Supporting Information

Fragment-based Exploration of Binding Site Flexibility in *Mycobacterium tuberculosis* BioA

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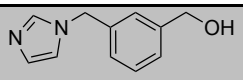
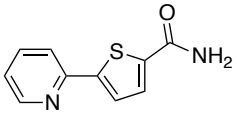
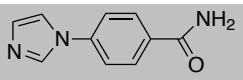
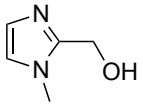
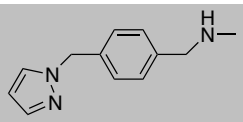
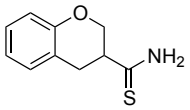
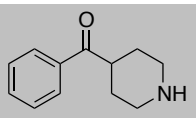
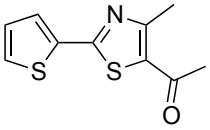
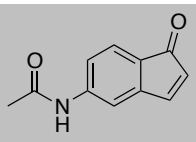
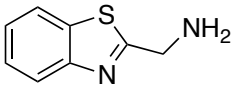
Table S1. Fragment hits from DSF

Fragment id	Maybridge Code	Structure	M. W. (Da)	T_m (°C)	ΔT_m shift (°C)
1	CC43209		188.23	90	+5
2	KM03152		204.25	90	+5
3	CC18528		187.20	89	+4
4	CC20809		112.13	89	+4
5	CC56046		201.27	89	+4
6	CC24118		193.27	88	+3
7	SEW04290		189.25	88	+3
8	SPB02598		223.31	88	+3
9	TL00757		189.21	88	+3
10	CC06013		164.23	78	-7

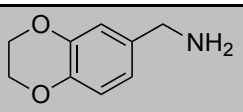
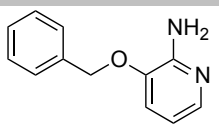
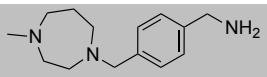
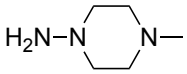
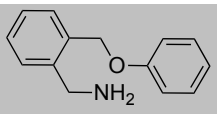
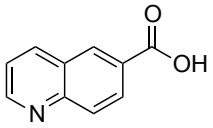
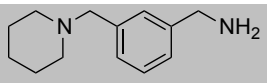
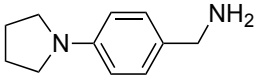
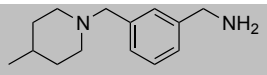
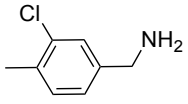
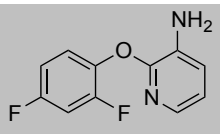
Fragment id	Maybridge Code	Structure	M. W. (Da)	T_m (°C)	ΔT_m shift (°C)
11	CC01313		165.19	76	-9
12	BTB08555		200.24	73	-12
13	CC55813		233.35	73	-12
14	AC10403		115.18	72	-13
15	CC58513		213.28	72	-13
16	CC04501		173.17	71	-14
17	MO01157		204.31	70	-15
18	CC30113		176.26	69	-16
19	MO01158		218.34	68	-17
20	TL00917		155.62	68	-17
21	BTB08015		222.19	65	-20

