Supporting Information

Conformational changes in alkyl chains determine the thermodynamic and kinetic binding profiles of Carbonic Anhydrase Inhibitors

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Table of Contents

Table S1 2

Figure S1 4

Figure S2 5

Table S2 6

Table S3 7

Table S4 8

Table S5 8

Thermograms, Isotherms and ETCs 9

Synthesis and Determination of Purity 25

References 26

Table S1: X-ray data collection and refinement statistics for **1a**-**e**.[a]

	hCAII-**1a**	hCAII-**1b**	hCAII-**1c**	hCAII-**1d**	hCAII-**1e**
Data collection and processing					
Beamline	14.2	14.2	14.2	14.1	14.2
Wavelength / Å	0.9184	0.9184	0.9184	0.9184	0.9184
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
a,*b*,*c* / Å	42.3, 41.4, 72.2	42.4, 41.5, 72.3	42.3, 41.4, 72.2	42.3, 41.5, 72.3	42.2, 41.5, 72.1
β / °	104.5	104.7	104.7	104.6	104.5
Matthews coefficient / $Å^3\ Da^{-1}$ [b]	2.1	2.1	2.1	2.1	2.1
Solvent content / % [b]	40.3	40.4	40.2	40.4	40.2
Diffraction data					
Resolution range / Å	41.4 – 1.08 (1.14 – 1.08)	41.5 – 1.09 (1.16 – 1.09)	41.4 – 1.10 (1.10 – 1.09)	41.5 – 1.02 (1.08 – 1.02)	41.5 – 1.12 (1.19 – 1.12)
Unique reflections	102486 (15973)	99517 (15508)	97587 (15119)	121413 (19412)	91347 (14347)
$CC_{1/2}$ / %[1]	99.9 (96.6)	99.8 (95.6)	99.9 (89.1)	99.9 (78.4)	99.9 (97.9)
R_{sym} / %[2]	4.2 (17.8)	4.5 (19.1)	4.8 (33.9)	4.9 (46.2)	4.2 (13.7)
Completeness~/~%	98.3 (95.1)	98.1 (95.0)	98.6 (95.1)	98.2 (97.5)	97.7 (95.6)
Wilson B factor / $Å^2$	9.1	8.2	10.7	9.8	9.3
Multiplicity	3.6 (3.5)	3.6 (3.5)	3.6 (3.6)	3.6 (3.5)	3.7 (3.7)
I / σ(I)	15.9 (5.1)	15.4 (5.2)	12.5 (2.9)	11.6 (2.0)	16.6 (6.4)
Refinement					
Resolution range / Å	41.0 – 1.08	41.0 – 1.09	40.9 – 1.1	40.0 – 1.02	40.9 – 1.12
Reflections used in refinement (work/free)[3] [c]	102486 (97361/5125)	99517 (94541/4976)	97587 (92707/4880)	121413 (115342/6071)	91339 (86772/4567)
Final *R* values for all reflections (work/free)[3] [c]	0.114/0.130	0.104/0.118	0.122/0.139	0.122/0.140	0.115/0.131
Protein residues	257	257	257	257	257
Inhibitor atoms	10/10/10	11/11	12/11	13	14
Water molecules	248	294	275	243	252
RMSD from ideality					
Bond lengths / Å	0.007	0.007	0.008	0.008	0.008
Bond angles / °	1.03	1.05	1.1	1.06	1.1
Ramachandran plot / % [d]					
Residues in most favored regions	89.4	88.9	89.4	89.4	89.4
Residues in additionally allowed regions	10.2	10.6	10.2	10.6	10.2
Regions in generously allowed regions	0.5	0.5	0.5	0	0.5
Residues in disallowed regions	0	0	0	0	0
Mean *B* factor / $Å^2$ [e]					
Protein non-hydrogen atoms	12.7	11.2	13.2	12.4	11.7
Inhibitor	9.1/14.6/16.2	8.3/14.1	12.4/19.4	14.1	11.5
Water molecules	24.3	23.7	27.9	23.9	23.5

[a] Values in brackets refer to the highest resolution shell unless specified differently. [b] Calculated using the program *Phaser Cell Content Analysis* from the *CCP4* suite.[4] [c] 5 % of all reflections were used for R_{free} calculation. [d] Calculated using the program *PROCHECK*.[5] [e] Calculated using the program MOLEMAN.[6]

Table S1 (continued): X-ray data collection and refinement statistics for **1f**-**2d**.[a]

	hCAII-**1f**	hCAII-**2a**	hCAII-**2b**	hCAII-**2c**	hCAII-**2d**
Data collection and processing					
Beamline	14.1	14.2	14.2	14.2	14.2
Wavelength / Å	0.9184	0.9184	0.9184	0.9184	0.9184
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
a,*b*,*c* / Å	42.3, 41.5, 72.2	42.4, 41.4, 72.3	42.3, 41.4, 72.2	42.2, 41.4, 72.0	42.5, 41.5, 71.9
β / °	104.5	104.6	104.5	104.3	104.2
Matthews coefficient / $Å^3\ Da^{-1}$ [b]	2.1	2.1	2.1	2.1	2.1
Solvent content / % [b]	40.3	40.3	40.2	40.0	40.4
Diffraction data					
Resolution range / Å	41.5 – 0.95 (1.01 – 0.95)	41.4 – 1.04 (1.10 – 1.04)	41.4 – 1.08 (1.15 – 1.08)	41.4 – 1.19 (1.26 – 1.19)	41.5 – 1.07 (1.13 – 1.07)
Unique reflections	142312 (21669)	113777 (17379)	102490 (15912)	72714 (11290)	101318 (15148)
$CC_{1/2}$ / %[1]	99.6 (85.2)	99.8 (75.1)	99.9 (94.2)	93.5 (90.1)	99.3 (81.7)
R_{sym} / %[2]	7.1 (36.1)	5.7 (51.2)	4.4 (24.0)	4.24 (16.9)	8.8 (46.7)
Completeness~/~%	93.2 (88.1)	97.7 (93.1)	98.6 (95.1)	93.5 (90.1)	94.5 (87.7)
Wilson B factor / $Å^2$	8.1	9.2	9.7	9.5	9.2
Multiplicity	3.8 (3.6)	3.6 (3.3)	3.6 (3.6)	3.8 (3.7)	3.8 (3.9)
I / σ(I)	9.3 (2.1)	11.0 (2.0)	14.5 (4.1)	17.7 (6.3)	7.9 (2.3)
Refinement					
Resolution range / Å	34.9 – 0.95	35.0 – 1.04	40.9 – 1.08	40.9 – 1.19	41.2 – 1.07
Reflections used in refinement (work/free)[3] [c]	142305 (135189/7116)	113766 (108078/5688)	102476 (97352/5124)	70978 (67429/3549)	101316 (96250/5066)
Final *R* values for all reflections (work/free)[3] [c]	0.120/0.134	0.119/0.132	0.116/0.133	0.116/0.139	0.129/0.147
Protein residues	257	257	257	257	257
Inhibitor atoms	15	12	13/12	14	15/14/12
Water molecules	227	266	255	282	234
RMSD from ideality					
Bond lengths / Å	0.008	0.008	0.008	0.010	0.007
Bond angles / °	1.06	1.08	1.09	1.16	1.01
Ramachandran plot / % [d]					
Residues in most favored regions	90.7	88.9	89.8	89.4	88.9
Residues in additionally allowed regions	8.8	10.6	9.7	10.2	11.1
Regions in generously allowed regions	0.5	0.5	0.5	0.5	0
Residues in disallowed regions	0	0	0	0	0
Mean *B* factor / $Å^2$ [e]					
Protein non-hydrogen atoms	10.6	11.5	12.1	12.3	10.9
Inhibitor	8.7	9.6	11.1/20.2	10.7	11.7/20.3/18.5
Water molecules	22.2	23.2	23.5	23.7	24.4

[a] Values in brackets refer to the highest resolution shell unless specified differently. [b] Calculated using the program *Phaser Cell Content Analysis* from the *CCP4* suite.[4] [c] 5 % of all reflections were used for R_{free} calculation. [d] Calculated using the program *PROCHECK*.[5] [e] Calculated using the program MOLEMAN.[6]

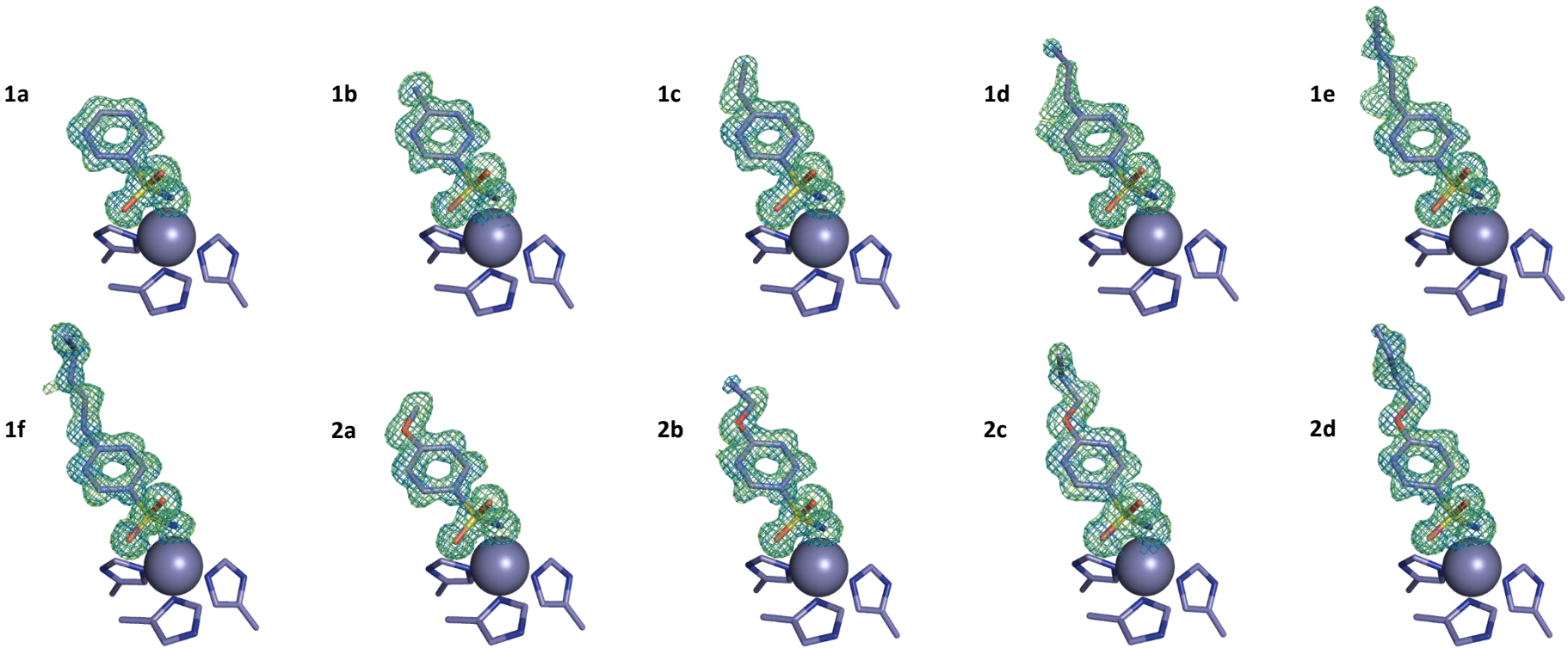


Figure S1: 2mFo-DFc maps at 1 σ in blue from the last refinement step and mFo-DFc omit maps at 3 σ in green for the investigated ligands. Omit maps were taken from a refinement run of the final model without ligand.

