Supporting Information

Paramagnetic relaxation enhancement for protein-observed ^{19}F NMR as an enabling approach for efficient fragment screening

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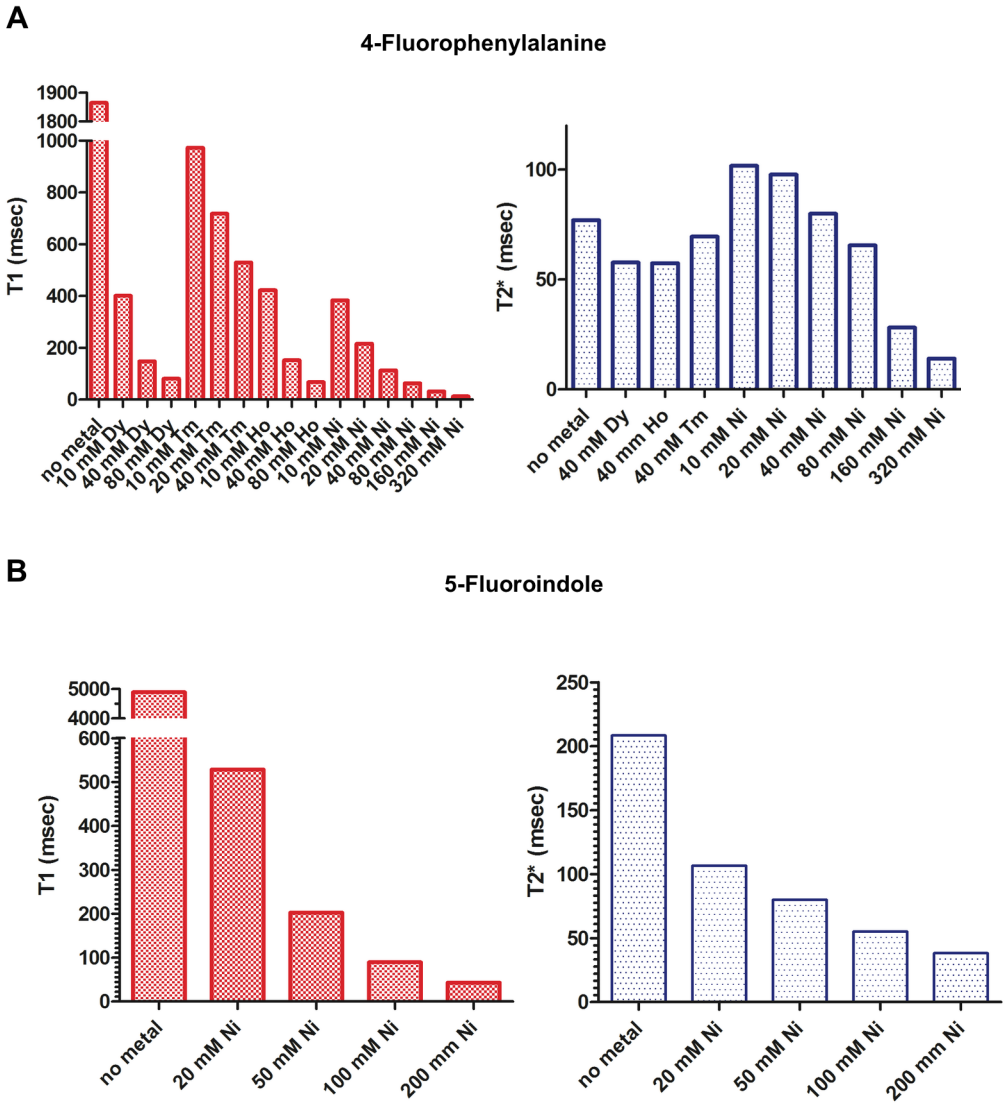


Figure S1. A. T1 and T2* measurements on 4-fluorophenylalanine upon the addition of Dy(III), Ho(III), Ni(II), and Tm(III) as EDTA chelates. All metals cause decreases in T1, but Ni(II) causes the largest and most rapid decreases for increasing Ni(II) concentrations. B. T1 and T2* measurements on 5-fluoroindole with increasing concentrations of Ni-DTPA.