Table S2. Crystallographic statistics for BioA ligand complexes

Ligand	F2	F3	F5	F7	F9
PDB code	4wya	4wyc	4wyd	4wye	4wyf
X-Ray Source	MicroMax HighFlux	APS 17-ID	APS 17-ID	APS 17-ID	APS 17-ID
Detector	Saturn 944+ CCD	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M
Wavelength (Å)	1.541	1.000	1.000	1.000	1.000
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
***a* (Å)**	62.65	63.29	63.10	62.69	63.09
***b* (Å)**	65.67	65.98	66.49	66.25	66.09
***c* (Å)**	201.34	204.00	204.65	202.33	203.19
β (°)	90.29	90	90	90	90
Protein chains per ASU	4	2	2	2	2
Resolution (Å)	29.9-2.50 (2.59-2.50)	204.0-1.70 (1.76-1.70)	102.3-1.35 (1.40-1.35)	202.3-1.75 (1.81-1.75)	101.6-2.25 (2.32-2.25)
R_{merge}	0.158 (0.238)	0.123 (0.460)	0.058 (0.429)	0.066 (0.342)	0.088 (0.171)
I/σ_I	4.1(2.0)	13.8(3.2)	18.7(4.1)	18.4(4.3)	15.4(10.3)
Completeness	87.7% (72.5%)	98.6% (94.4%)	99.0% (99.2%)	99.0% (99.2%)	98.5% (96.7%)
Multiplicity	2.0 (1.9)	6.4 (6.5)	6.4 (6.1)	6.0 (6.3)	4.9 (4.3)
Observations	101966	609249	1219705	516385	91412
Unique reflections	50058	94878	189301	85959	38797
Refinement					
Resolution (Å)	29.9-2.50	39.7-1.70	32.8-1.35	39.4-1.75	101.6-2.25
R_{work}	23.3	17.57	12.58	17.58	19.03
R_{free}	31.0	21.34	15.76	20.62	23.56
Atoms	12665	7539	7800	7296	6787
Water sites	49	779	936	546	206
Bound Ligands	2	1	2	2	1
PLP molecules	4	2	2	2	2
Other Solvent	0	0	2	0	0
Favored	89.5%	96.6%	97.25%	96.6%	95.2%
Allowed	8.6%	2.9%	2.1%	2.7%	2.8%
Disallowed	1.9%	0.6%	0.7%	0.8%	2.0%
Bond dist. (Å)	0.010	0.007	0.006	0.007	0.009
Bond angles(°)	1.36	1.17	1.09	1.16	1.32

Table S2. Crystallographic statistics for BioA ligand complexes (continued)

Ligand	**F5.1**	**W1**
PDB code	4wyg	4xew
X-Ray Source	APS 17-ID	MicroMax HighFlux
Detector	PILATUS 6M	Saturn 944+ CCD
Wavelength (Å)	1.000	1.541
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell axis lengths		
***a* (Å)**	63.12	62.76
***b* (Å)**	66.43	66.15
***c* (Å)**	205.32	203.26
Protein chains per ASU	2	2
Resolution (Å)	205.3-1.62 (1.625-1.62)	101.6-2.47 (2.51-2.46)
R_{merge}	0.058 (0.190)	0.084 (0.083)
I/σ_I	22.3 (8.7)	12.3 (6.2)
Completeness	96.2% (94.7%)	94.08% (79.4%)
Multiplicity	6.8 (6.4)	2.9 (1.7)
Observations	725282	81740
Unique reflections	106327	28186
Refinement		
Resolution (Å)	46.40-1.62	101.6-2.47
R_{work}	0.152	0.165
R_{free}	0.180	0.220
Atoms	7951	6821
Water sites	879	212
Bound Ligands	2	2
PLP molecules	2	2
Other Solvent	11	3
Ramachandran Plot Characteristics		
Favored	96.6%	95.5%
Allowed	2.9%	3.4%
Disallowed	0.6%	1.0%
RMS deviations		
Bond dist. (Å)	0.007	0.010
Bond angles(°)	1.17	1.14

Table S3. Whole-cell Inhibition of *Mtb* Growth

Compounds	IC_{50} (μM)		
	BioA-UE	WT-biotin	WT+biotin
F2	39.2	249.3	>1000
F3	>1000	>1000	>1000
F5	>1000	>1000	>1000
F7	259.5	339.8	356.6
F9	>1000	>1000	>1000
F10	367.6	250.5	303.9

Figure S1.