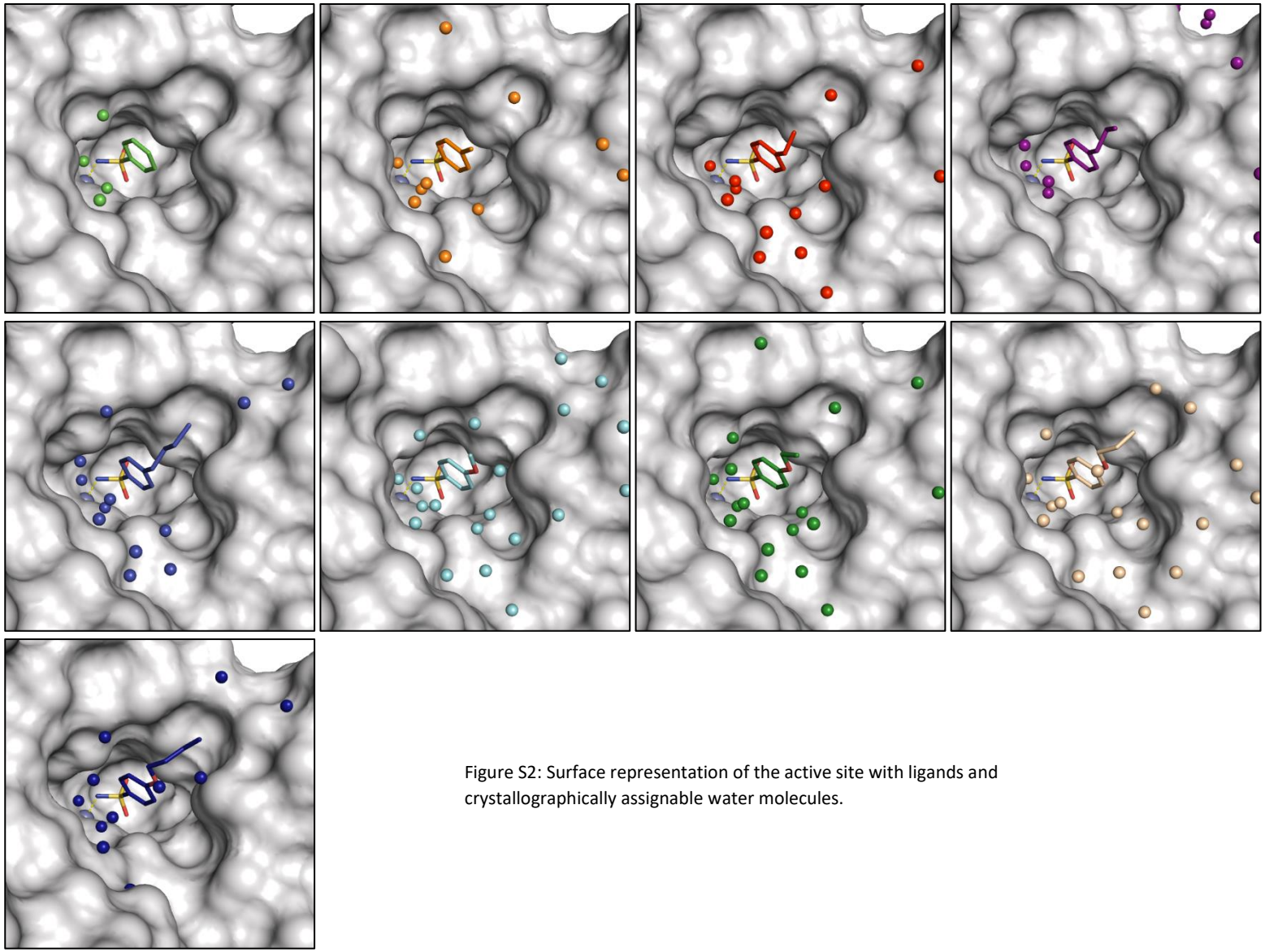


Figure S2: Surface representation of the active site with ligands and crystallographically assignable water molecules.

Thermodynamic data

Table S2: Individual thermodynamic data for fitting of the raw data of three ITC measurements per compound. **1f** is not included, as a displacement approach was applied for this compound.

Compound	χ^2	ΔG / kJ mol^{-1}	ΔH / kJ mol^{-1}	$-T\Delta S$ / kJ mol^{-1}	K_a / 10^6 M^{-1}
	0.12	-36.14	-39.54	3.40	2.15
1a	0.18	-35.78	-39.20	3.42	1.86
	0.51	-36.07	-39.86	3.79	2.08
	0.17	-38.12	-41.02	2.91	4.76
1b	0.15	-37.58	-40.57	2.99	3.84
	0.71	-38.76	-40.19	1.42	6.18
	0.68	-40.91	-41.13	0.22	14.71
1c	0.36	-40.24	-41.43	1.19	11.21
	0.52	-39.82	-42.17	2.34	9.46
	0.26	-40.80	-37.97	-2.83	14.05
1d	0.28	-40.36	-39.54	-0.81	11.76
	0.43	-40.95	-39.24	-1.71	14.95
	0.39	-42.17	-44.73	2.58	24.41
1e	0.28	-42.34	-43.35	1.02	26.04
	0.37	-43.43	-44.73	1.26	40.83
	0.15	-37.28	-46.02	8.73	3.40
2a	0.27	-37.13	-45.15	8.00	3.20
	0.29	-37.55	-45.35	7.82	3.78
	0.59	-39.63	-46.74	7.09	8.77
2b	0.25	-39.76	-46.36	6.62	9.22
	0.24	-39.11	-46.44	7.32	7.10
	0.56	-42.05	-50.04	8.00	23.29
2c	0.54	-42.76	-49.25	6.45	31.24
	0.51	-43.35	-50.12	6.77	39.07
	0.40	-44.48	-51.80	7.32	62.09
2d	0.30	-44.31	-51.51	7.21	57.81
	0.20	-42.89	-51.97	9.08	32.56

Values for Δ*H*° were globally fitted. Δ*G*° was calculated according to equation (1) from the globally fitted values of K_a. -*T*Δ*S*° was calculated with equation (2).

Table S3: Thermodynamic data.

					Standard error [d]				Global fit error			
Compound	**Δ*G*° [a] kJ mol^{-1}**	**Δ*H*° [b] kJ mol^{-1}**	**-*T*·Δ*S*° [c] kJ mol^{-1}**	**K_a [b] M^{-1}**	**Δ*G*° kJ mol^{-1}**	**Δ*H*° kJ mol^{-1}**	**-*T*·Δ*S*° kJ mol^{-1}**	**K_a M^{-1}**	**Δ*G*° [e] kJ mol^{-1}**	**Δ*H*° [f] kJ mol^{-1}**	**-*T*·Δ*S*° [e] kJ mol^{-1}**	**K_a [f] M^{-1}**
1a	-36.0	-39.6	3.6	1.99E+06	0.1	0.2	0.1	8.89E+04	0.02	0.03	0.04	1.84E+04
1b	-38.0	-40.8	2.8	4.46E+06	0.3	0.2	0.5	6.81E+05	0.07	0.04	0.08	1.23E+05
1c	-40.4	-41.5	1.1	1.18E+07	0.3	0.3	0.6	1.54E+06	0.04	0.05	0.06	1.79E+05
1d	-40.7	-38.6	-2.1	1.34E+07	0.2	0.5	0.6	9.50E+05	0.05	0.06	0.08	2.87E+05
1e	-42.6	-44.2	1.6	2.97E+07	0.4	0.5	0.5	5.22E+06	0.06	0.04	0.07	6.76E+05
1f	-45.0	-43.0	-2.0	7.73E+07					0.36	0.02	0.36	1.11E+07
2a	-37.3	-45.5	8.2	3.48E+06	0.1	0.3	0.3	1.70E+05	0.02	0.03	0.03	2.70E+04
2b	-39.5	-46.5	7.0	8.41E+06	0.2	0.1	0.2	6.45E+05	0.04	0.03	0.05	1.21E+05
2c	-42.6	-49.8	7.2	2.90E+07	0.4	0.3	0.5	4.56E+06	0.06	0.06	0.08	6.77E+05
2d[g]	-43.6	-51.8	8.3	4.27E+07	0.5	0.1	0.6	9.21E+06	0.12	0.05	0.13	2.15E+06

[a] Calculated using equation (1) with the globally fitted K_a. [b] Globally fitted value. [c] Calculated with equation (2). [d] Standard error of individual measurements. Due to its high affinity, **1f** could not be fitted individually and had to be characterized by a displacement setup as described in the Methods section of the main article, which was globally fitted. [e] Calculated from the global fit error of K_a assuming error propagation (equations (3) and (4)). [f] Global fit error. [g] Values do not fully add up due to rounding.

$$\Delta G° = -\mathrm{R} \cdot T \cdot ln(K_a) \quad (1)$$

$$-T\Delta S° = \Delta G° - \Delta H° \quad (2)$$

$$e_{\Delta G°} = \left| -\mathrm{R} \cdot T \cdot \frac{e_{K_a}}{K_a} \right| \quad (3)^7$$

$$e_{-T\Delta S°} = \sqrt{e_{\Delta G°} + e_{\Delta H°}} \quad (4)^7$$

Kinetic data

Table S4: Individual kinetic data for fitting of the raw data of three ITC measurements per compound before and after adjustment with globally fitted thermodynamic values. **1f** is not included, as a displacement approach was applied, which does not enable kinetic data extraction.

Before global adjustment				After global adjustment			
Compound	**χ^2**	**k_{on} / 10^4 M^{-1} s^{-1}**	**k_{off} /10^{-2} s^{-1}**	**Compound**	**χ^2**	**k_{on} / 10^4 M^{-1} s^{-1}**	**k_{off} /10^{-2} s^{-1}**
	0.55	1.71	0.79		0.54	1.62	0.81
1a	0.95	2.08	1.12	**1a**	1.39	2.17	1.09
	1.35	2.28	1.09		1.29	2.22	1.11
	0.98	2.24	0.47		0.89	2.17	0.49
1b	0.84	2.84	0.74	**1b**	1.06	3.02	0.68
	0.69	2.48	0.40		0.81	2.15	0.48
	1.50	7.53	0.51		1.47	6.90	0.59
1c	2.48	5.27	0.47	**1c**	2.54	5.41	0.46
	0.68	7.04	0.74		0.74	7.71	0.65
	0.34	8.56	0.61		0.32	8.41	0.63
1d	0.81	4.82	0.41	**1d**	0.84	5.00	0.37
	1.31	8.43	0.56		1.18	8.08	0.60
	0.69	12.43	0.51		0.71	13.32	0.45
1e	1.33	16.74	0.64	**1e**	1.36	17.45	0.59
	0.47	14.18	0.35		0.52	13.00	0.44
	1.22	2.31	0.68		1.22	2.33	0.67
2a	2.01	1.91	0.60	**2a**	2.15	2.02	0.58
	0.83	2.74	0.72		0.74	2.61	0.75
	1.30	6.75	0.77		1.28	6.62	0.79
2b	3.93	5.65	0.61	**2b**	4.60	5.27	0.63
	1.38	5.99	0.84		1.46	6.62	0.79
	2.35	6.01	0.26		2.74	6.58	0.23
2c	2.50	7.76	0.25	**2c**	2.38	7.47	0.26
	2.10	5.64	0.14		1.80	5.15	0.18
	0.95	36.16	0.58		0.96	31.68	0.74
2d	1.59	10.90	0.19	**2d**	1.47	9.67	0.23
	2.15	12.01	0.37		2.26	13.30	0.31

Table S5: Kinetic data.

			Standard error	
Compound	**k_{on}[a] 10^4 M^{-1} s^{-1}**	**k_{off}[a] 10^{-3} s^{-1}**	**k_{on}[b] 10^4 M^{-1} s^{-1}**	**k_{off}[b] 10^{-3} s^{-1}**
1a	2.00	10.0	0.19	0.96
1b	2.45	5.48	0.29	0.64
1c	6.68	5.66	0.67	0.57
1d	7.16	5.34	1.08	0.81
1e	14.6	4.91	1.43	0.48
2a	2.32	6.67	1.72	0.49
2b	6.17	7.34	0.45	0.53
2c	6.40	2.21	0.68	0.23
2d	18.2	4.27	6.81	1.60

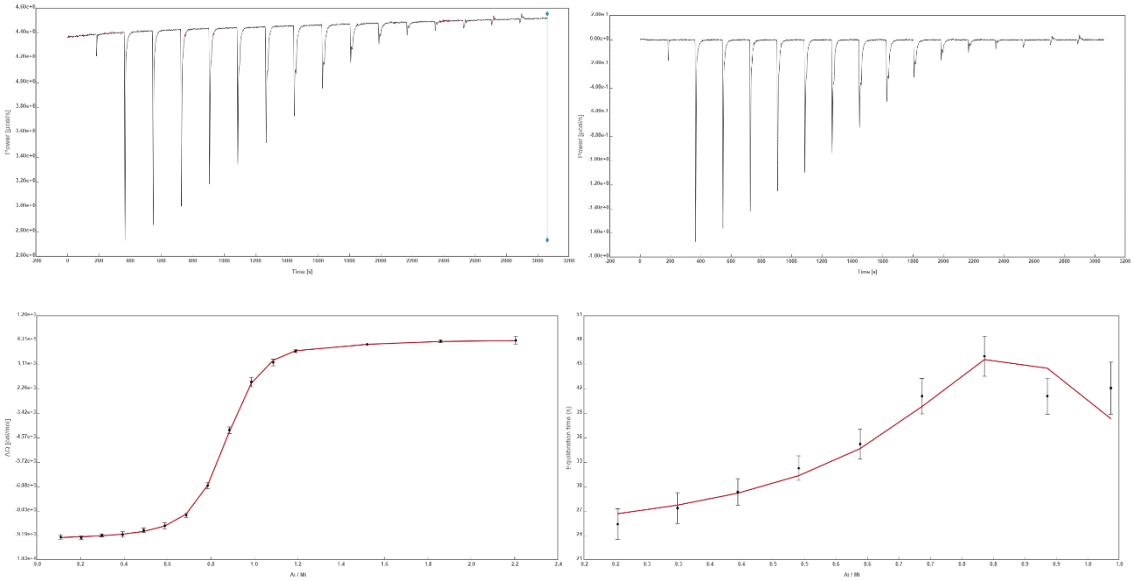
[a] Kinetic data were obtained after renewed processing of the raw data with $\Delta H°$ and K_a values from the global fitting.[b] Standard error of measurement after adjustment with global data.