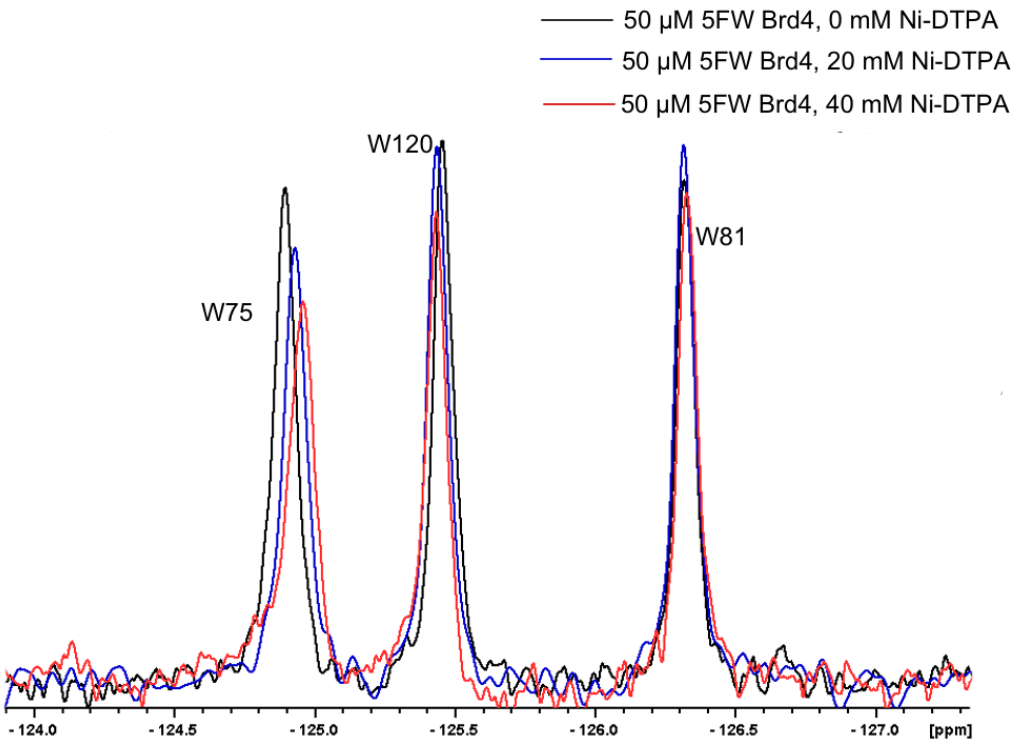
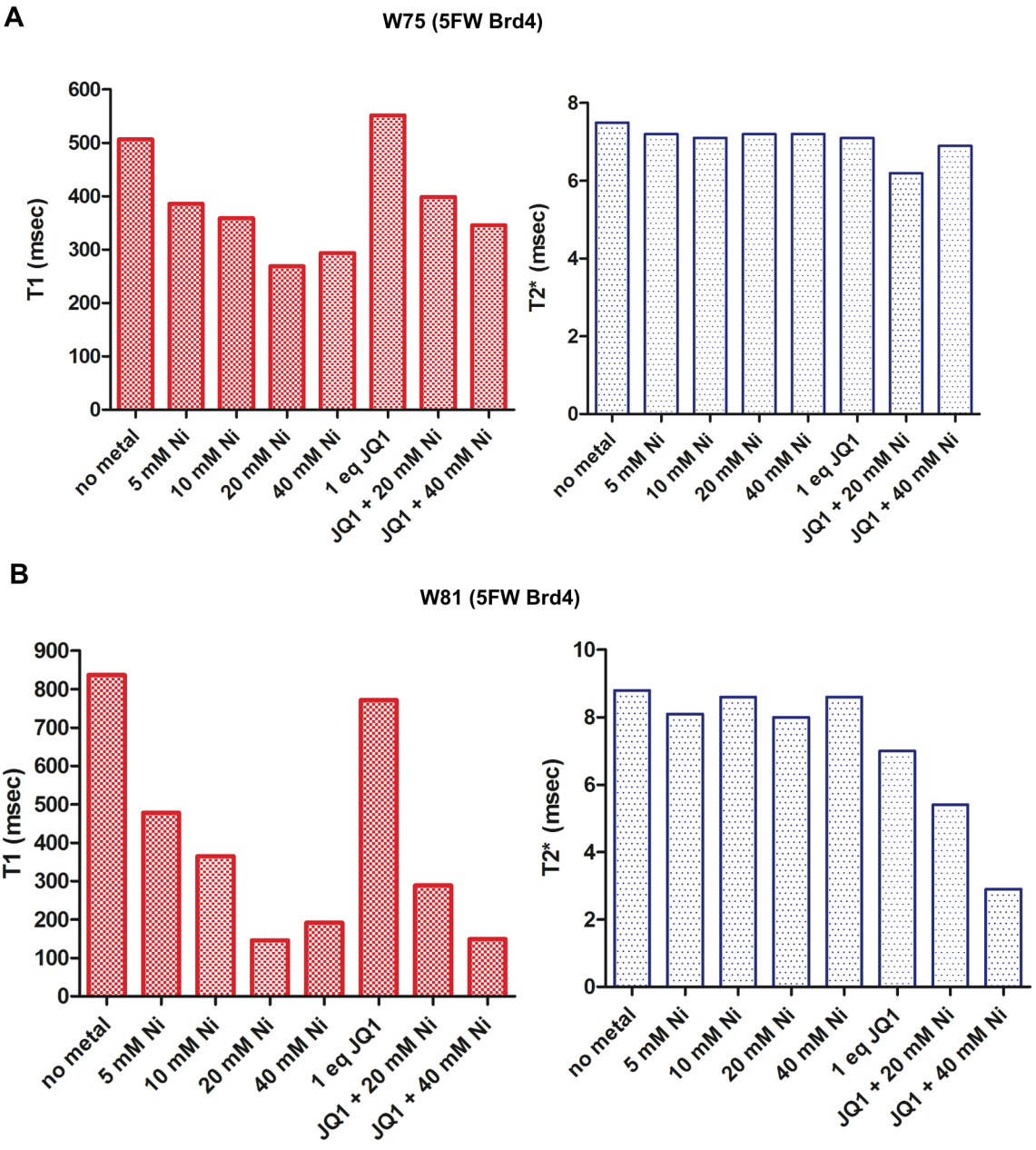