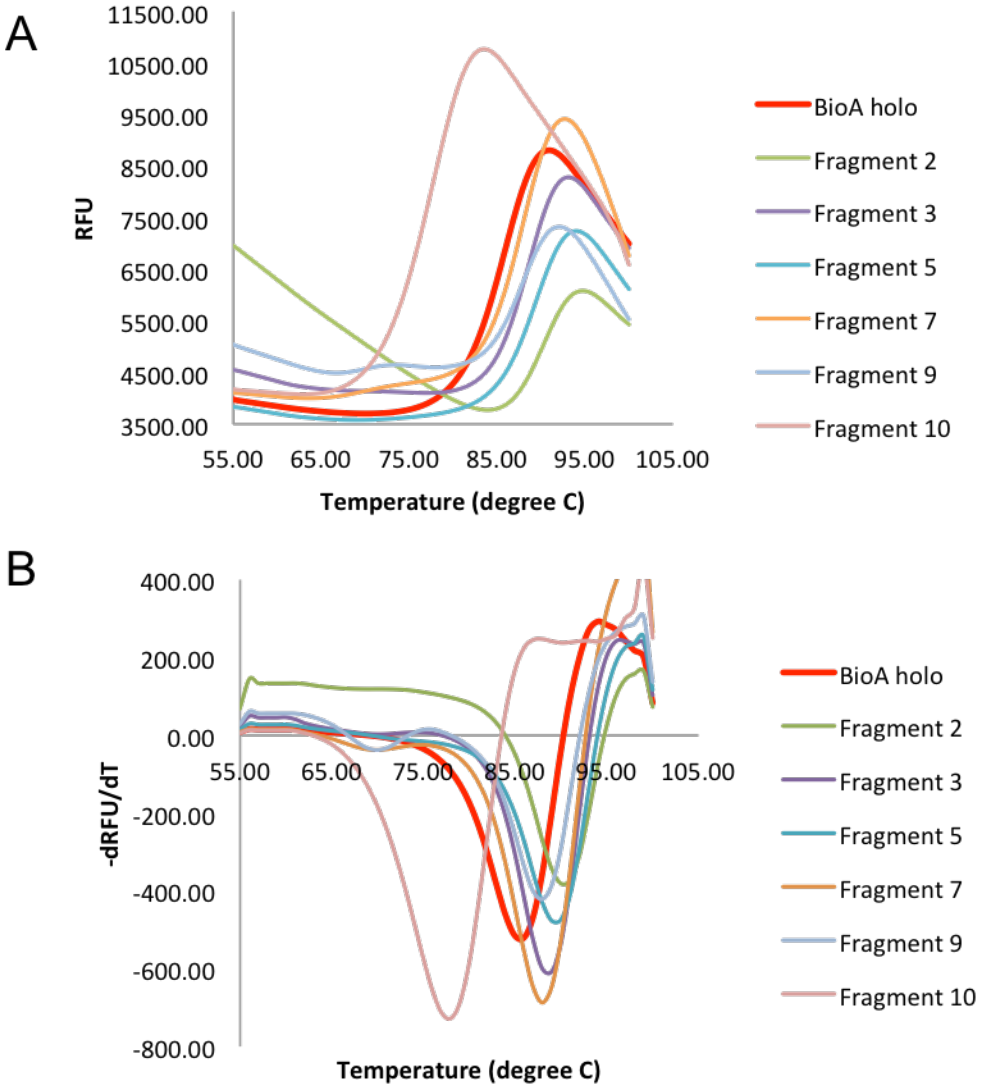


Figure S1. DSF results for F2, F3, F5, F7, F9, and F10. A) DSF melting curves from initial screening for crystallized fragment hits. **B**) First derivative curves from which the T_m for each compound is determined.

Figure S2.

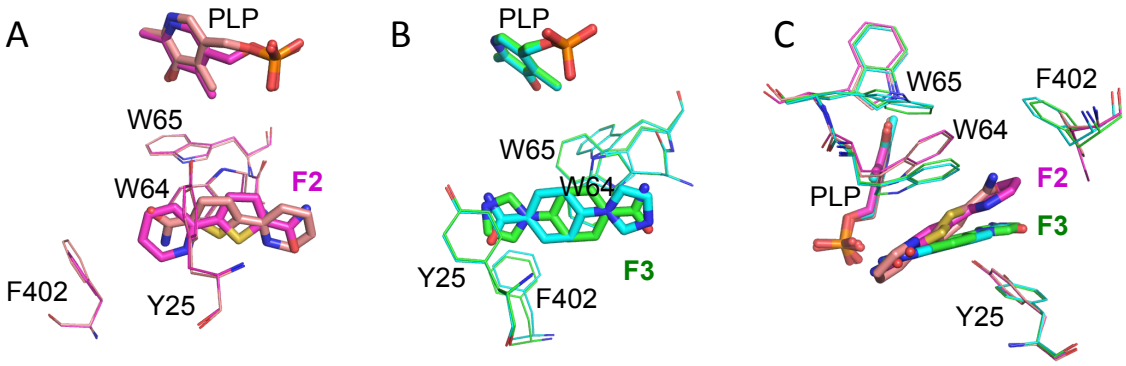


Figure S2. Comparison of alternate binding modes of F2 and F3. F2 and **F3** are modeled in different orientations in different binding sites in the asymmetric unit. A) Alternate F2 orientations are magenta (chain A) and salmon (chain C); B) Alternate F3 orientations are cyan (chain A) and green (chain B). Even with the flip in orientation, the ligands occupy the same general position and induce the same localized side chain conformational shifts (C).