Isothermal Titration Calorimetry data

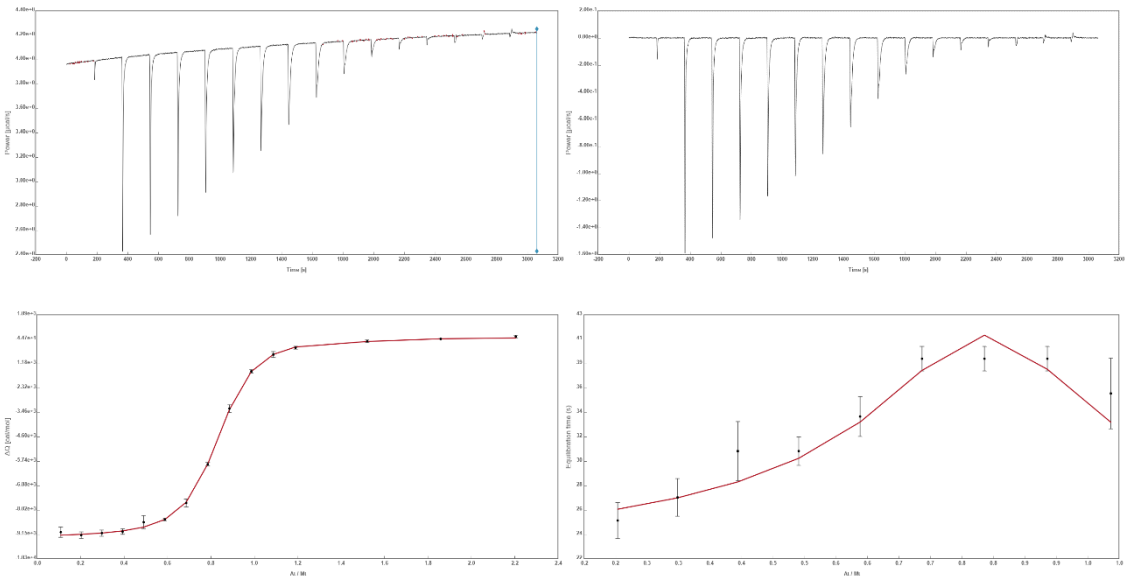
Raw and processed thermograms. calculated isotherms and equilibration-time curves for every measurement for every compound are provided as issued by the AFFINImeter cloud software. For **1f**, raw and processed thermograms as well as the globally fitted isotherms are provided.

1a

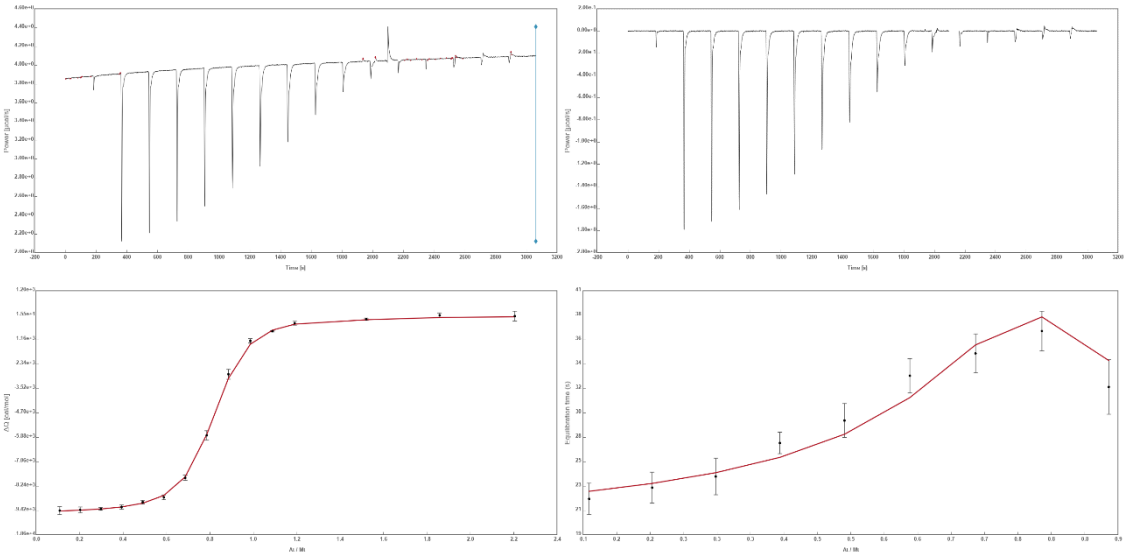
Measurement 1



Measurement 2

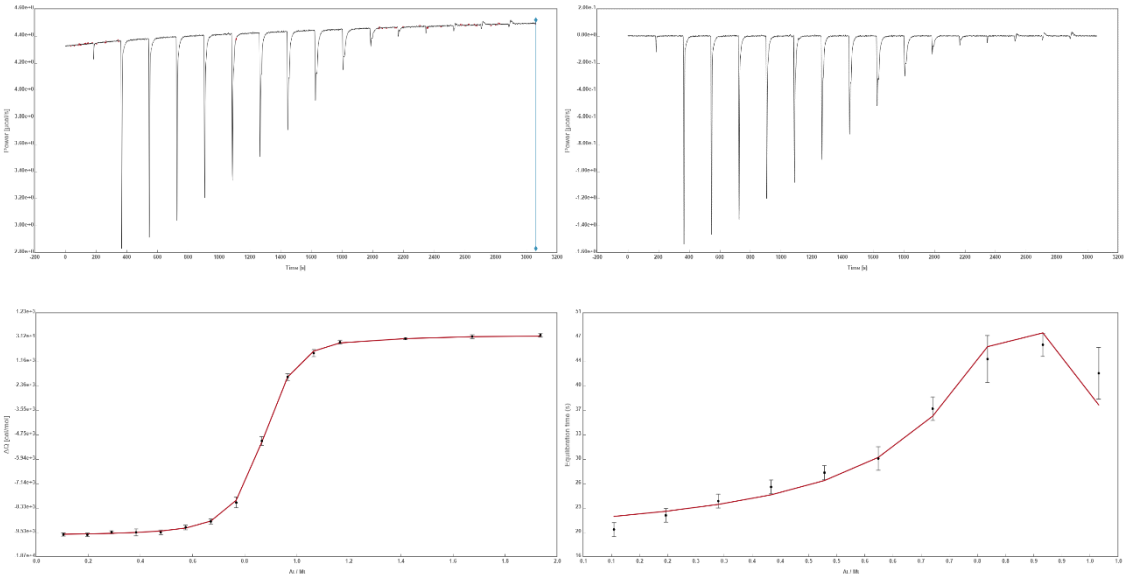


Measurement 3

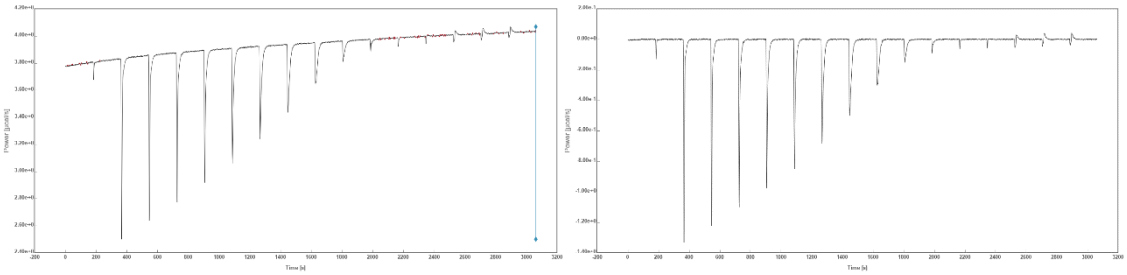


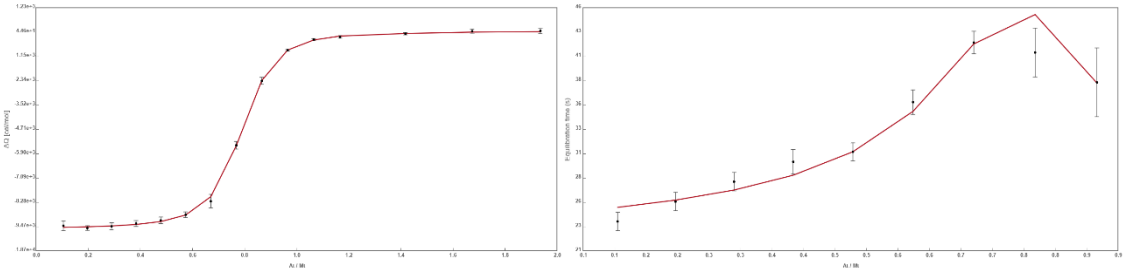
1b

Measurement 1



Measurement 2





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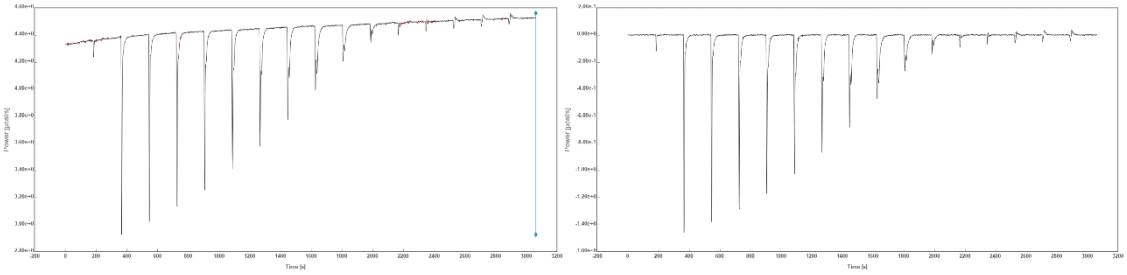
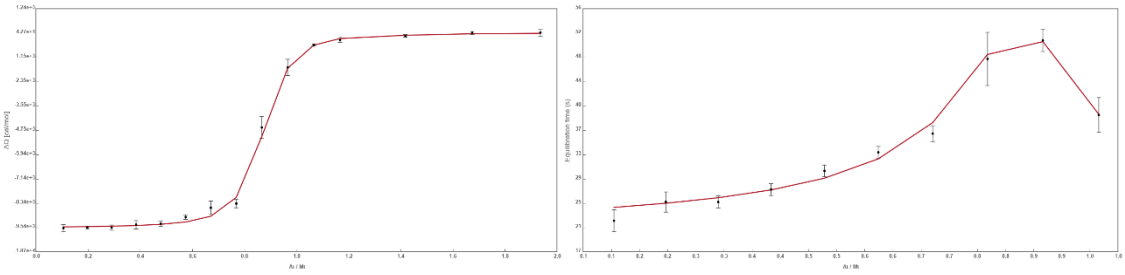
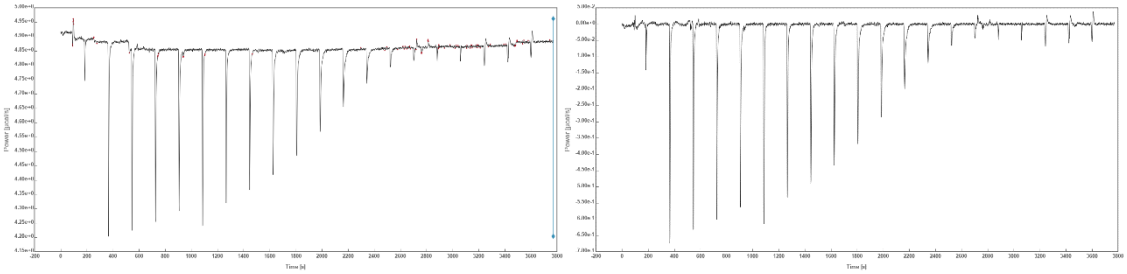
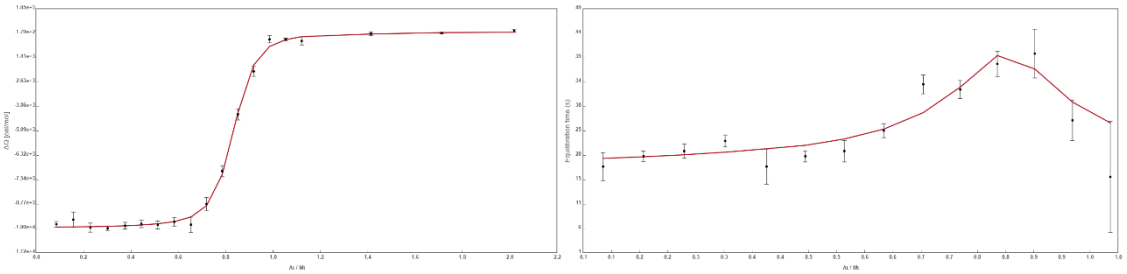




