Electronic Supplementary Material (ESI) for RSC Advances.

Supplemental Information

Discovery of fragments inducing conformational effects in dynamic proteins using a second-harmonic generation biosensor

Edward A. FitzGerald,[1, 2] Margaret Butko,[3] Pierre Boronat,[4] Daniela Cederfelt,[1] Mia Abramsson,[1] Hildur Ludviksdottir,[1] Jacqueline E. van Muijlwijk-Koezen,[4] Iwan J.P. de Esch,[4] Doreen Dobritzsch,[1] Tracy Young,[3] and U. Helena Danielson.[1,5,*]

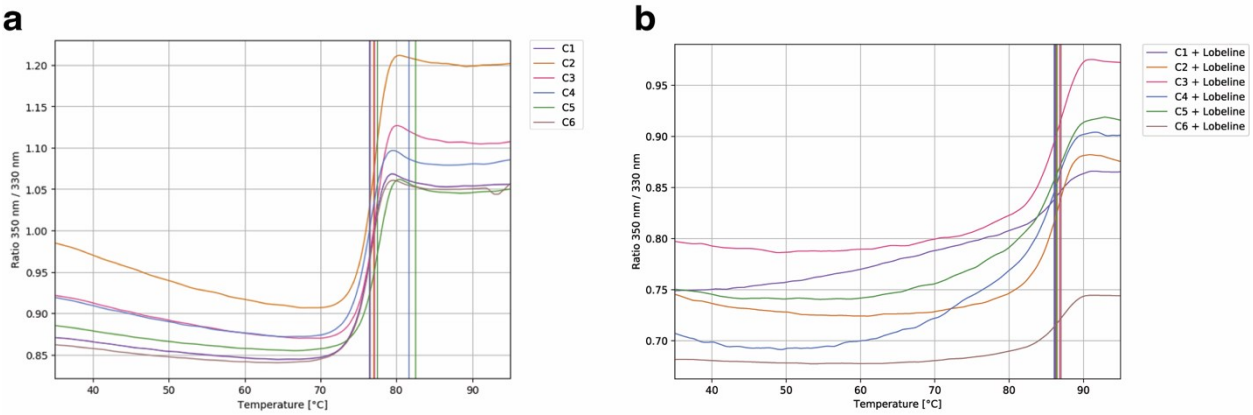

Figure S1. *NanoDSF analysis of single cysteine mutants C1-C6 of AChBP at (a) 25 µM, in the absence of a ligand, and (b) 1 µM in the presence of 1 mM lobeline. Plotted as the ratio of the intrinsic fluorescence detected at 350 and 330 nm as a function of temperature. An Increase in Ti (inflection temperature) indicated by the colored vertical line noted in the presence of compound.*

Table S1. *Summary of Ti observed with APO and ligand bound single cysteine AChBP mutants*

		Protein	**Ti#1**	**Ti#2**		**Protein + Lig**	**Ti#1**
Reference:		AChBP Wt	77.5			AChBP Wt	77.5
	1	C1	76.5		**1**	C1 + Lobeline	86.2
	2	C2	77.0		**2**	C2 + Lobeline	86.3
	3	C3	77.1		**3**	C3 + Lobeline	86.8
	4	C4	76.4	81.7	**4**	C4 + Lobeline	86.1
	5	C5	77.4	82.5	**5**	C5 + Lobeline	86.5
	6	C6	76.5		**6**	C6 + Lobeline	87.0