Figure S3. ITC titration results for fragments **F2**, **F3**, **F5**, **F5.1**, **F7**, **F9**, and **F10** represented as a function of heat exchanged per injection on the following pages. The upper figures show the time dependence of the electric power (μcal/sec) to maintain constant temperature of the sample after each injection. The lower figures show the heat per mole of injectant vs. the molar ratio of the ligands and protein in the system. Each titration was repeated to three final inhibitor concentrations of 1.0, 1.5 and 2.0 mM.

Figure S3. (Continued)

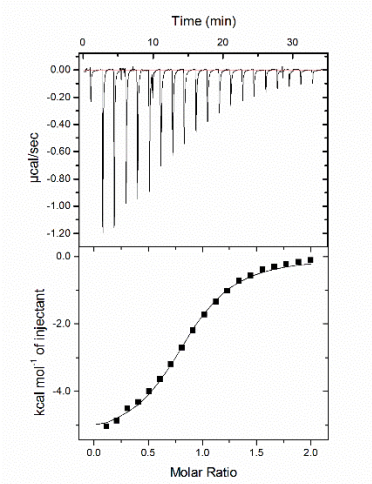
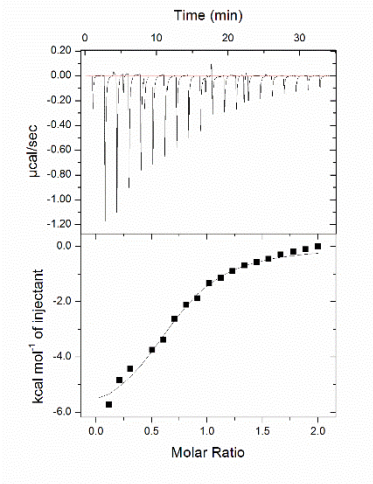
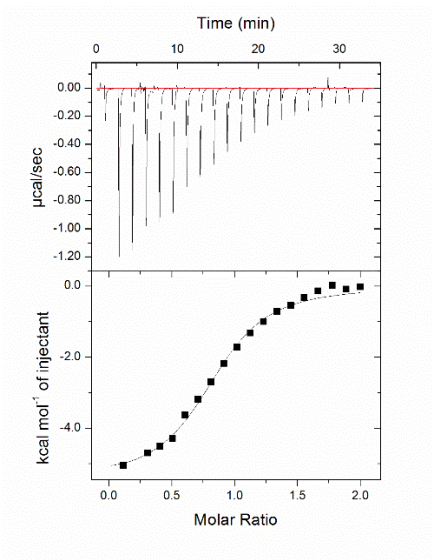
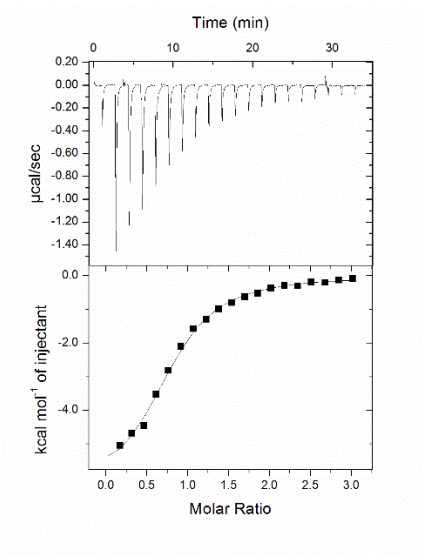
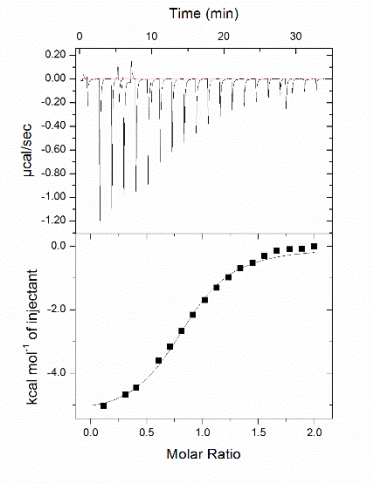
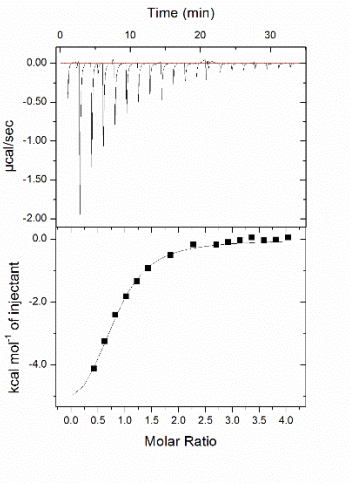
	F-2	F-3
1.0 µM		
1.5 µM		
2.0 µM		