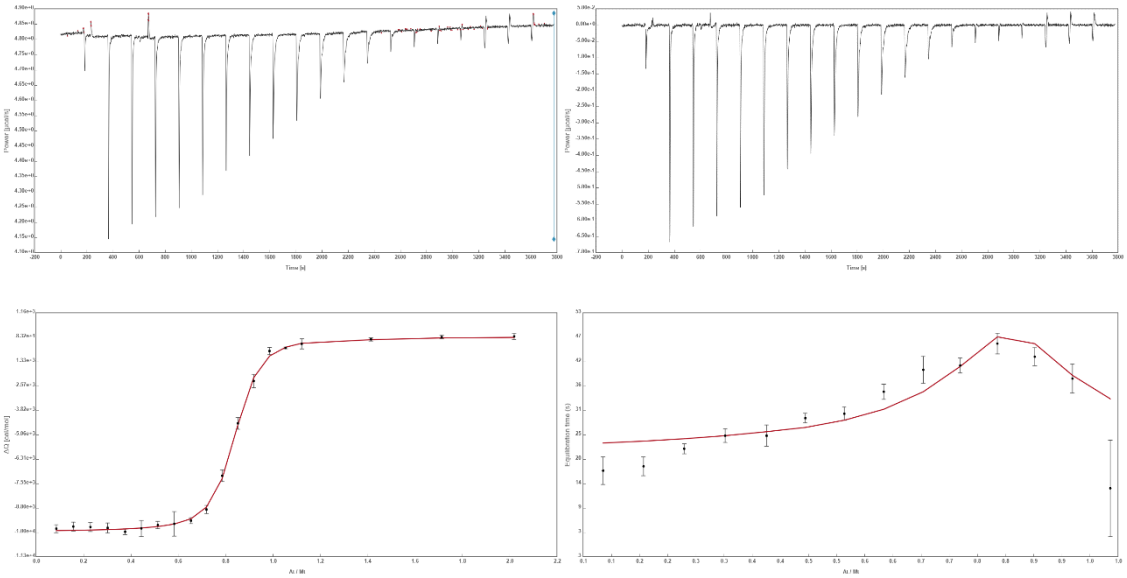
1c

Measurement 1

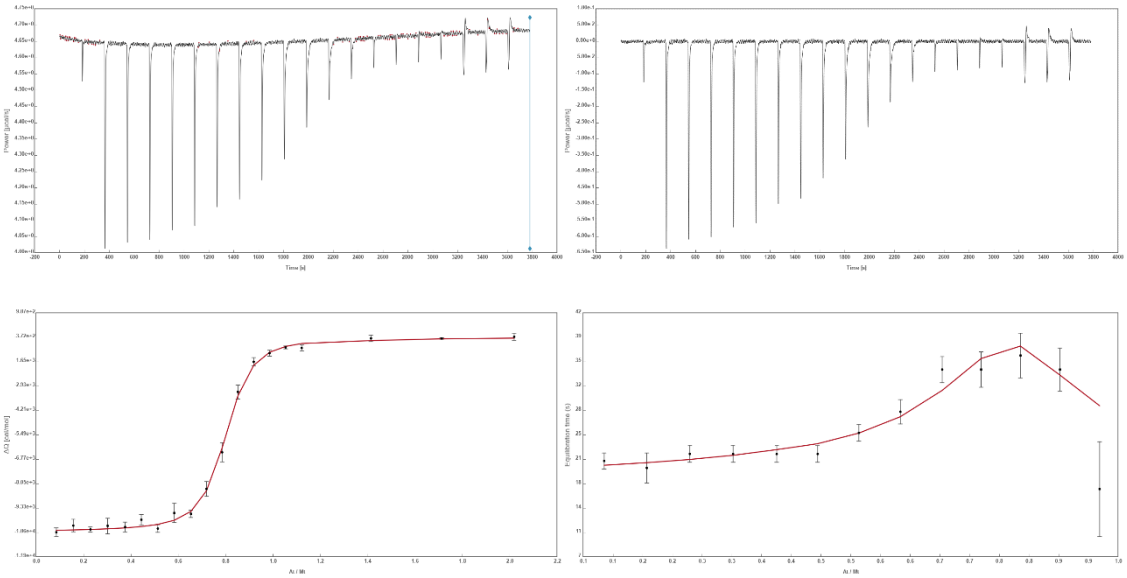




Measurement 2

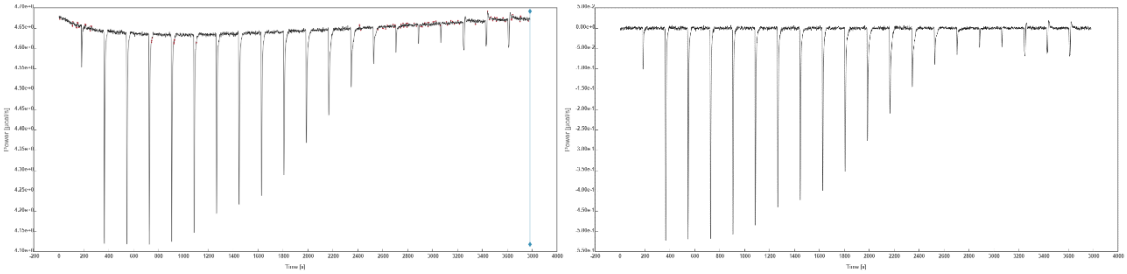


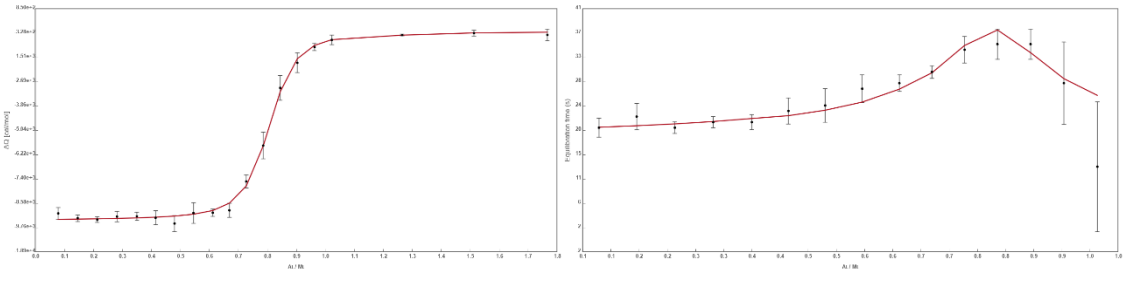
Measurement 3



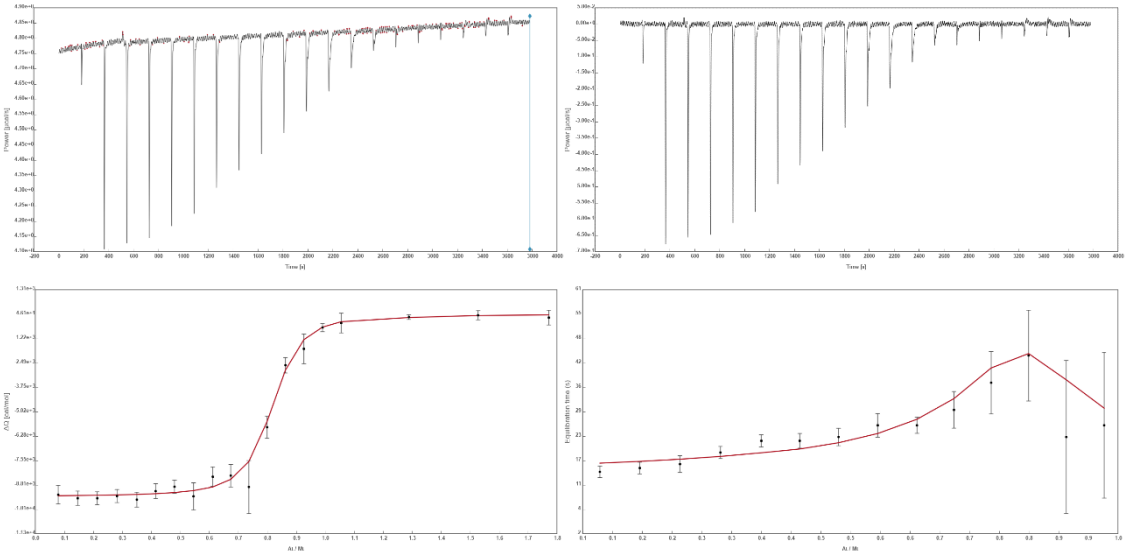
1d

Measurement 1

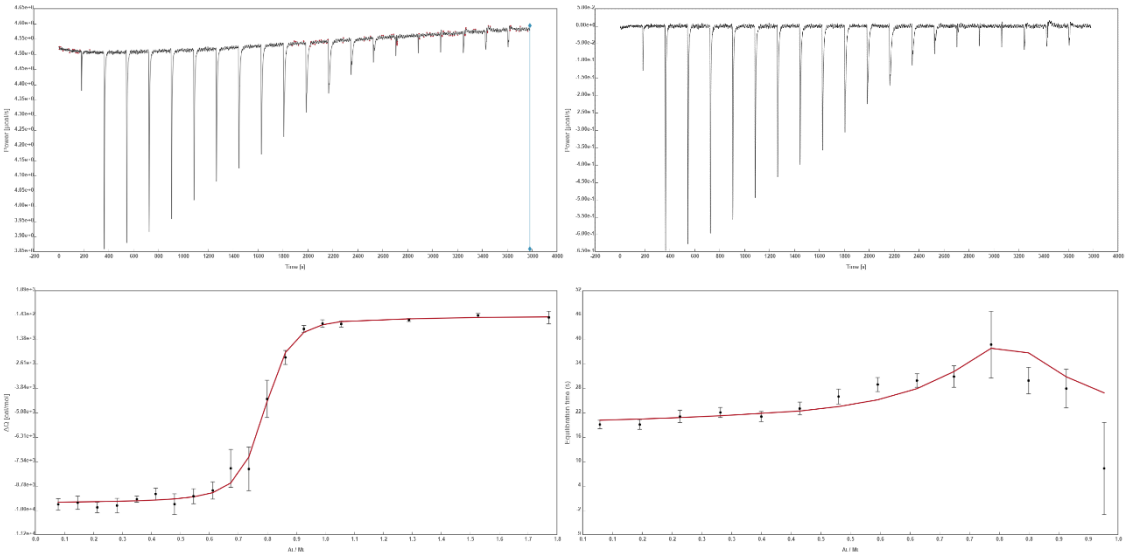




Measurement 2

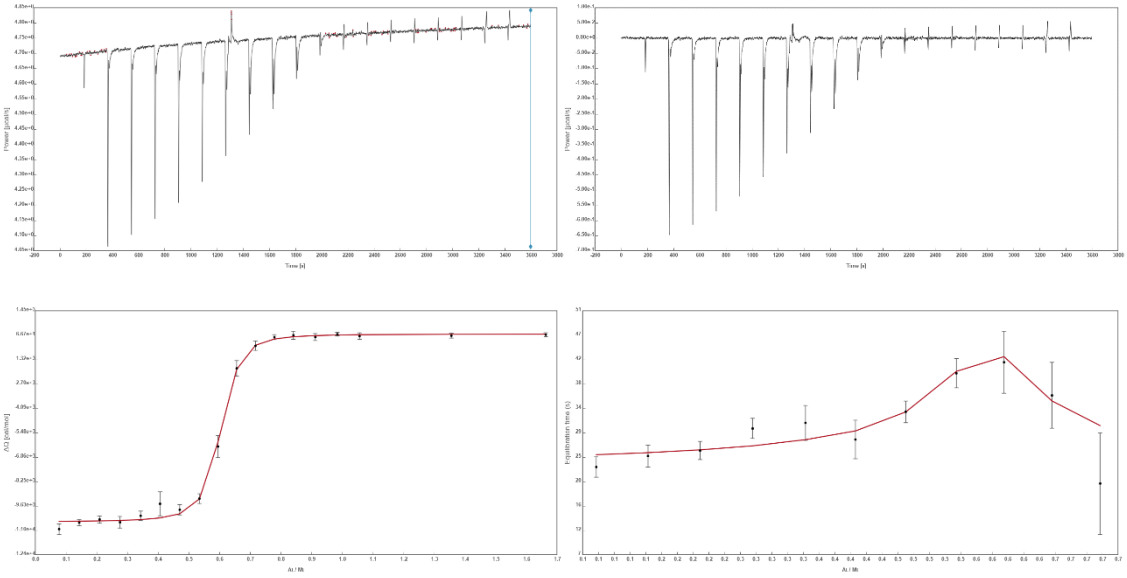


Measurement 3

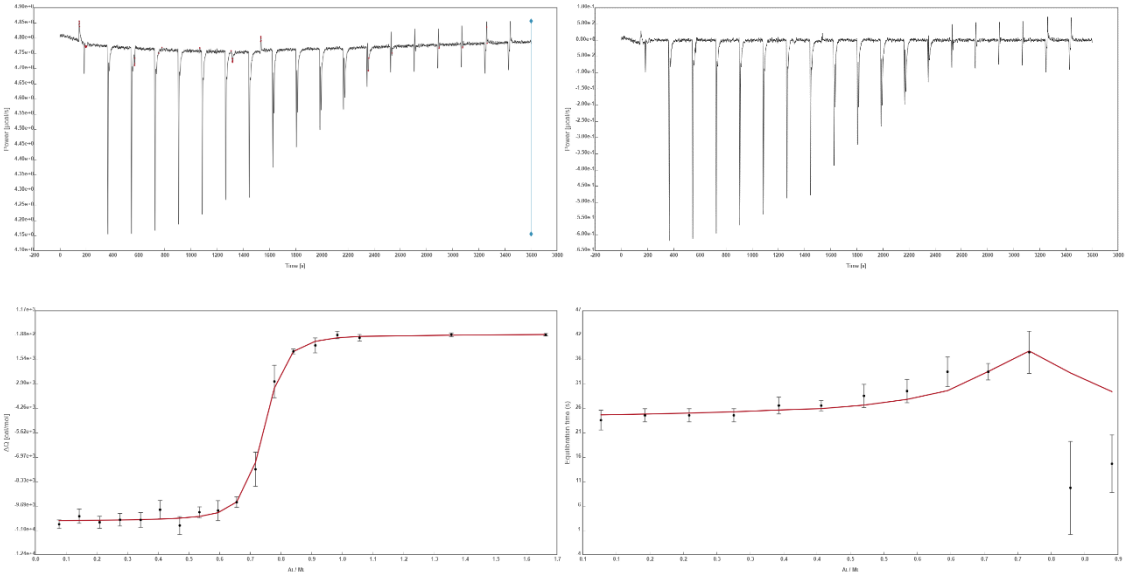


1e

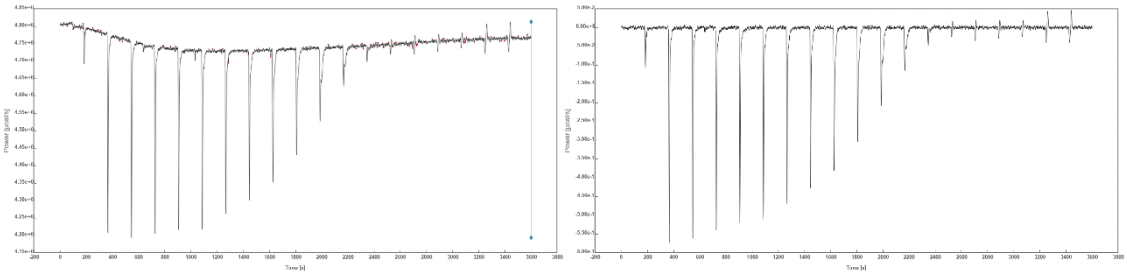
Measurement 1

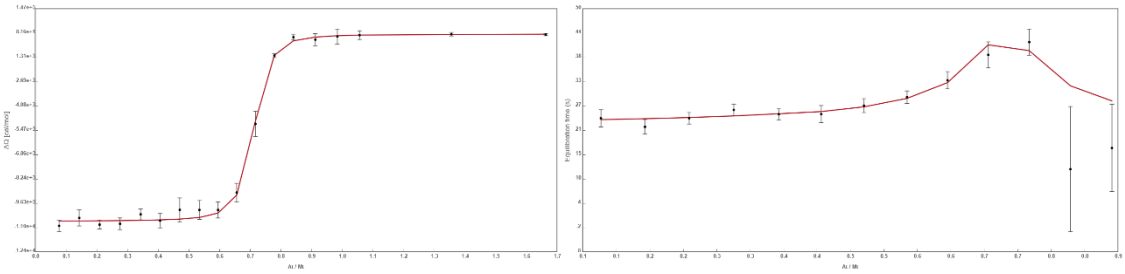


Measurement 2



Measurement 3

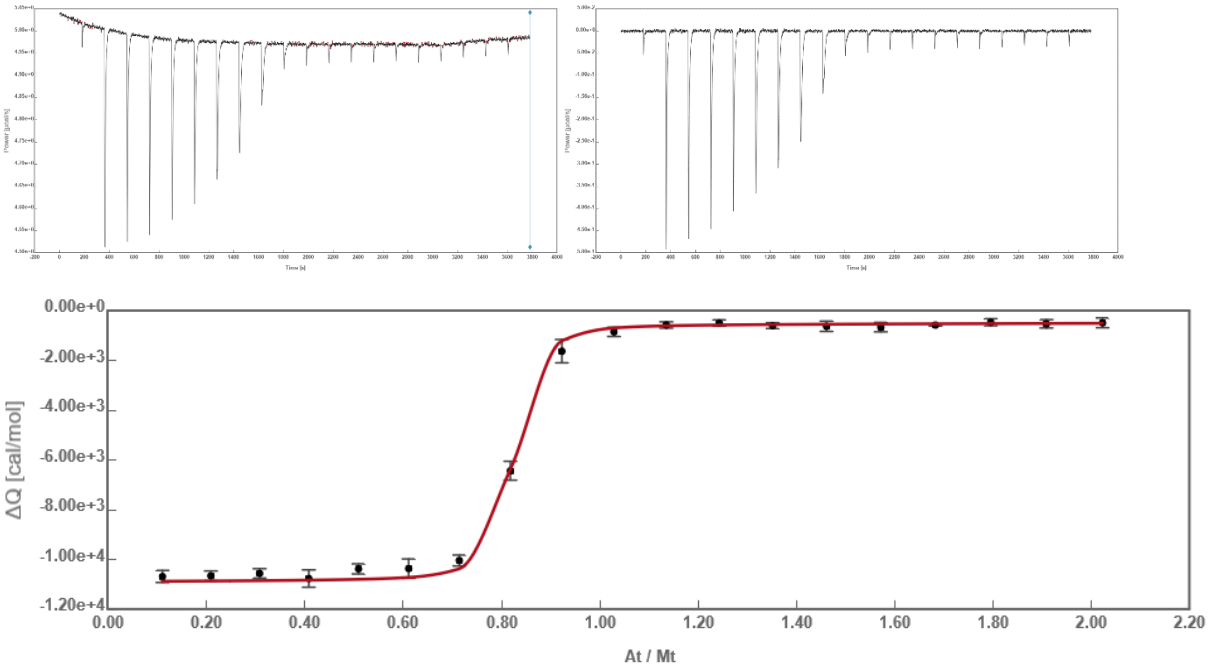




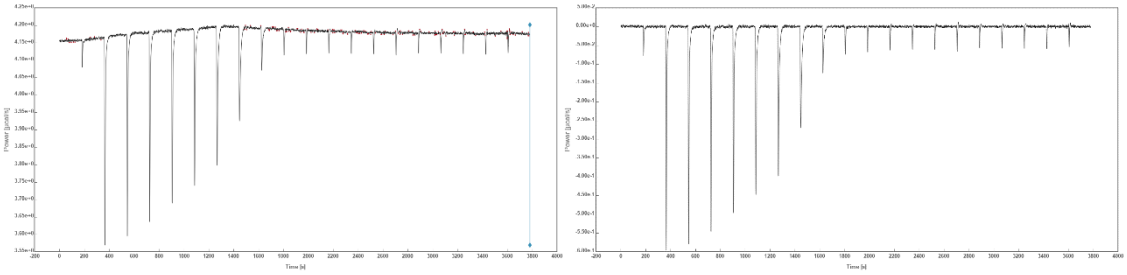
1f

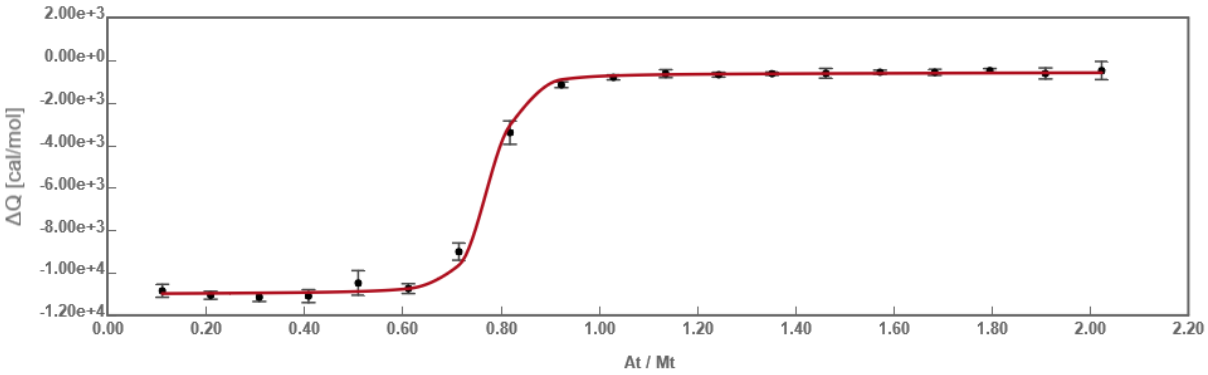
Direct titration **1f** in hCAII

Measurement 1



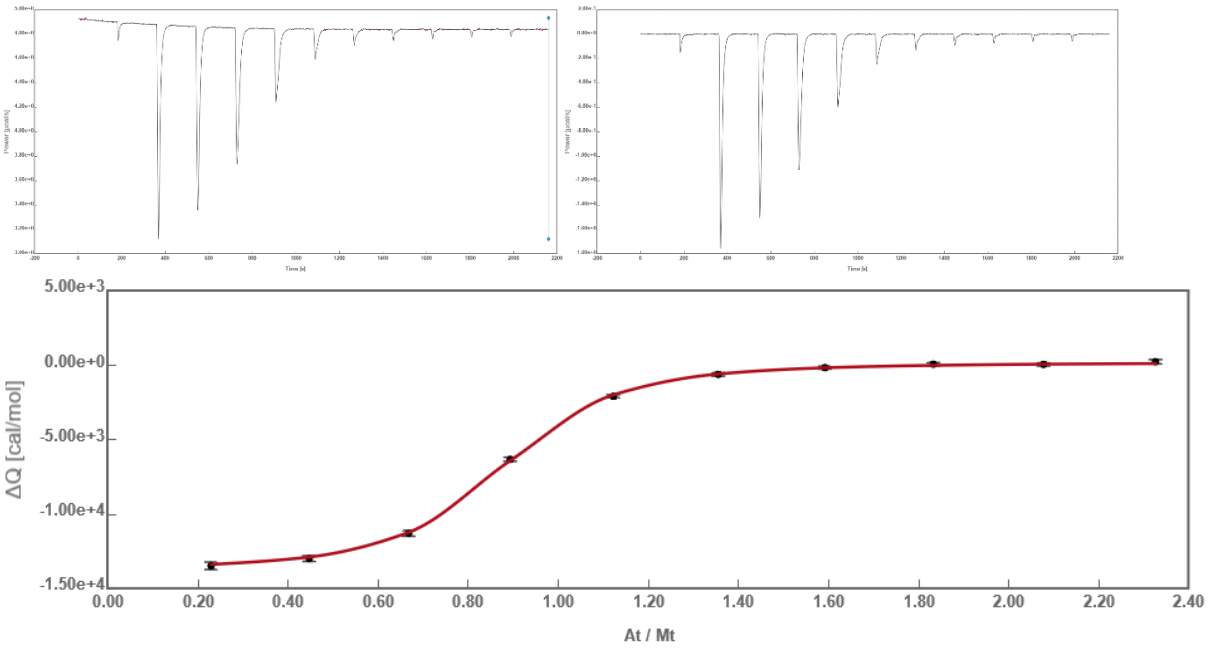
Measurement 2



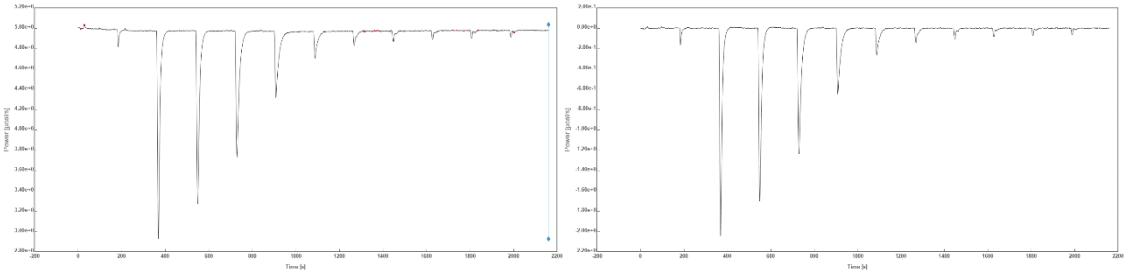


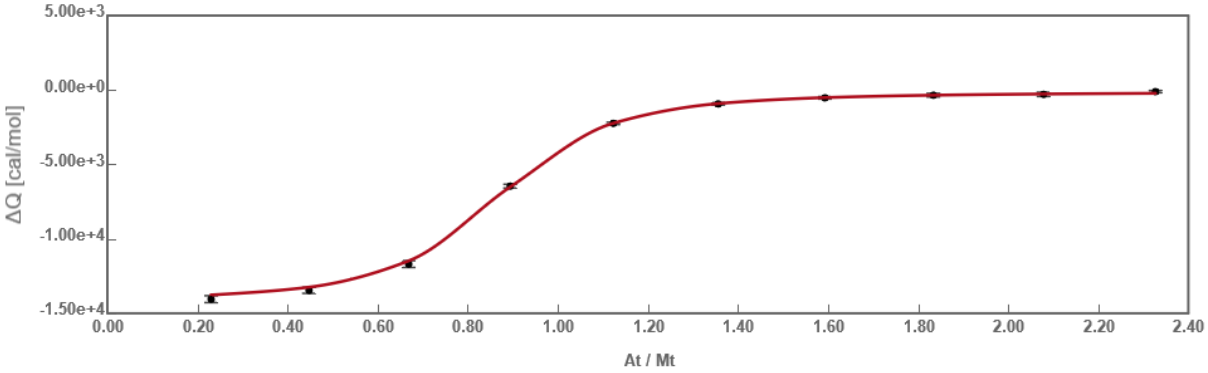
Reference titration of 4CBS

Measurement 1

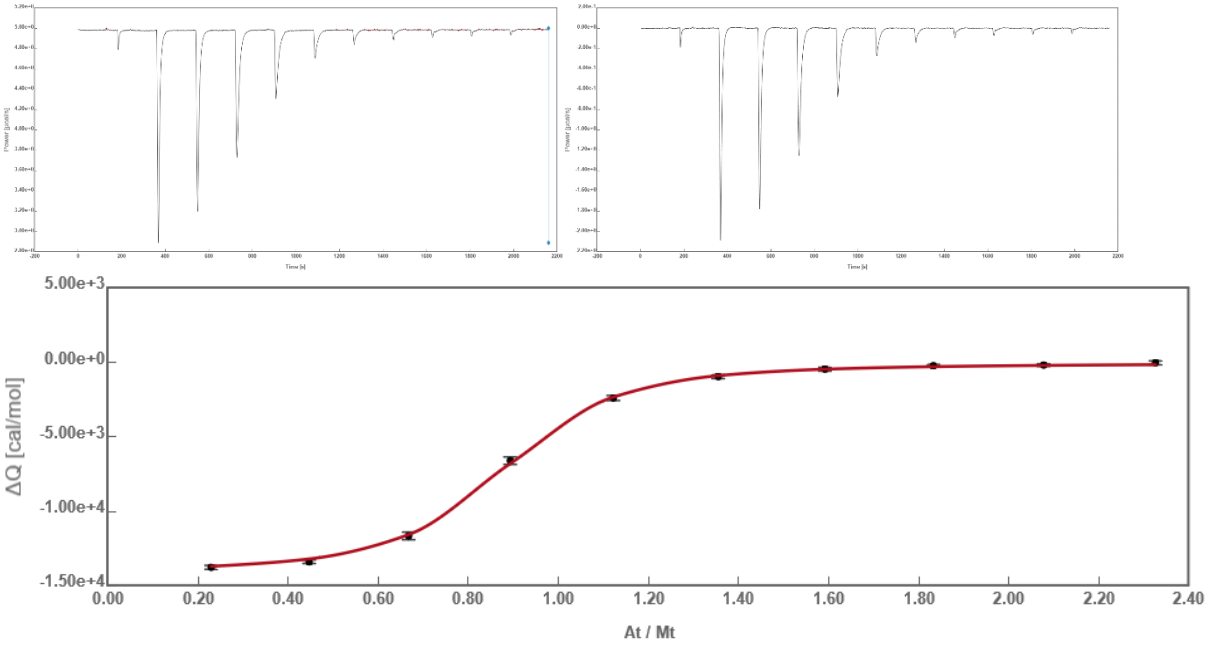


Measurement 2



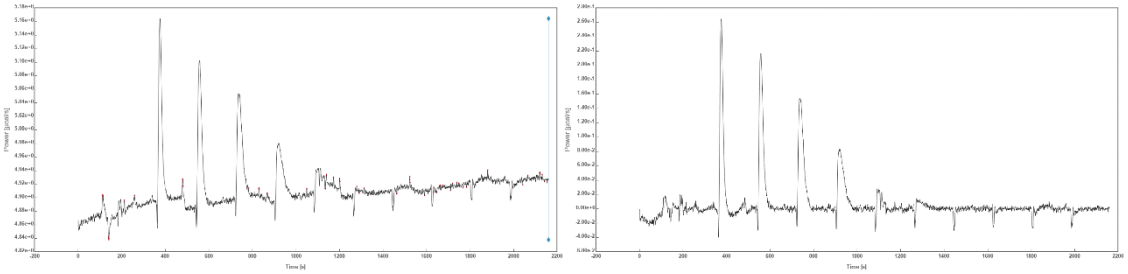


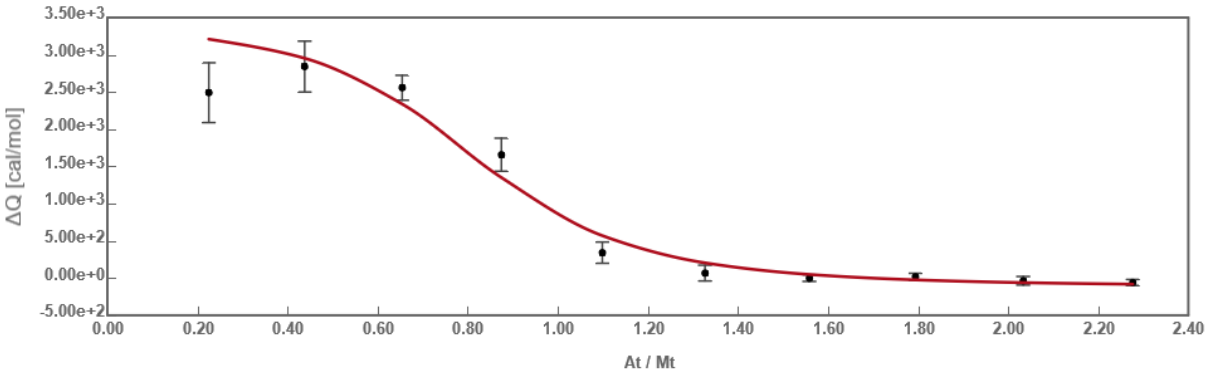
Measurement 3



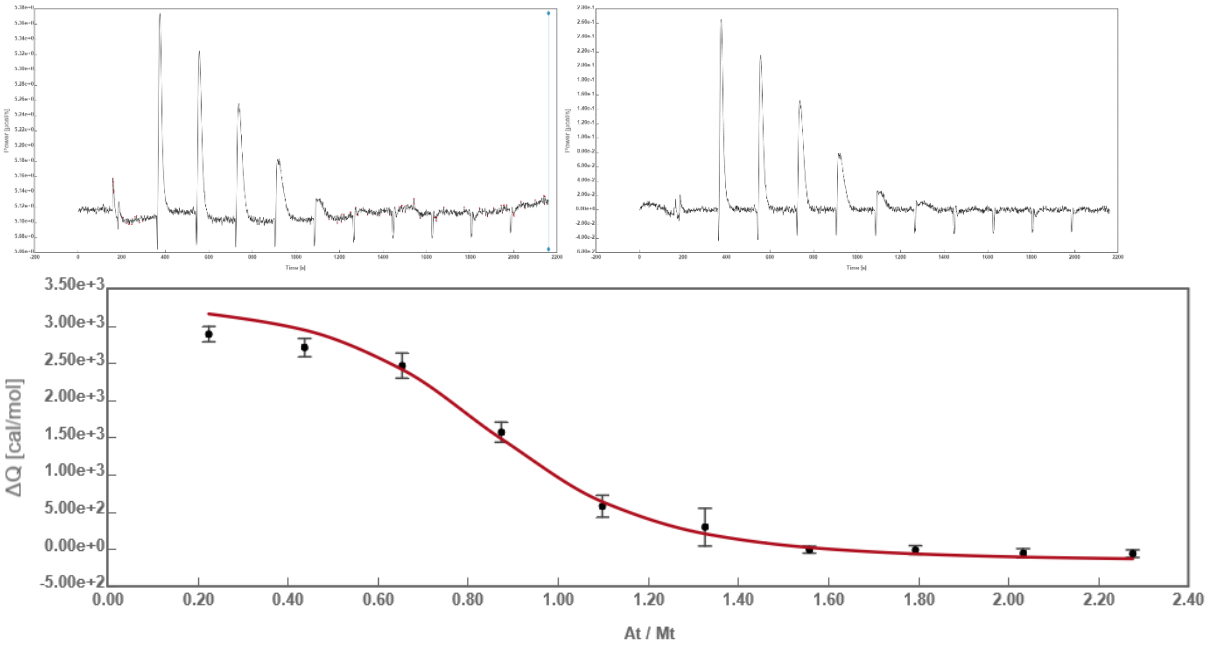
1f vs. 4CBS

Measurement 1