Figure S2. The linewidths of 5FW Brd4 are unchanged upon titration of Ni-DTPA. All spectra were acquired with 1000 scans and a 1 second recycle delay.


A
W75 (5FW Brd4)
T1 (msec)
600
500
400
300
200
100
0
T2* (msec)
8
6
4
2
0
no metal
5 mM Ni
10 mM Ni
20 mM Ni
40 mM Ni
1 eq JQ1
JQ1 + 20 mM Ni
JQ1 + 40 mM Ni
B
W81 (5FW Brd4)
T1 (msec)
900
800
700
600
500
400
300
200
100
0
T2* (msec)
10
8
6
4
2
0
no metal
5 mM Ni
10 mM Ni
20 mM Ni
40 mM Ni
1 eq JQ1
JQ1 + 20 mM Ni
JQ1 + 40 mM Ni

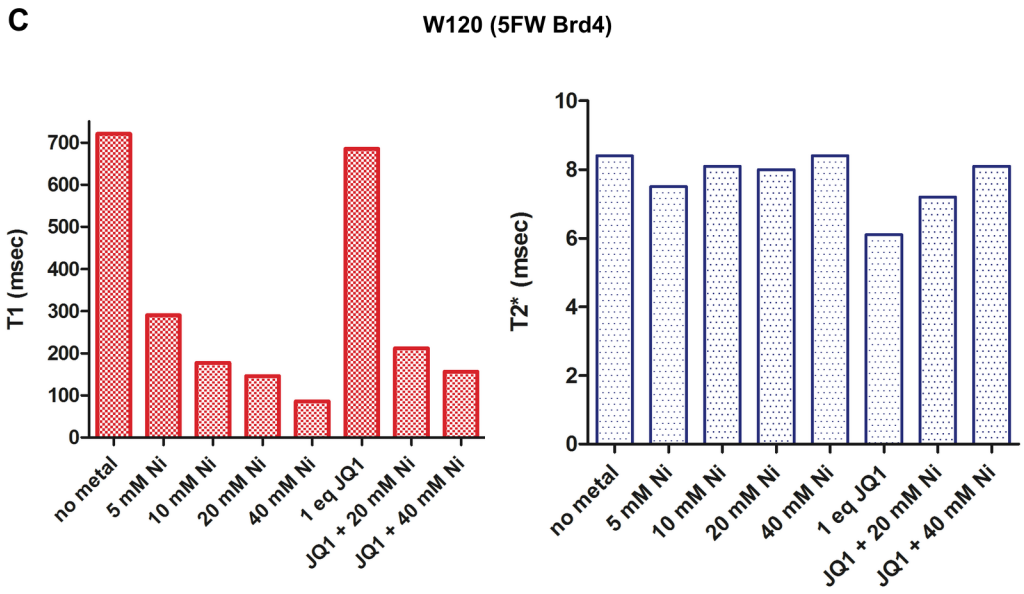


Figure S3. T1 and T2* measurements for ^{19}F resonances 5FW labeled residues in Brd4 at increasing concentrations of Ni-DTPA in the presence and absence of JQ1.