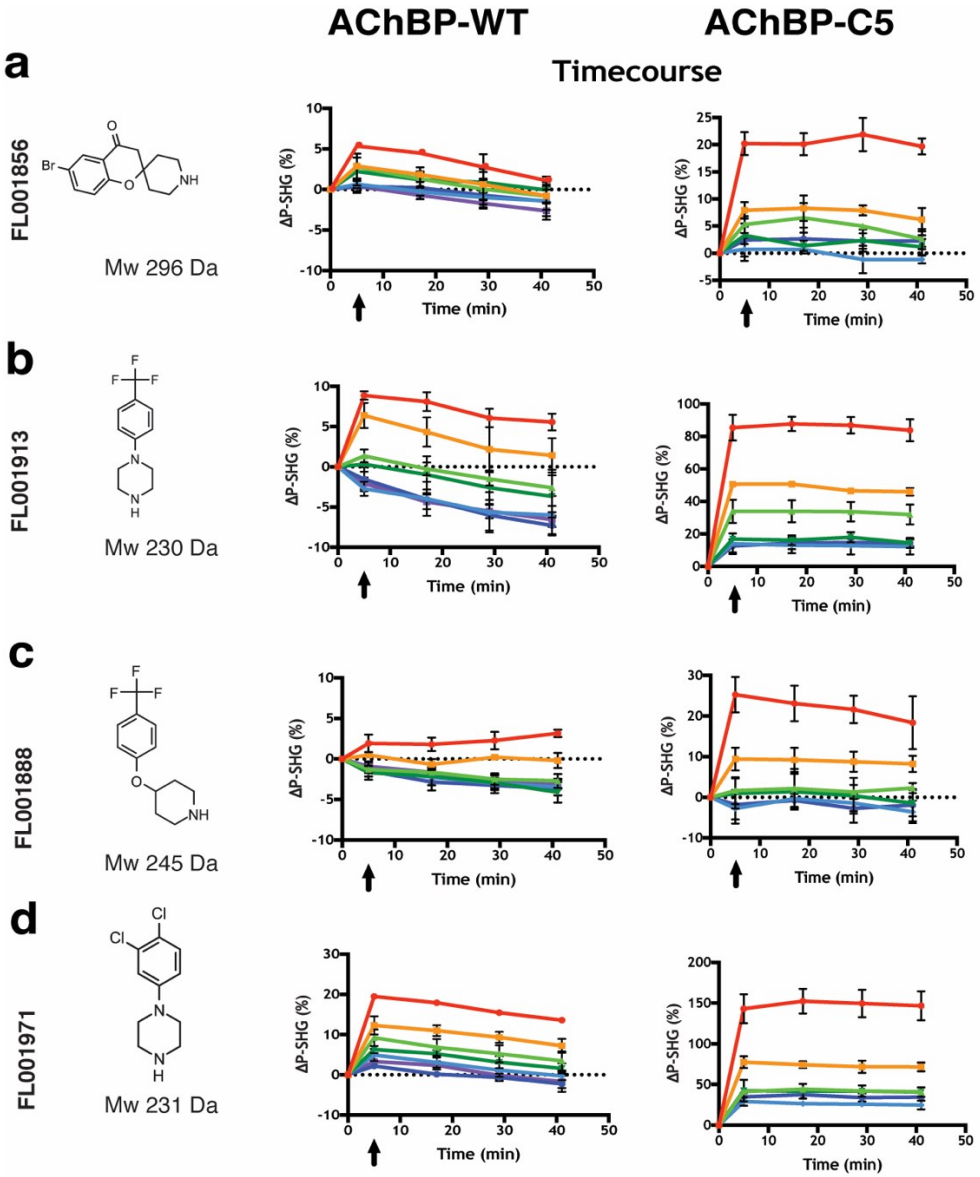

Figure S2. *Fragments which overlapped between WT and C5 assays showing time courses reaching steady state shown at a highest concentration of 250 µM in a two-fold concentration series in rows; (a) FL001856 (b) FL001913 (c) FL001888 (d) FL001971*

