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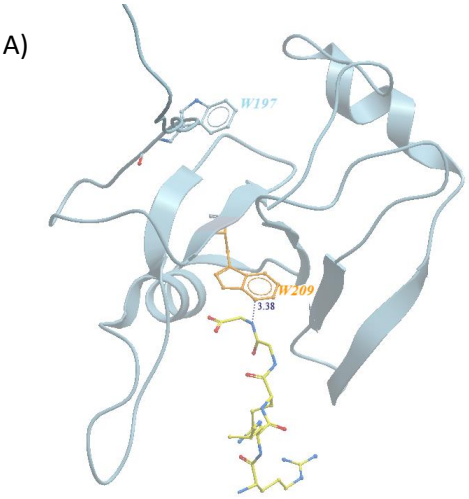
Compounds of interest identified by screening a focused library against USP5 Zf-UBD with a 19F NMR assay

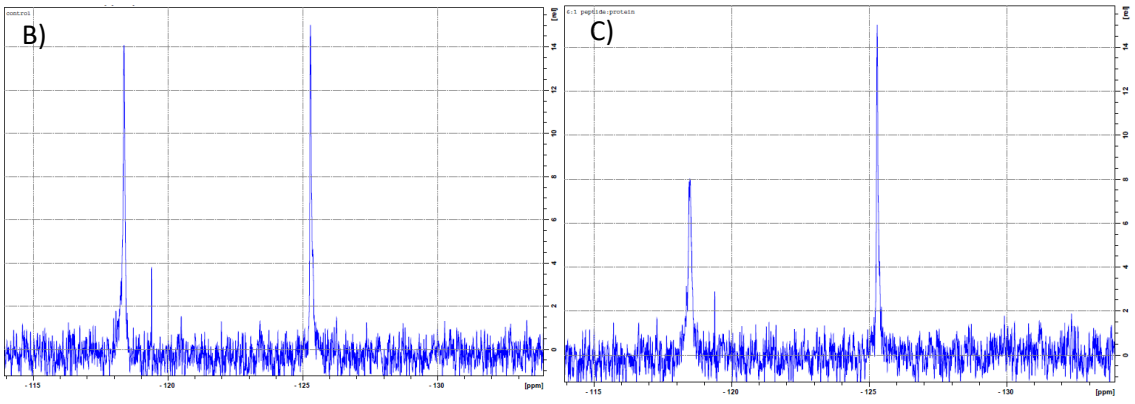
Objective: To screen a set of commercial compounds selected from computational docking studies against USP5 zinc finger ubiquitin binding domain (Zf-UBD) using ^{19}F NMR spectroscopy. You can find details of preliminary ^{19}F NMR experiments here.

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 2 well-resolved peaks of equal intensity with peak 1 at 118.2 and peak 2 at 125.3 ppm. In my previous post, we could see that the intensity of peak 1 was significantly reduced in the presence of a ubiquitin peptide, while peak 2 was unaffected, strongly suggesting that peak 1 corresponds to the tryptophan W209 at the binding pocket, while peak 2 corresponds to W197, at a distant site (Figure 1A-C).





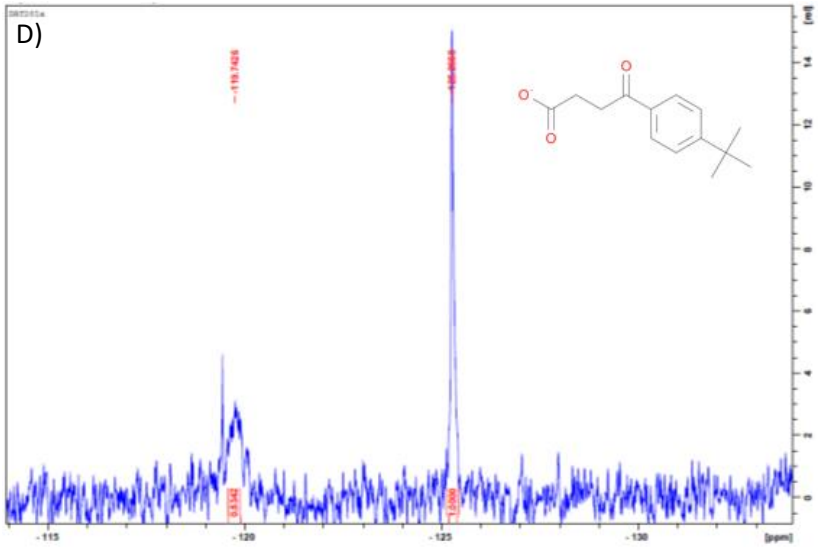
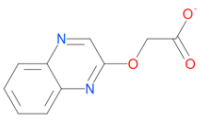