Figure S3. (Continued)

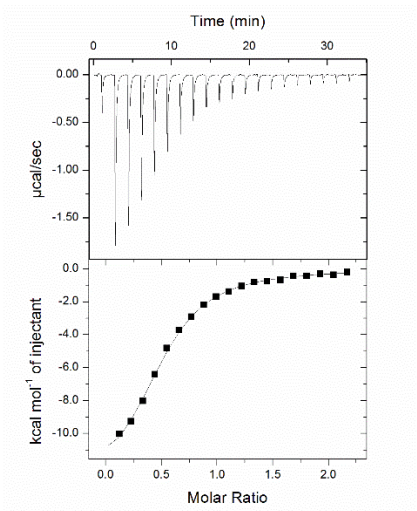
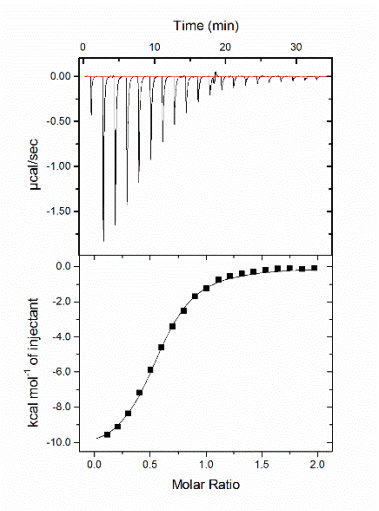
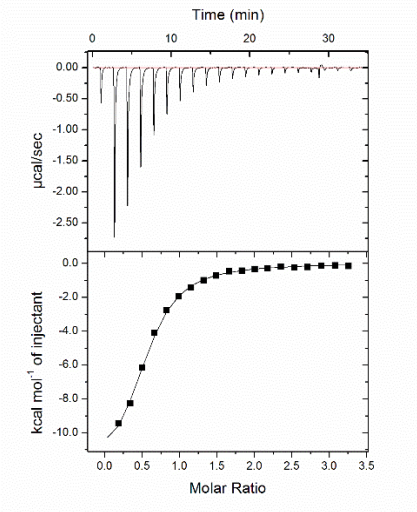
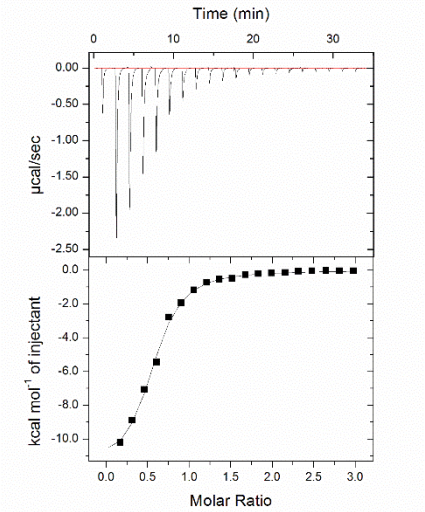
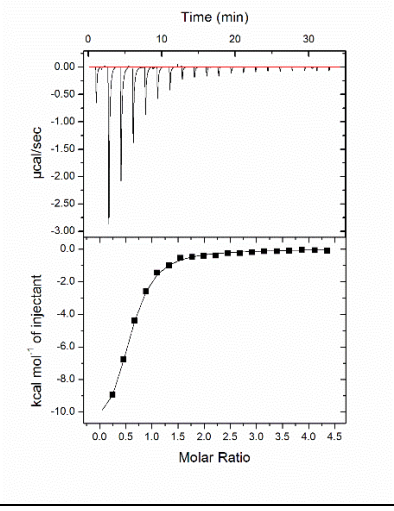
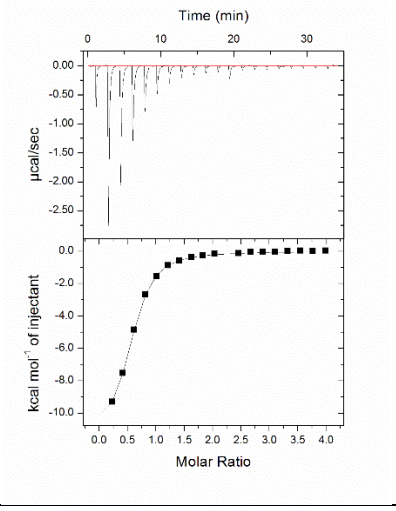
	F-5	F-5.1
1.0 µM		
1.5 µM		
2.0 µM		