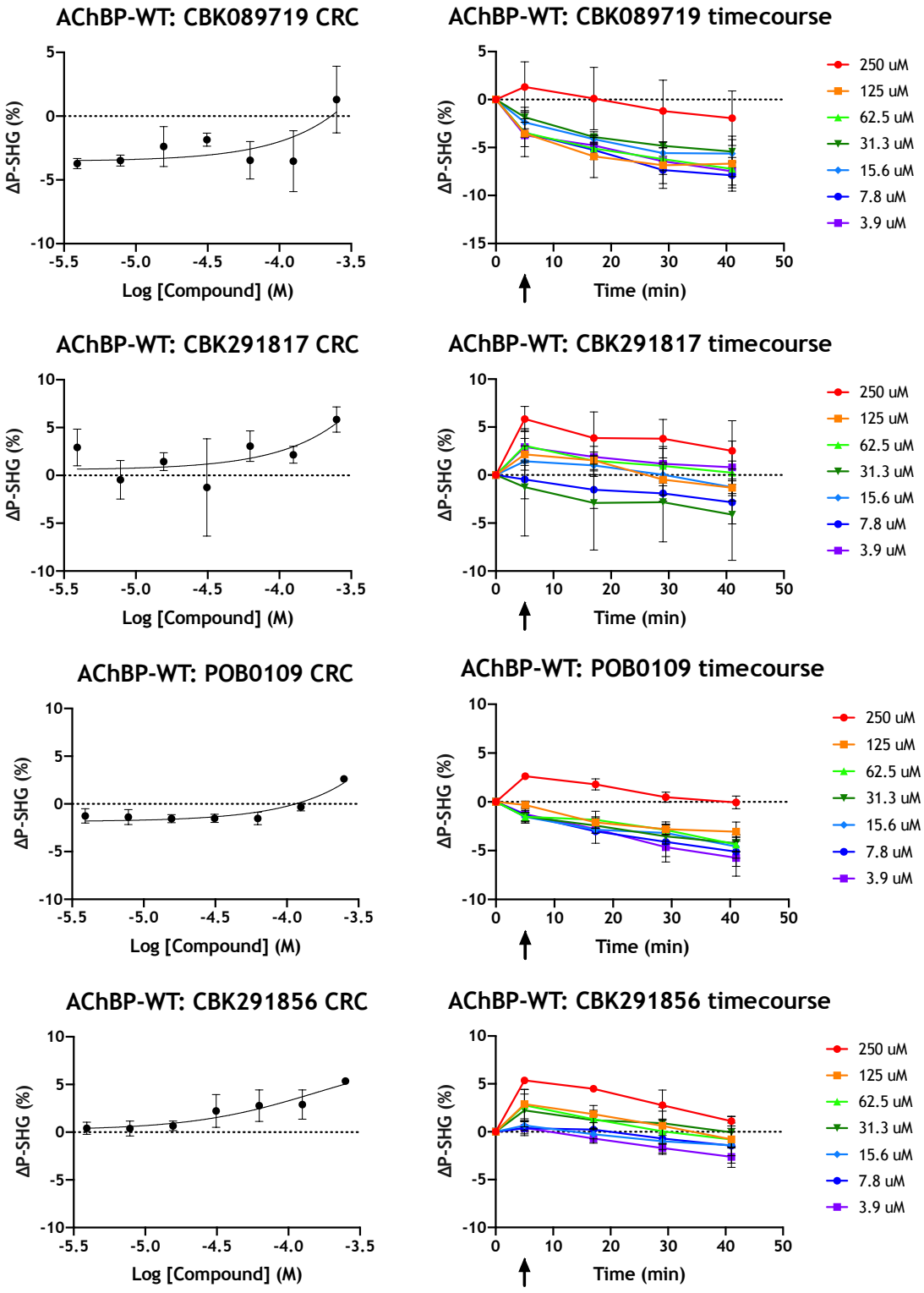

Figure S3. *Example data-set for non-selected hits in the wild-type assay.*

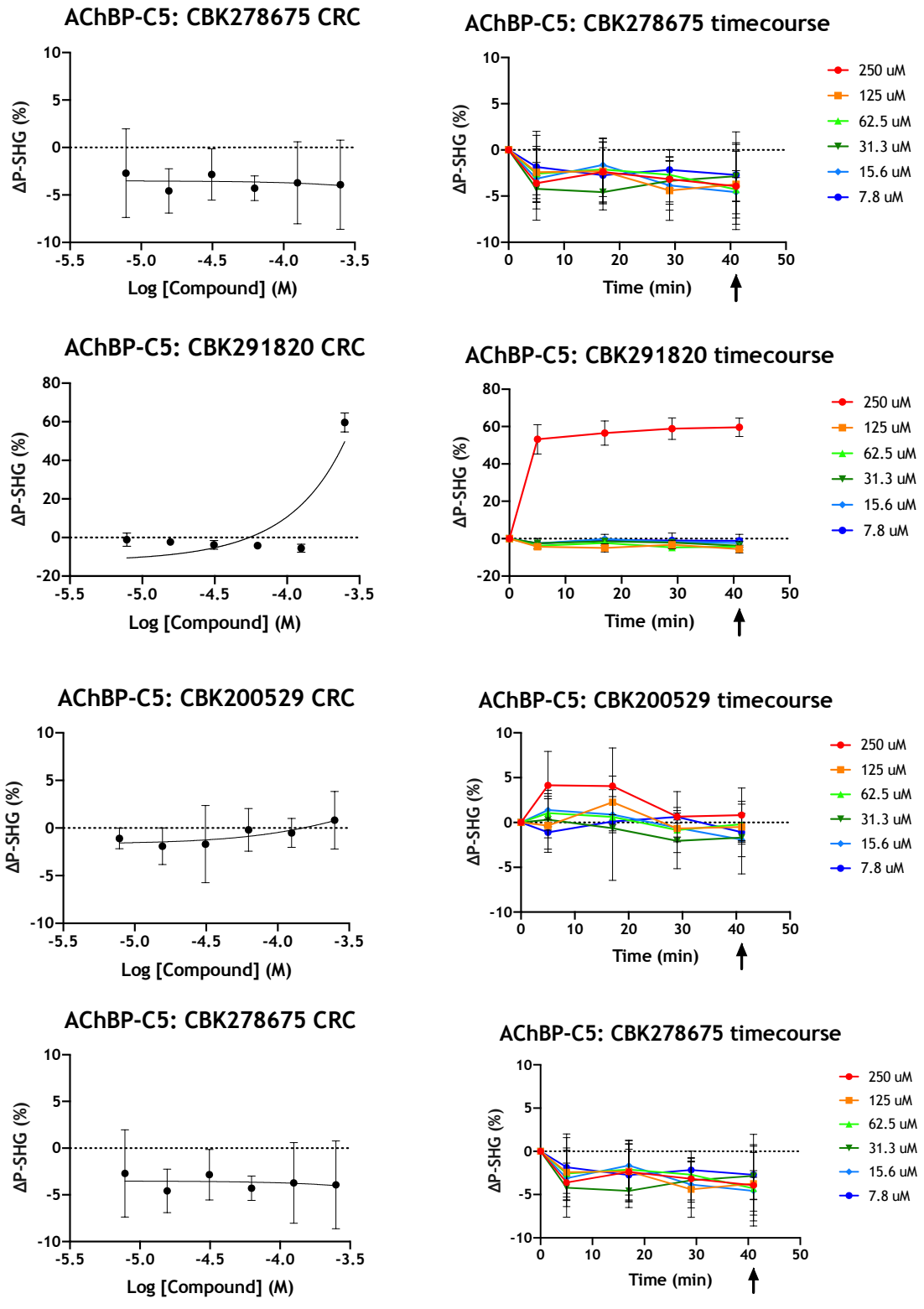

Figure S4. *Example data-set for non-selected hits in the C5 assay.*

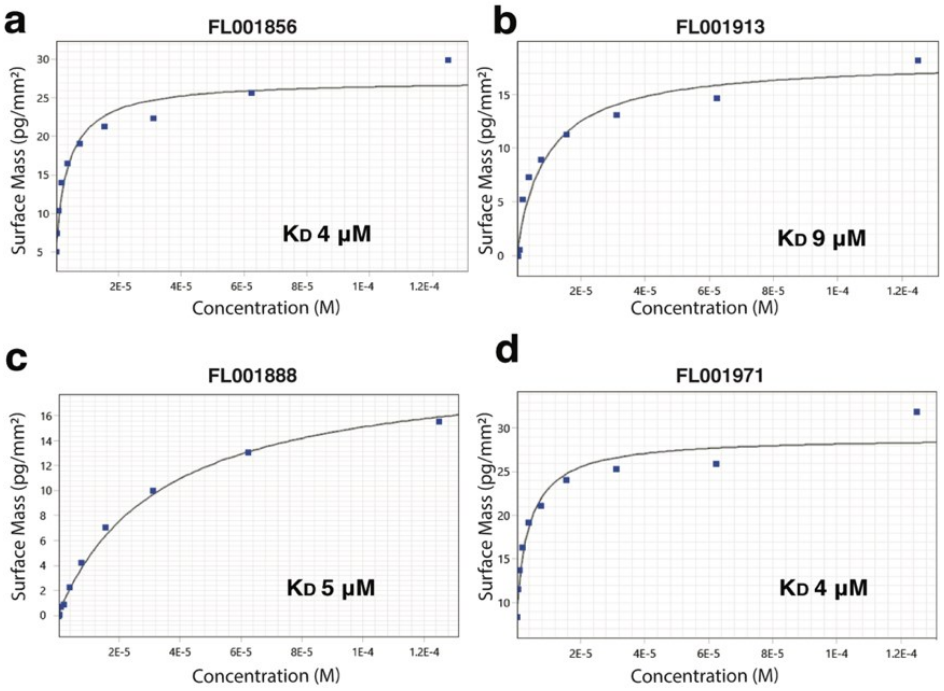

Figure S5. *Steady state analysis of data in Figure 6 and estimation of KD values by fitting a reversible 1:1 interaction model to 10-point dose–response curves (up to 125 µM) generated by extracting report points at steady state for each concentration. This is a less reliable procedure than the global regression analysis shown in Figure 6, considering that a more limited data set used*

