Mandeep Mann
2018/09/10

Screening Compounds against USP5 Zf-UBD with a ^{19}F NMR assay #2

Objective: to screen compound against USP5 zinc finger ubiquitin binding domain (Zf-UBD) using ^{19}F NMR spectroscopy. You can find previous ^{19}F NMR experiments here.

Experiment & Results:

500 µL of 40 µM USP5$^{171-290}$ and 1 mM compound solutions (1:25) were prepared in 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 5% D2O (v/v), 0.5% DMSO (v/v). ^{19}F measurements were taken with a Bruker 600 MHz NMR and IconNMR software, with the solvent: H_2O + D_2O. Raw data was processed using TopSpin (Bruker), LB=10, Pick Peaks and Integrate functions.

The control spectra of 40 µM USP5$^{171-290}$ showed two well resolved peaks of equal intensity at 118 and 125 ppm (Figure 1).

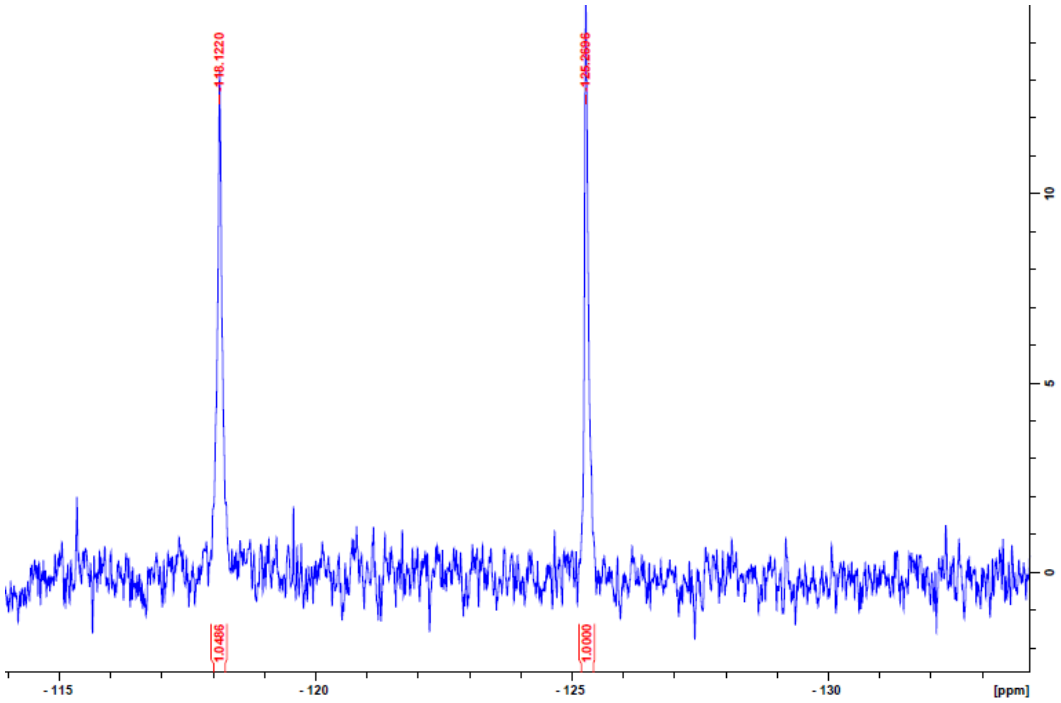


Figure 1. NMR spectra of control: 40 µM USP5$^{171-290}$

28 compounds were selected from the SGC database by substructure search for molecules that were similar to previous hits which contained an aromatic ring and a propanoate moiety (Table 1).