Figure S3. (Continued)

	F-7	F-9

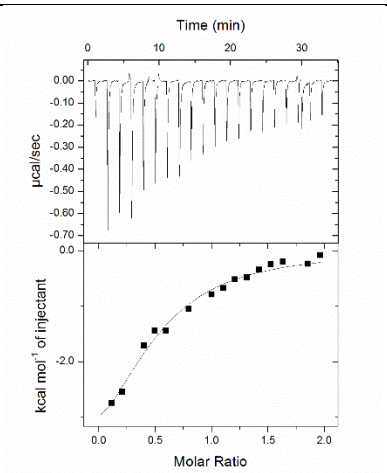
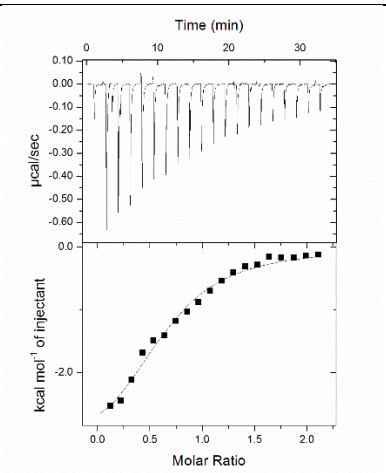
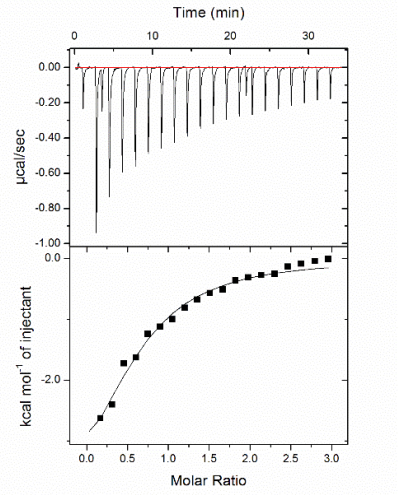
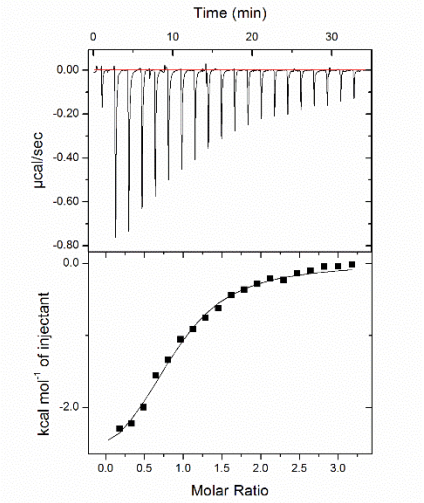
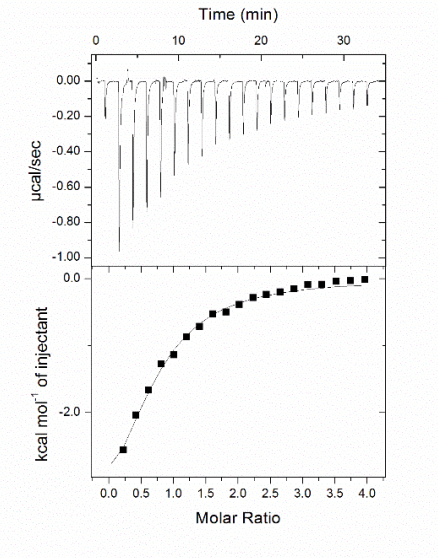
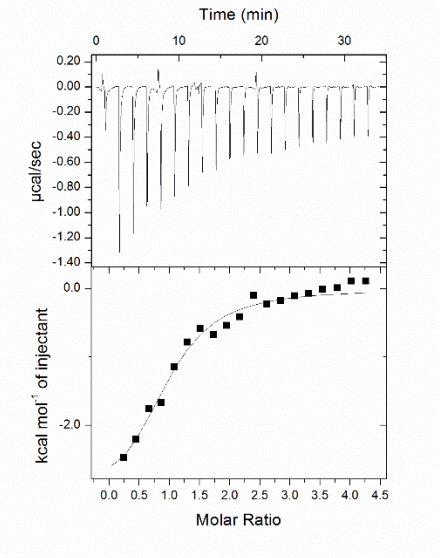
1.0 µM		
1.5 µM		
2.0 µM		