Table S2: *Data collection and refinement statistics.*

Values given in parentheses are for the highest resolution shell.

	AChBP + 1888	**AChBP + 1856**
Data collection		
Beamline	BioMAX	BioMAX
Space group	$P2_12_12_1$	$P2_12_12_1$
Wavelength (Å)	0.9762	0.9762
a, b, c (Å)	76.7, 121.3, 239.3	76.3, 121.0, 242.1
Resolution range (Å)	50.0-1.7 (1.8-1.7)	50.0-2.0 (2.1-2.0)
Unique reflections	244709 (38136)	150840 (20197)
Multiplicity	13.7 (13.7)	13.7 (13.6)
Completeness (%)	100.0 (100.0)	51.3 (99.1)
R_{meas} (%)	9.5 (304.5)	10.7 (220.6)
Mean (I)/σ(I)	14.0 (0.9)	12.8 (1.3)
CC(1/2)	0.999 (0.413)	0.999 (0.529)
Wilson B-factor (Å^2)	42.2	55.3
Refinement		
Resolution (Å)	48.27-1.70	47.53-2.00
R-factor	0.2059	0.2125
R_{free}	0.2356	0.2432
No. non-hydrogen atoms/ average B-factor (Å^2)		
all	17804/42.6	16961/57.5
H_2O	1127/44.5	490/52.4
Ligand (1888 resp. 1856)	153/50.0	136/86.6
r.m.s.d.		
bond lengths (Å)	0.0095	0.0084
bond angles (°)	1.61	1.56
Ramachandran plot, no. residues in		
favored region	1991 (99.2%)	1971 (99.4%)
allowed region	15 (0.7%)	11 (0.6%)
outlier region	1 (0.05%)	1 (0.05%)
PDB accession code	7NDV	7NDP