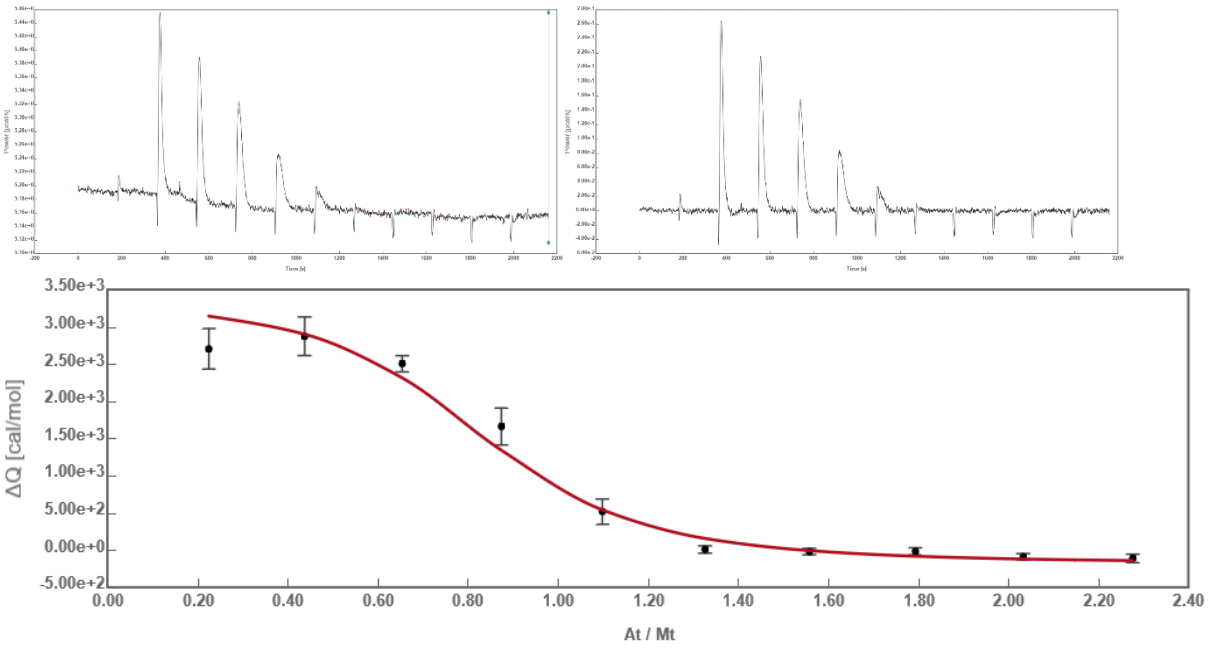




Measurement 2

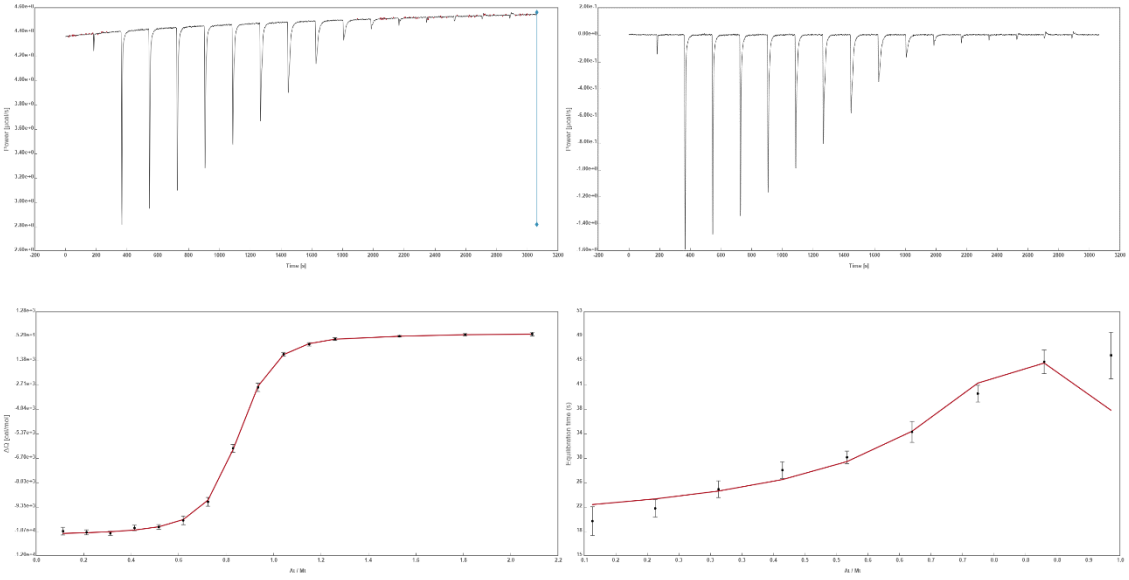


Measurement 3

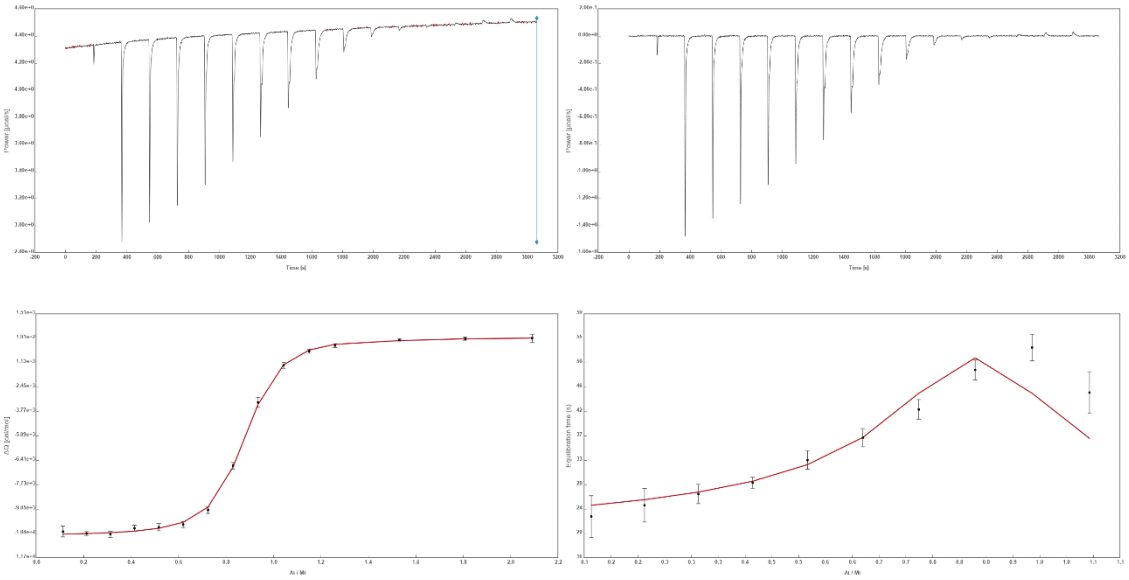


2a

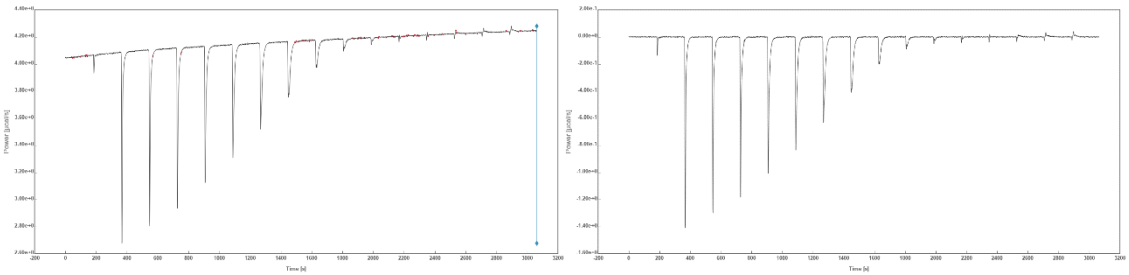
Measurement 1

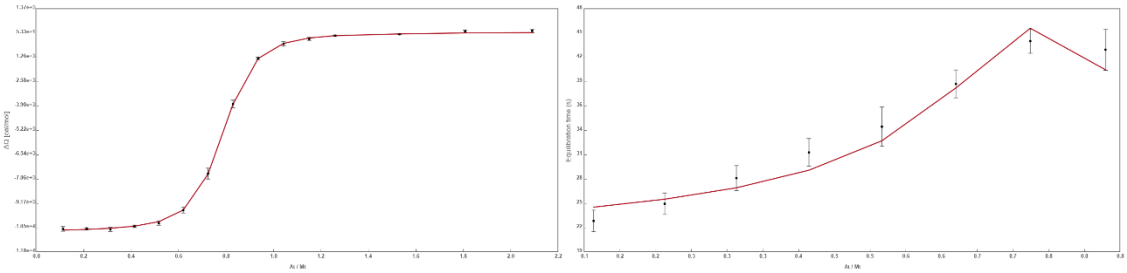


Measurement 2



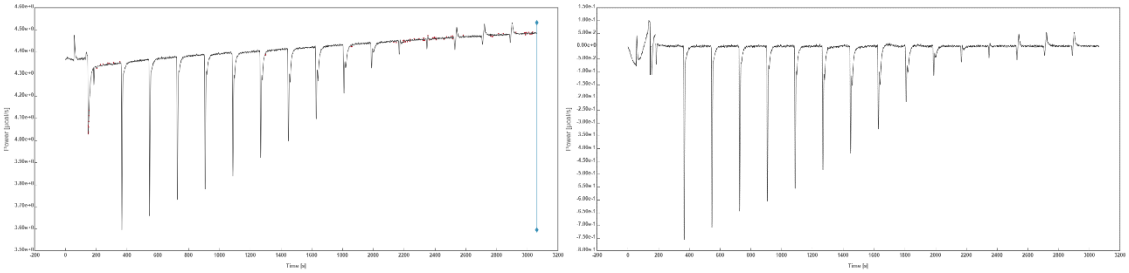
Measurement 3

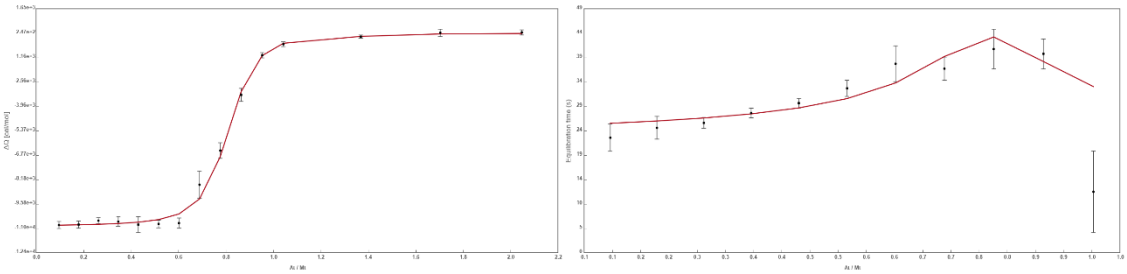




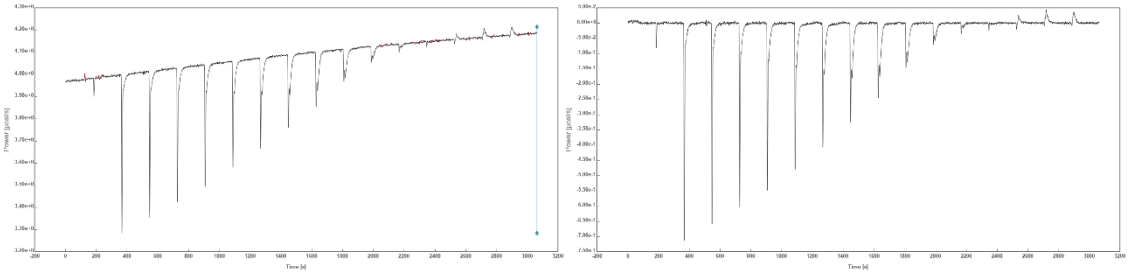
2b

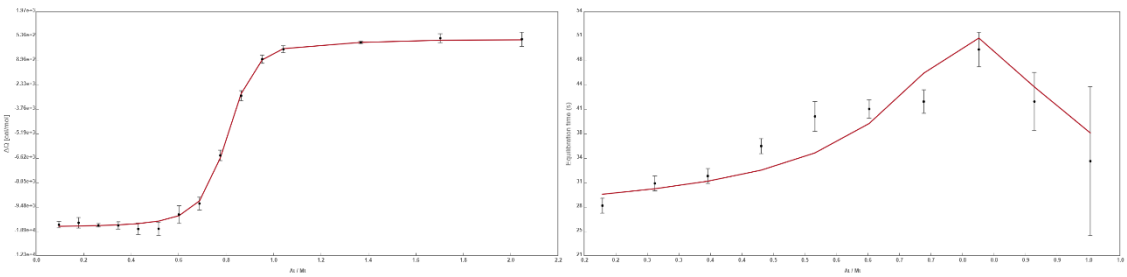
Measurement 1



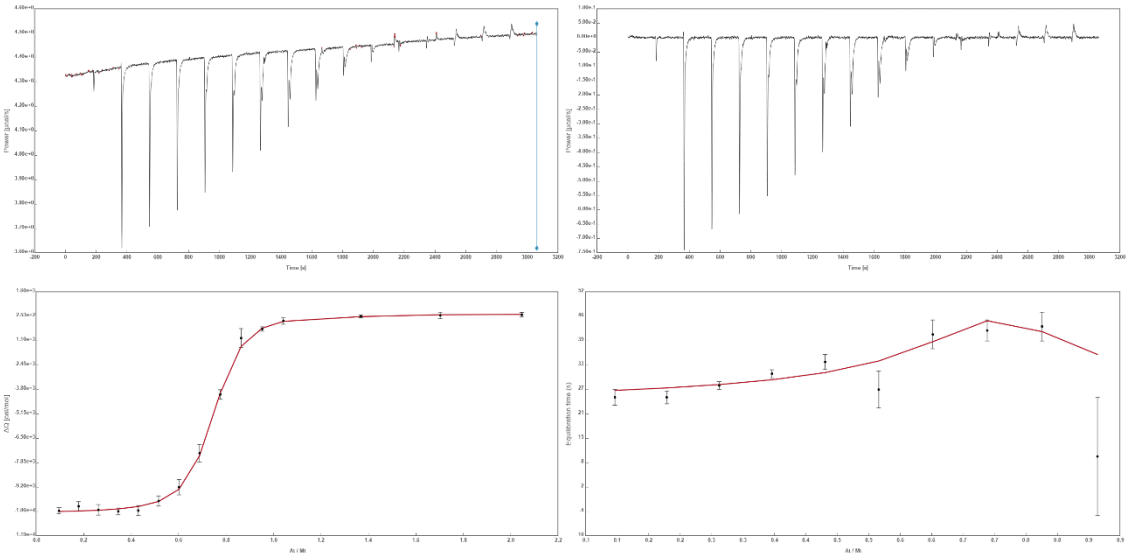


Measurement 2



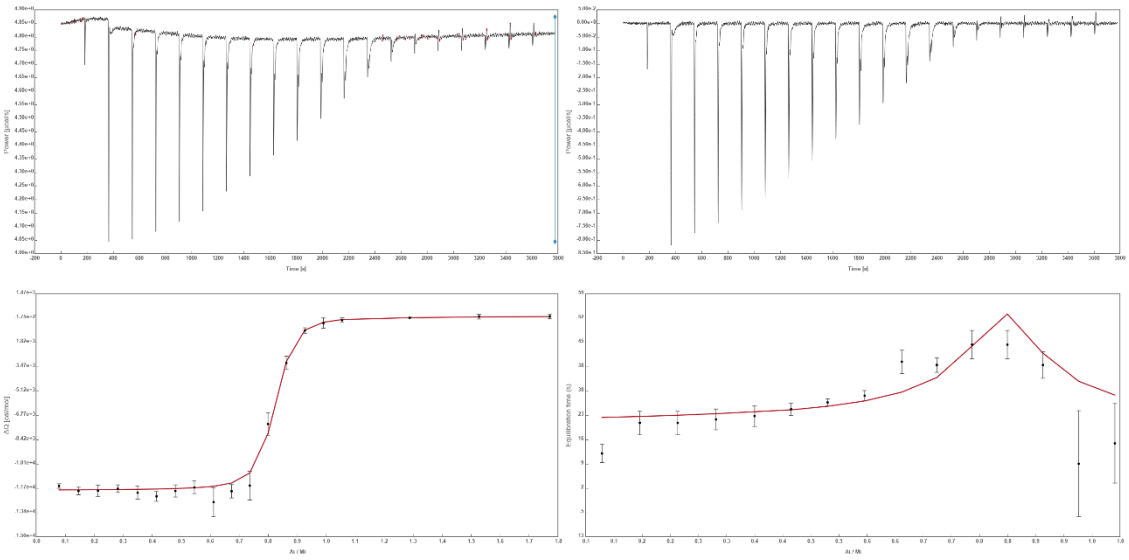


Measurement 3

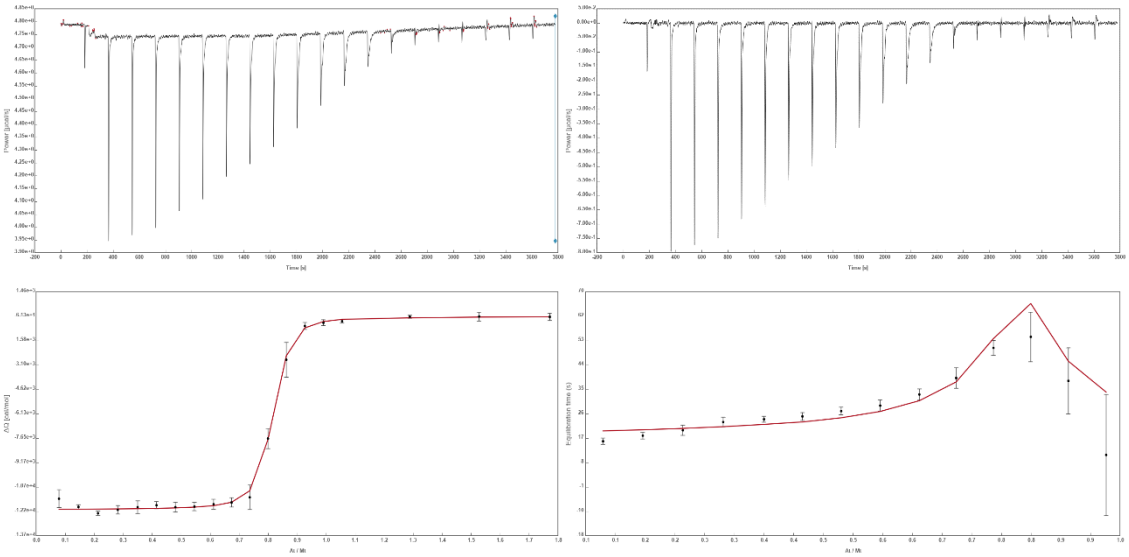


2c

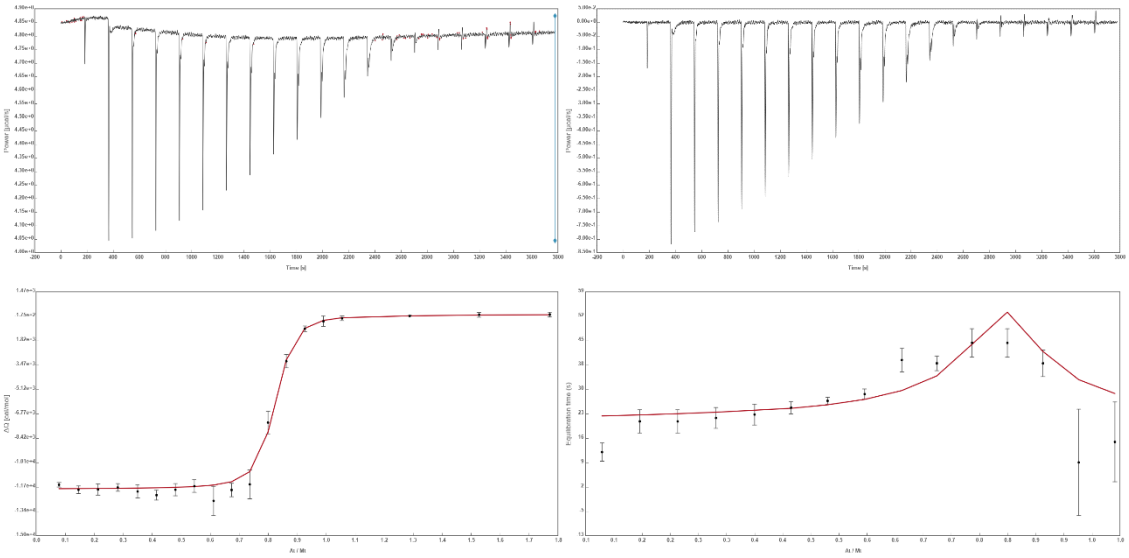
Measurement 1



Measurement 2

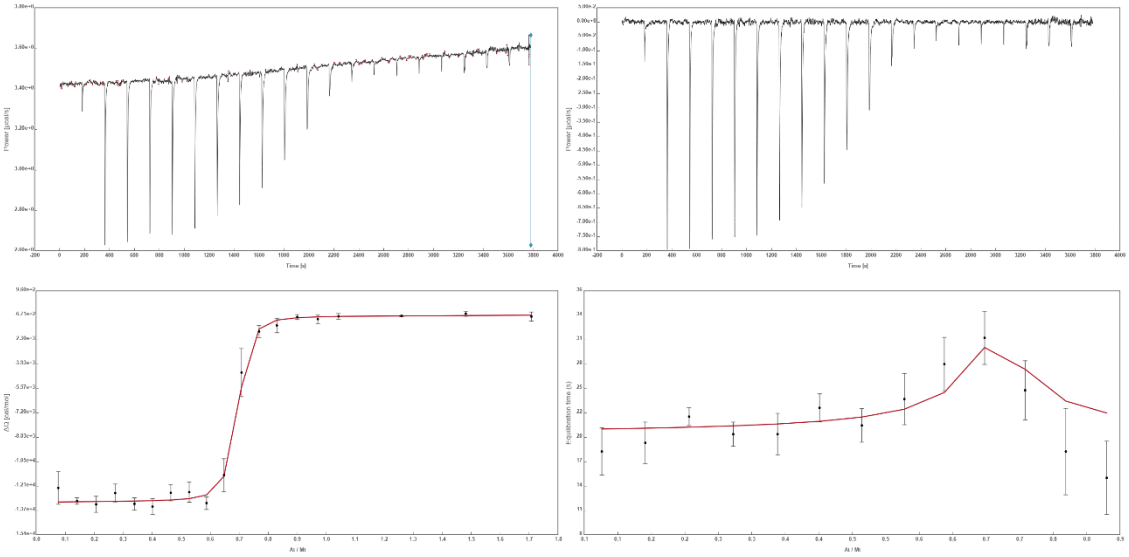


Measurement 3

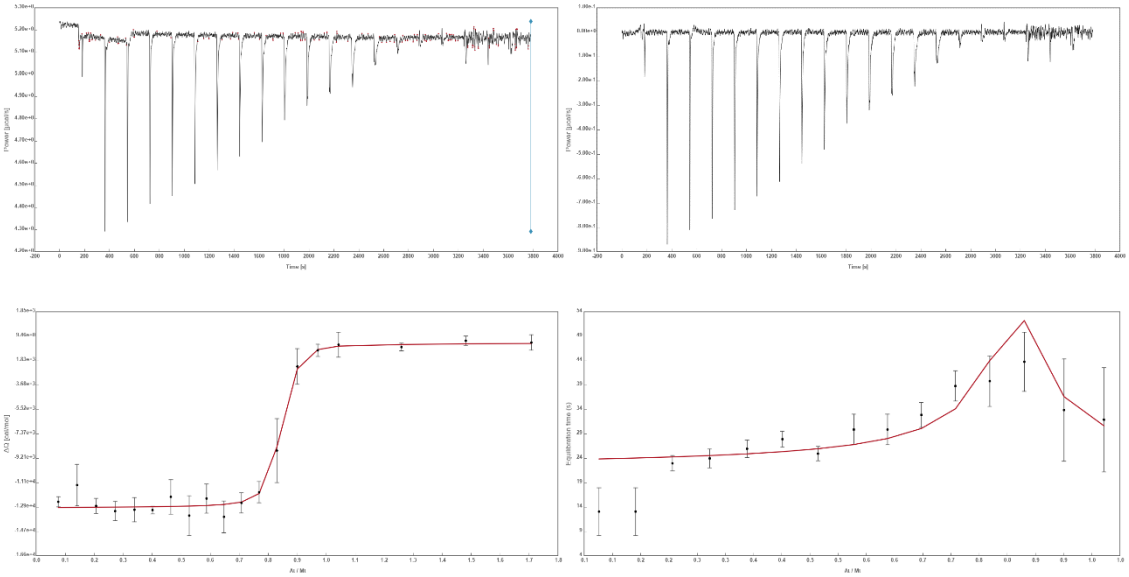


2d

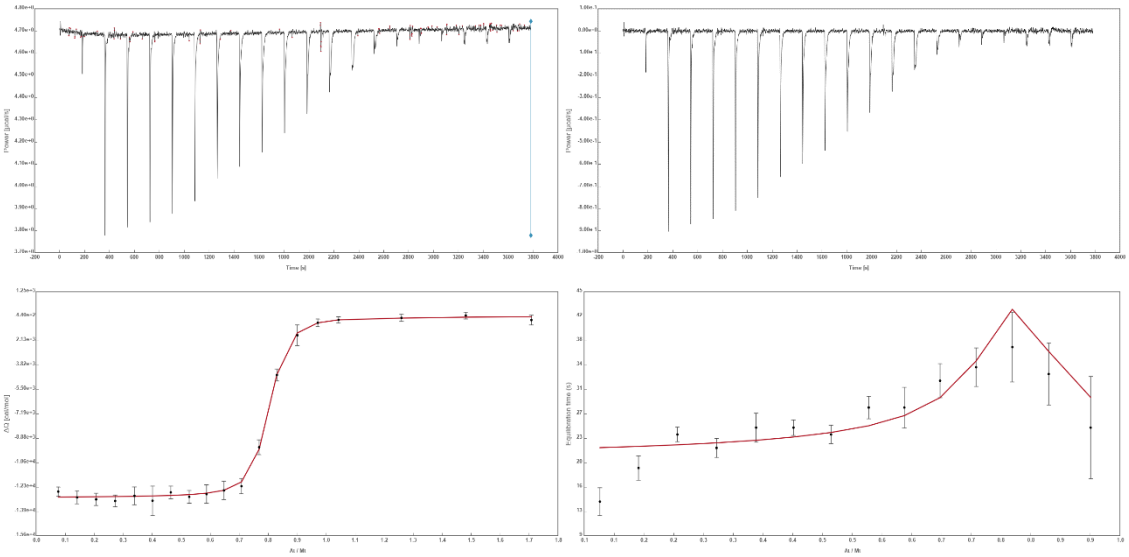
Measurement 1



Measurement 2



Measurement 3



Synthesis and Determination of Purity

The purity of all ligands was determined by analytical HPLC with a Shimadzu LC-10A system (reversed-phase column: Nucleodur C18, 5 µm, 100 Å, 4.6 x 250 mm, Macherey-Nagel, Düren, Germany). All solvents were HPLC grade and in