample cell volume (approximate)		1.4	mL										
number of injections		10											
injection volume		10	uL										
dilution factor for single injection (d)		0.992857142857143											
dilution factor after final injection (d^n)		0.930824157149805											
		desired						actual		error		percent error	
cell concentration		10.00	uM					7.99	uM	0.21	uM	2.67%	
c-value		10.00						9.99		0.04		0.42%	
optimal Rm		5.34						5.34					
syringe concentration		718.29	uM					698.41	uM	7.94	uM	1.14%	
LIGAND													
compound name	CBSCarboxybenzenesulfonamide (CBS)												
description	4-sulfonylbenzoic acid at 97% purity												Color key
vendor	Sigma-Aldrich												fill in before experiment
product no.	000944798												fill in during experiment
lot no.	#MKBF3323V												automatically computed
purity		97.00%											
molecular weight		201.2	g/mol										
solubility		453	mg/L	2,251.49	uM								
Stock solution preparation													
		desired		typical error		typical percent error		actual		error		percent error	
target compound mass	(most balances need min 10 mg)	10	mg	0.1	mg	1.00%		10.1	mg	0.1	mg	0.99%	used marked balance uncertainty (min: 10 mg +- 0.1 mg)
buffer volume needed for target mass	(for planning buffer usage)	24.11	mL										
buffer volume needed for actual mass	(use this for actual preparation)	24.35	mL	0.49	mL	2.00%		25.099	mL	0.067	mL	0.27%	used P5000 multiple times to avoid accuracy loss of serological pipettes
purity-corrected stock solution concentration	(be careful not to exceed solubility)	2,000.00	uM	44.72	uM	2.24%		1,940.03	uM	19.89	uM	1.03%	
Syringe solution preparation													
		desired		typical error		typical percent error		actual		error		percent error	
dilution factor from stock to titrant solution		0.370		0.003		0.89%		0.36000		0.00176		0.49%	
stock solution volume		1.851	mL	0.02	mL	1.00%		1.8	mL	0.012	mL	0.67%	use systematic error from Gilson P5000, 2000 uL transfer
buffer volume		3.149	mL	0.03	mL	1.00%		3.2	mL	0.012	mL	0.38%	used systematic error from Gilson P5000, 2000 uL transfer
total volume for titrant	(need min 2.1 mL)	5	mL	0.04	mL	0.73%		5	mL	0.017	mL	0.34%	
titrant concentration		718.29	uM	18	uM	2.48%		698	uM	8	uM	1.14%	(this error is included in the 'actual error' in thermodynamic parameters below)
PROTEIN													
protein name	carbonic anhydrase II (CAII)												
source	Sigma-Aldrich C2522-25mg												
lot no.													
molar extinction coefficient		50070	M-1 cm-1										
protein purity (or 100% if unknown)		80.00%											
Stock solution preparation													
Dilution for UV-Vis absorbance measurement		desired		typical error				actual		error		percent error	NanoDrop is linear over very large A280 due to short path length
dilution factor for absorbance measurement		0.0625		0.001				0.06250		0.00086		1.37%	
volume of stock solution		10	uL	0.1	uL			10	uL	0.1	uL	1.00%	used systematic error for Gilson P20, 20 uL transfer
volume of buffer solution		150	uL	1.5	uL			150	uL	1.6	uL	1.07%	used systematic error for Gilson P200, 100 uL transfer
total volume of dilution		160	uL	1.50	uL			160	uL	1.603	uL	1.00%	
absorbance measurement								0.66844	A	0.01	A	1.50%	used error from spec manual (for 200-350nm)
concentration of dilution								13.35	uM	0.20	uM	1.50%	
concentration of stock solution								213.60	uM	4.33	uM	2.03%	
purity-corrected concentration of stock solution								170.88	uM	3.47	uM	2.03%	
Cell solution preparation													Add a section for UV-VIS measurement of protein final dilution
		desired		typical error				actual		error		percent error	
dilution factor		0.05852		0.00078				0.04676		0.00081		1.74%	
stock solution volume		0.293	mL	0.003	mL			0.234	mL	0.004	mL	1.71%	used systematic error for Gilson P1000uL transfer
buffer volume		4.71	mL	0.05	mL			4.77	mL	0.03	mL	0.63%	used systematic error from Gilson P5000 transfer
total volume for titrate	(need ca. 2.1 mL/experiment)	5.00	mL	0.05	mL			5.00	mL	0.030	mL	0.60%	
titrate concentration		10.00	uM	0.13	uM			7.99	uM	0.213	uM	2.67%	(this error is absorbed into the n parameter in the ITC fit)
Parameters from Origin fit													
		reported from fit		error from fit		percent error from fit		actual		error		percent error	
n (stoichiometry, purity, and V0 correction)		0.926		0.005		0.51%		0.926		0.006		0.61%	Origin fit used purity-corrected protein concentration in cell
K (association constant)		1.25E+06	M-1	3.99E+04	M-1	3.19%		1.25E+06	M-1	4.24E+04	M-1	3.39%	
Kd (dissociation constant)		8.00E-07	M	2.55E-08	M	3.19%		8.00E-07	M	2.71E-08	M	3.39%	
DeltaH		-10.8	kcal/mol	0.07304	kcal/mol	0.68%		-10.80	kcal/mol	0.14	kcal/mol	1.32%	
TDeltaS		-2.48	kcal/mol	0.08	kcal/mol	3.04%		-2.48	kcal/mol	0.14	kcal/mol	5.81%	
DeltaG		-8.32	kcal/mol	0.02	kcal/mol	0.23%		-8.32	kcal/mol	0.02	kcal/mol	0.24%	