Figure S3. (Continued)

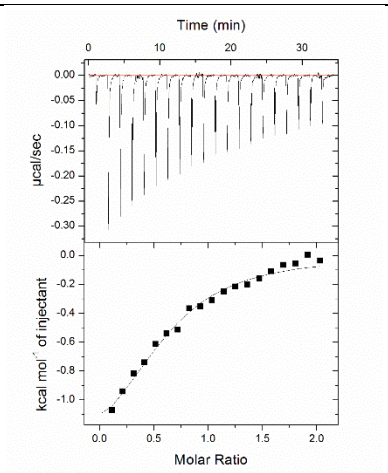
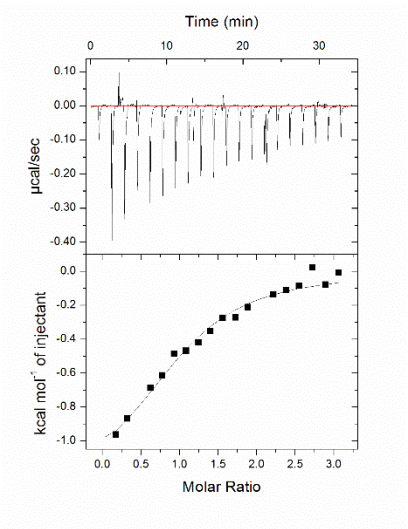
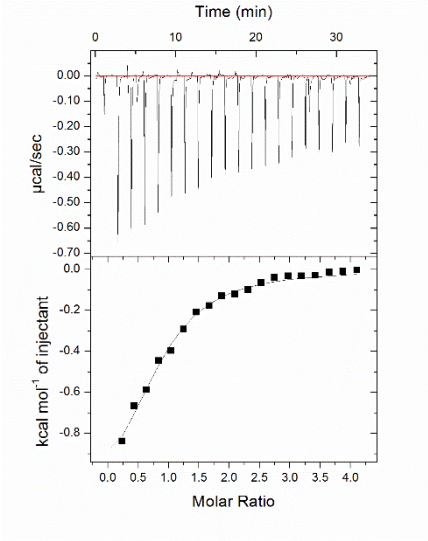
1.0 µM		
1.5 µM		
2.0 µM		

Figure S4.