a gradient run the percentage of acetonitrile was increased 1% solvent min^{-1} at a flow rate of 1 mL min^{-1}. The detection was recorded at a wavelength of 220 nm. ^{1}H and ^{13}C NMR spectra were measured on a JEOL ECX-400 instrument. Chemical shifts are reported in ppm using residual peaks for the deuterated solvent as internal standard:[8] DMSO-d_6, 2.50 ppm (^{1}H NMR), 39.5 ppm (^{13}C NMR). The multiplicity of the signals is described with the following abbreviations: s = singlet, d = doublet, t = triplet and m = multiplet. The coupling constants *J* are given in Hz. MS spectra were measured on a Q-Trap 2000 system with an electrospray interface (ESI). **2c** and **2d** were synthesized according to a procedure by *Carta et al*.[9]

*4-Pentylbenzenesulfonamide (**1f**)*: 4-Pentylbenzenesulfonyl chloride (1.50 g, 6.08 mmol) was dissolved in chloroform (10 mL) and 25% (w/v) aqueous ammonia solution was added. The mixture was stirred for 4 h at rt and the layers were separated. The aqueous layer was extracted with chloroform (3 x 20 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. **1f** (1.08 g, 4.73 mmol, 78%) was obtained as a white solid without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.73 (d, *J* = 8.5 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.24 (s, 2H), 2.64 (m, 2H), 1.62-1.55 (m, 2H), 1.35-1.22 (m, 4H), 0.86 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 146.5, 141.5, 128.6, 125.6, 34.7, 30.7, 30.2, 21.8, 13.8. MS (ESI+) *m/z* calculated for $C_{12}H_{21}N_2O_2S$ $[M+NH_4]^+$: 245.13; found: 245.23.

*4-n-Propoxybenzenesulfonamide (**2c**)*: 4-Hydroxybenzenesulfonamide (0.70 g, 4.03 mmol) and potassium carbonate (0.83 g, 6.04 mmol) were dissolved in dried DMF (10 mL). The suspension was stirred for 20 min at rt under nitrogen atmosphere. 1-Bromopropane (0.55 mL, 6.04 mmol) was added and the mixture was stirred for 22 h at rt. The reaction was quenched with water (10 mL) and the forming precipitate was collected by filtration. The crude product was purified by flash column chromatography over silicagel (cyclohexane/EtOAc, 2:1). **2c** (0.39 g, 1.79 mmol, 44%) was obtained as a white-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.74 (d, *J* = 8.3 Hz, 2H), 7.17 (s, 2H), 7.07 (d, *J* = 8.5 Hz, 2H), 4.00 (t, *J* = 6.5 Hz, 2H), 1.79-1.70 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 161.0, 136.0, 127.6, 114.3, 69.3, 21.8, 10.2. MS (ESI+) *m/z* calculated for $C_9H_{17}N_2O_3S$ $[M+NH_4]^+$: 233.09; found: 233.12.

*4-n-Butoxybenzenesulfonamide (**2d**)*: 4-Hydroxybenzenesulfonamide (0.70 g, 4.03 mmol) and potassium carbonate (0.83 g, 6.04 mmol) were dissolved in dried DMF (10 mL). The suspension was stirred at rt for 20 min under nitrogen atmosphere. 1-Iodobutane (0.46 mL, 6.04 mmol) was added and the mixture was stirred for 24 h at rt. The reaction was quenched with water (10 mL) and the forming precipitate was collected by filtration. The crude product was purified by flash column chromatography over silicagel (cyclohexane/EtOAc, 2:1). **2d** (0.75 g, 3.28 mmol, 81%) was obtained as a white-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.73 (d, *J* = 8.9 Hz, 2H), 7.17 (s, 2H), 7.07 (d, *J* = 8.9 Hz, 2H), 4.04 (t, *J* = 6.5 Hz, 2H), 1.74-1.67 (m, 2H), 1.48-1.39 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 161.0,

136.0, 127.6, 114.3, 67.6, 30.5, 18.6, 13.6. MS (ESI+) *m/z* calculated for $C_{10}H_{19}N_2O_3S$ $[M+NH_4]^+$: 247.11; found: 247.17.

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