Mandeep Mann
2018/09/10

Table 1. Compound hits from previous ^{19}F NMR screen

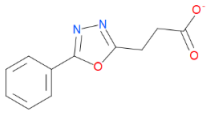
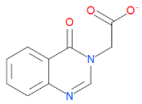
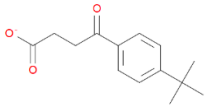
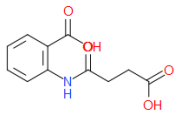
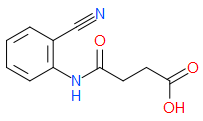
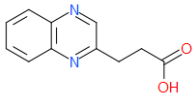
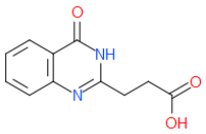
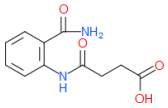
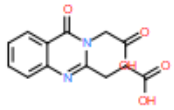
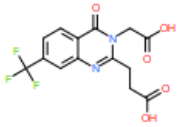
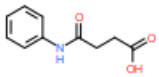
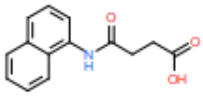
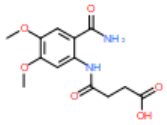
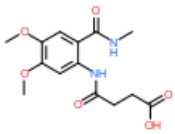
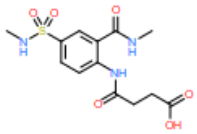
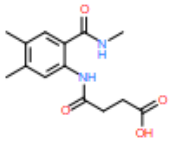
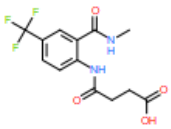
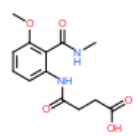
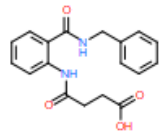
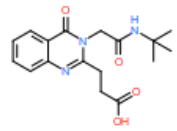
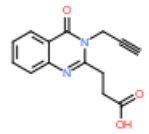
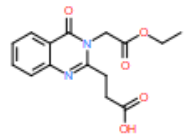
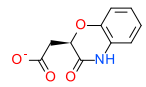
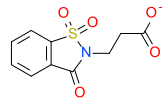
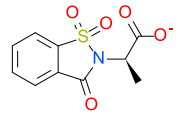
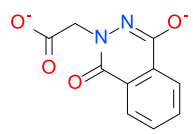
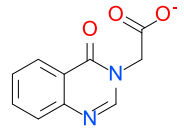
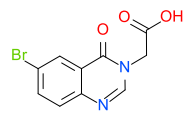
Compound Name	Compound Structure
DAT180	
DAT194	
DAT201	

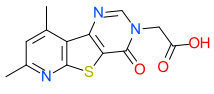
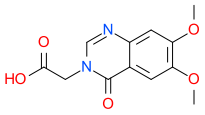
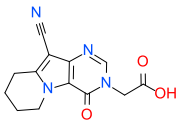
Table 2 summarizes the results of the NMR spectra for the compounds screened against USP5 Zf-UBD. Peak 1, corresponding to the tryptophan lining the ubiquitin binding pocket, was used as a relative reference for chemical shift and peak 2 was used a relative reference for integral calculation. Samples 9-29 have a large contaminating peak at approximately 119.5 ppm, due to the contamination in the lining of new NMR tubes that were used; however, changes in the resonance of the fluorine is still applicable (Figure 2). For this reason, the chemical shift and peak area could not be calculated for samples 18, 20, 24, 25, and 29 due to overlap with the contaminating peak. For these compounds, the spectra was visually analyzed and assigned as 'hit' or 'no hit'. Please see the attached zip file for the NMR spectra of the compounds.

Table 2. Summary of change in chemical shift and peak area of ^{19}F NMR spectra

Sample	**Compound Name**	**Compound Structure**	**δ (ppm)**	**Δδ (ppm) [rel to control peak 1]**	**Integral [rel to peak 2]**	**Hit (Y/N)**
1	Control		118.12	0.00	1.05	
2	UBXML54		118.31	0.19	0.99	N
3	UBXML55		118.25	0.13	0.98	N
4	UBXML56		118.68	0.56	0.95	N

5	UBXML57		118.36	0.24	0.98	N
6	UBXML58		118.43	0.31	1.08	N
7	UBXML70		120.05	1.93	0.74	Y
8	UBXML78		120.12	2.00	0.89	Y
9	UBXML84		118.28	0.16	1.13	N
10	UBXML85		118.47	0.35	1.10	N
11	UBXML86		118.80	0.68	1.01	Y
12	UBXML87		118.56	0.44	0.79	Y
13	UBXML88		N/A	N/A	N/A	Y
14	UBXML89		118.51	0.39	0.84	Y
15	UBXML90		118.57	0.45	0.52	Y