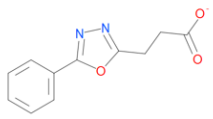
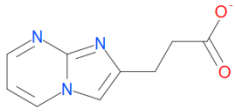
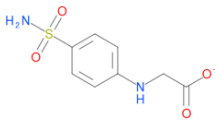
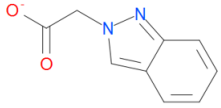
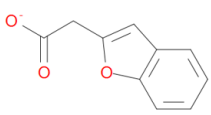
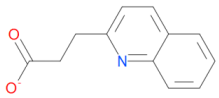
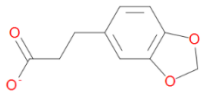
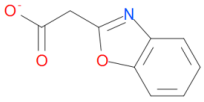
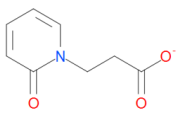
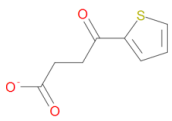
Figure 1. a) Ribbon representation of tryptophan residues of USP5 Zf-UBD (grey) and ubiquitin (yellow) b) NMR spectra of control USP5$^{171-290}$ c) NMR spectra of 1:6 USP5$^{171-290}$: LRLRGG ubiquitin peptide d) NMR spectra of 1:25 USP5$^{171-290}$:DAT201

33 compounds selected by virtual screening by Ivan Franzoni and Renato de Freitas, post-docs at the Unviersity of Toronto and SGC respectively, were screened against USP5 Zf-UBD using ^{19}F NMR. Twelve of the 33 compounds screened generated a shift and decrease in intensity of peak 1! Peak 2 remained unaffected. Figure 2D shows a representative response. Table 1 summarizes the results of the NMR spectra for the screened compounds against USP5 Zf-UBD. Peak 1 was used a relative reference for chemical shift and peak 2 was used as a relative reference for integral calculation, as there is no chemical shift, or significant change in peak area with peak 2. Please see the attached zip file for the NMR spectra of all the compounds, detailing peak chemical shift, intensity and integral data. The spectra can be viewed using TopSpin 3.6.0.

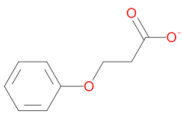
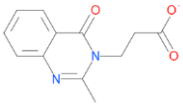
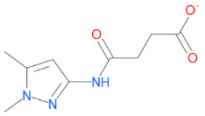
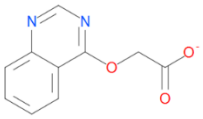
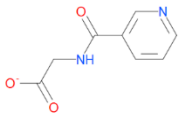
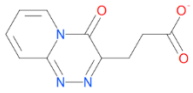
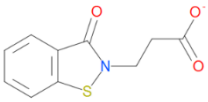
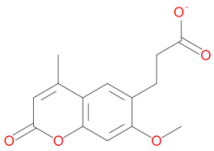
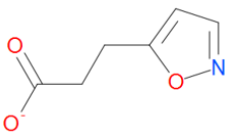
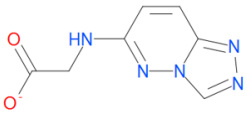
Table 1. Summary of change in chemical shift and peak area of ^{19}F NMR spectra

Compound Name	**Compound Structure**	**δ (ppm)**	**Δδ (ppm) [rel to control peak 1]**	**Integral [rel to peak 2]**
Control		118.20	0.00	1.16
DAT179		118.32	0.12	1.15