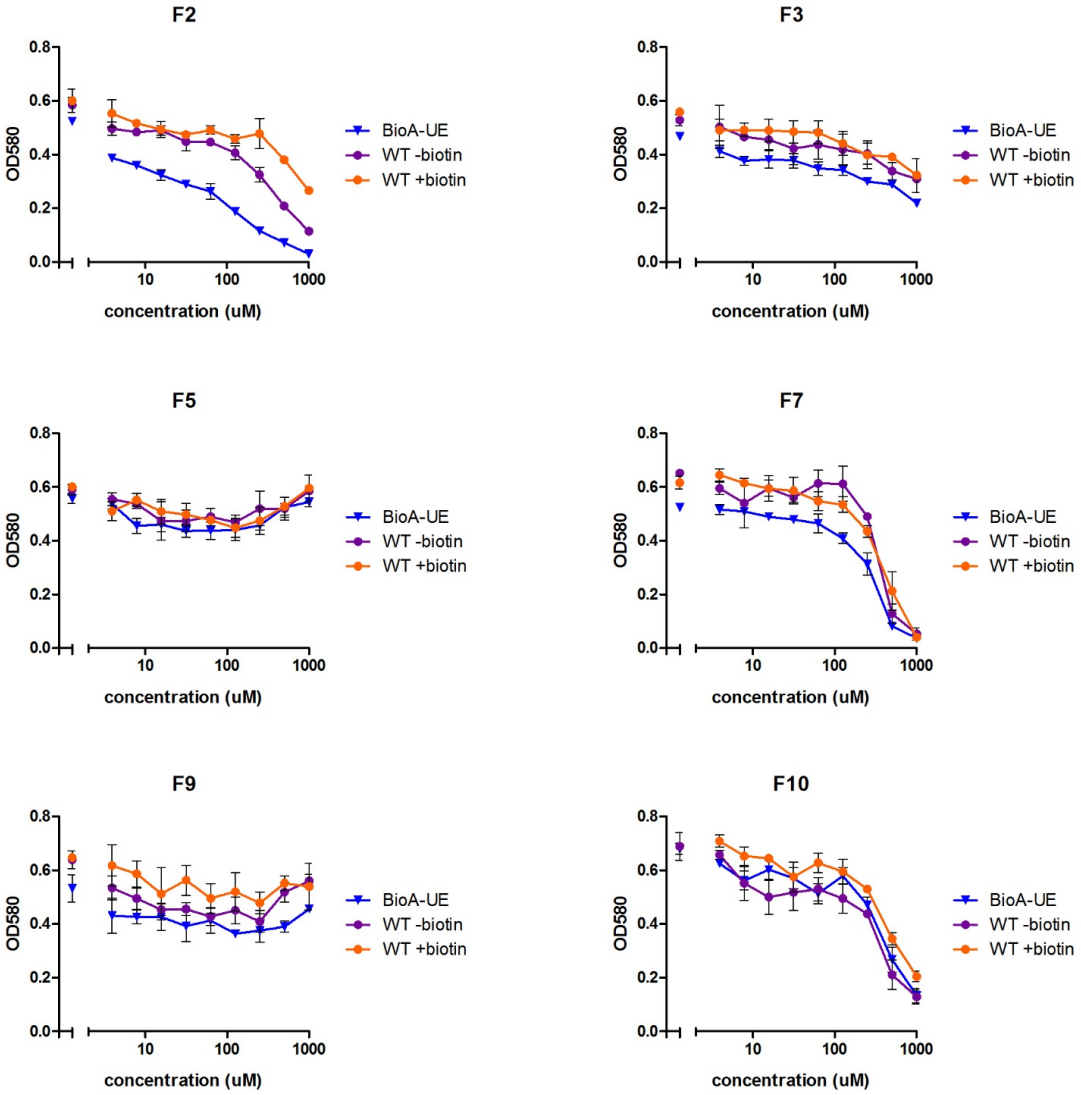


Figure S4. Whole cell growth dose response curves at 0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µM compound.

Figure S5.

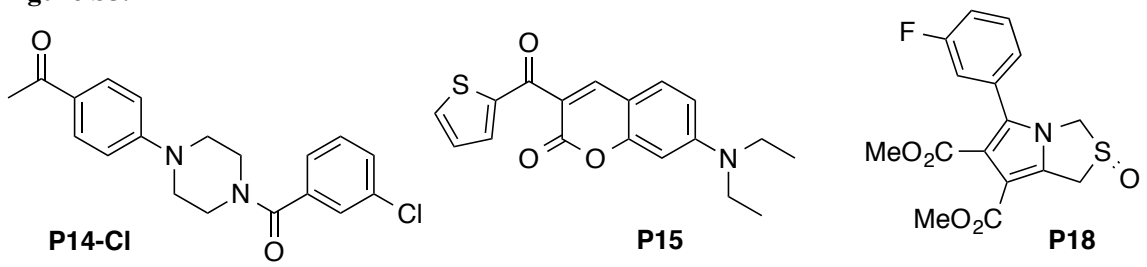


Figure S5. Non-covalent inhibitors of Mtb BioA with structurally characterized binding modes. **P14-Cl**, **P15**, and **P18** are compounds 14-Cl, 15 and 18 as described by Park *et al*.(*Chem Biol* **2015**, 22, pp76-87)