16	UBXML91		118.16	0.04	1.02	N
17	UBXML92		118.36	0.24	0.97	N
18	UBXML93		N/A	N/A	N/A	Y
19	UBXML94		119.90	1.78	0.62	Y
20	UBXML95		N/A	N/A	N/A	Y
21	DAT8		118.28	0.16	1.05	N
22	DAT9		118.21	0.09	0.99	N
23	DAT11		118.50	0.38	1.10	N
24	DAT19		N/A	N/A	N/A	Y
25	DAT22		N/A	N/A	N/A	Y
26	DAT53		119.41	1.29	0.69	Y

27	DAT61		119.41	1.29	0.64	Y
28	DAT76		119.73	1.61	0.36	Y
29	DAT80		N/A	N/A	N/A	Y

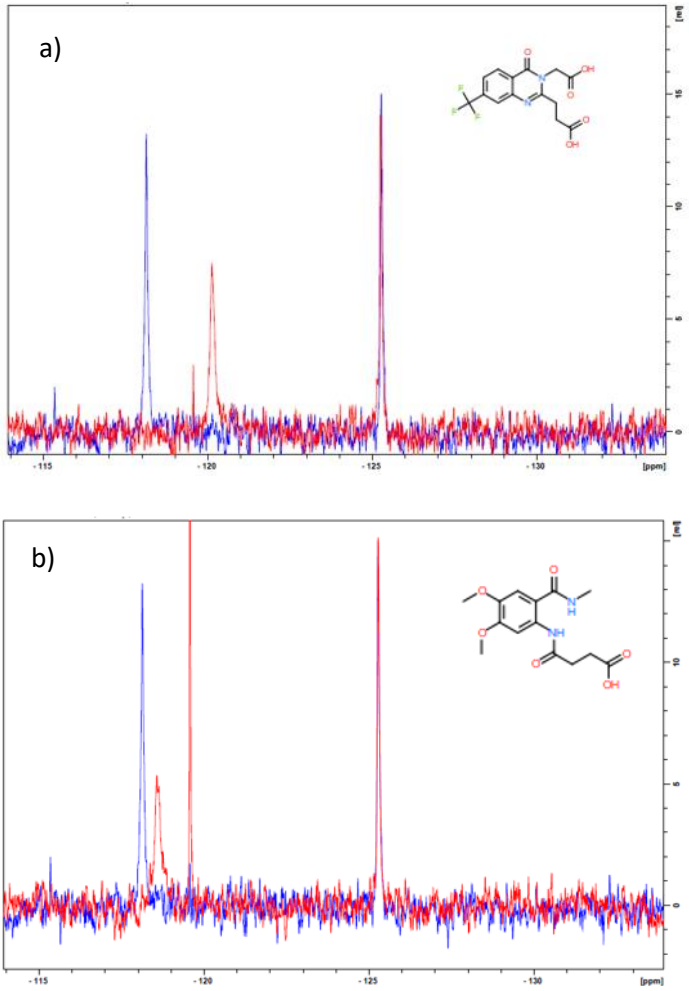


Figure 2. a) overlay of ^{19}F NMR control spectra 40 µM USP5$^{171-290}$ (blue) and 40 µM USP5$^{171-290}$ and 1 mM UBXML78 (red) b) overlay of ^{19}F NMR control spectra 40 µM USP5$^{171-290}$ (blue) and 40 µM USP5$^{171-290}$ and 1 mM UBXML87 (red); contaminating peak at 119.5 ppm

Mandeep Mann
2018/09/10

Conclusions & Future Directions:

Of the 28 compounds tested, 16 compounds showed a significant decrease in peak 1 area and a chemical shift to the right in the 19F NMR spectra (Table 1). The high hit rate can be attributed to the high compounds concentration in the NMR assay. As this assay is more qualitative than quantitative, next, I will be using a SPR assay to determine the binding affinity of the hits from the 19F NMR screen. I will also see if I can decrease compound concentration in future 19F NMR screens.