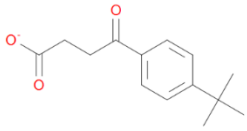
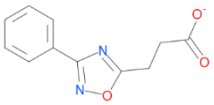
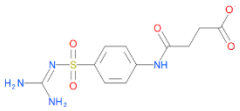
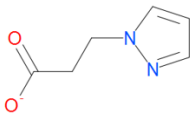
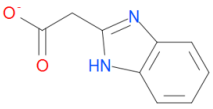
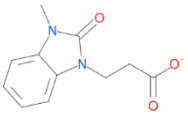
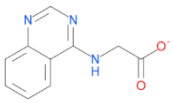
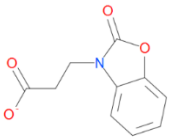
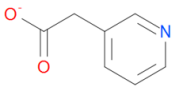
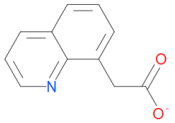
Mandeep Mann
2018/06/05

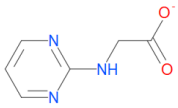
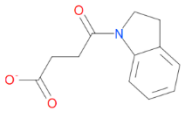
DAT180		119.33	1.13	0.88
DAT181		118.31	0.11	1.05
DAT182		118.40	0.20	1.17
DAT183		118.99	0.79	1.03
DAT184		118.57	0.37	1.05
DAT185		118.28	0.08	1.10
DAT186		118.32	0.12	1.16
DAT187		118.83	0.63	0.87
DAT188		118.28	0.08	1.35
DAT190		119.67	1.47	0.86

Mandeep Mann
2018/06/05

DAT191		118.59	0.39	1.15
DAT192		118.31	0.11	1.11
DAT193		118.32	0.12	1.13
DAT194		119.42	1.22	0.65
DAT195		118.36	0.16	1.16
DAT196		119.42	1.21	0.65
DAT197		118.24	0.04	1.01
DAT198		119.41	1.21	0.12
DAT199		118.32	0.12	1.12
DAT200		118.36	0.16	1.17

Mandeep Mann
2018/06/05

DAT201		119.74	1.54	0.53
DAT202		118.67	0.47	0.87
DAT203		118.84	0.64	0.91
DAT204		118.24	0.04	1.19
DAT205		118.25	0.05	1.25
DAT206		118.29	0.09	1.22
DAT207		118.36	0.16	1.11
DAT208		118.80	0.60	0.89
DAT209		118.33	0.13	1.10
DAT210		118.34	0.14	1.09

DAT211		118.40	0.20	1.19
DAT212		119.42	1.22	0.37

Conclusions & Future Directions:

The ^{19}F NMR spectra of the screened compounds were evaluated based on a) chemical shift of peak 1 and b) change in resonance area of peak 1 normalized over peak 2. In general, the compounds that resulted in the largest chemical shift to the right from the control fluorine resonance also had the greatest decrease in peak area relative to peak 2. Of the 33 compounds screened, 12 of the compounds showed significant changes in peak 1 fluorine resonance. 7 of these 12 compounds had the largest change in chemical shifts and change in peak area relative to the controls: DAT180, DAT190, DAT194, DAT196, DAT198, DAT201 and DAT212, indicated in red in Table 1. The high "hit" rate is likely due to the high compound concentration, the sensitivity of the ^{19}F NMR assay and the biased pool of compounds that were predicted to be favorable for USP5 binding. The purity of these compounds of interest was >95% as measured by UPLC-MS. Please see attached UPLC-MS reports for the best hit compounds.

Overall, ^{19}F NMR gives insight into structural perturbations of the USP5 binding pocket when small molecules bind. I will have to use a secondary assay to confirm these compounds of interest and a more quantitative method to determine binding affinities, such as a surface plasmon resonance assay (SPR). Once I have a better understanding of the K_d of these compounds, I can prioritize the best binding compounds for crystallization studies, from which I can then guide molecular designs of the ligands to increase binding affinity.