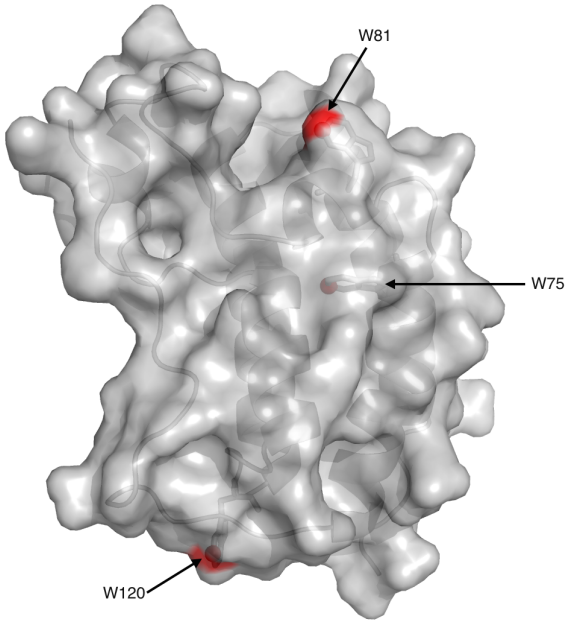


Figure S4. Brd4 with the three tryptophan residues highlighted with red spheres where the fluorine is appended in 5FW Brd4. The fluorines of W120 and W81 are relatively solvent exposed, and the fluorine on W75 appears buried.

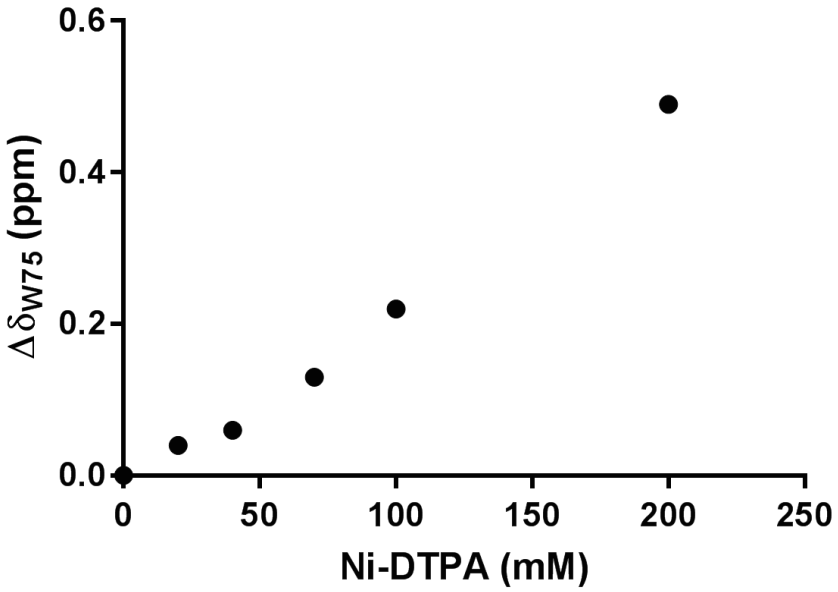


Figure S5. Change in chemical shift of W75 upon the titration of Ni-DTPA. The roughly linear trend of the data is consistent with nonspecific binding.

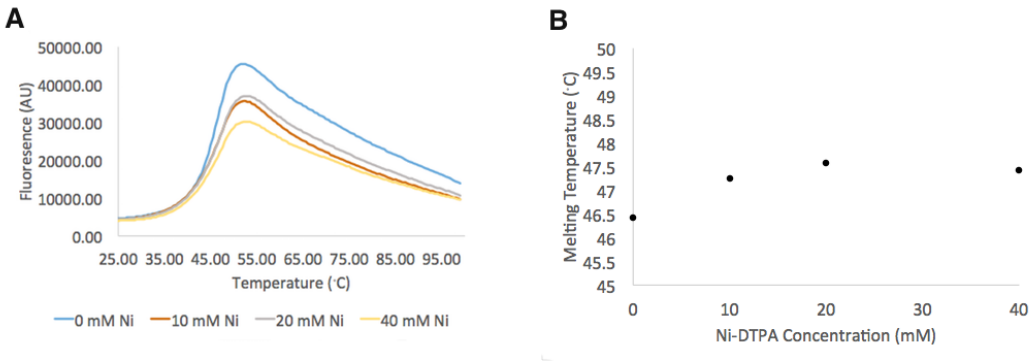


Figure S6. Differential scanning fluorimetry performed on Brd4 at increasing Ni-DTPA concentrations. Only small changes are seen in the melting temperature. (A) Melting curves at three different Ni-DTPA concentrations showing changes in fluorescence upon increasing temperature. (B) Melting temperature of Brd4 plotted against Ni-DTPA concentration. The melting temperature was 46.4 °C in the absence of Ni-DTPA, 47.3 °C in the presence of 10 mM Ni-DTPA, 47.6 °C with 20 mM Ni-DTPA, and 47.4 °C with 40 mM Ni-DTPA.

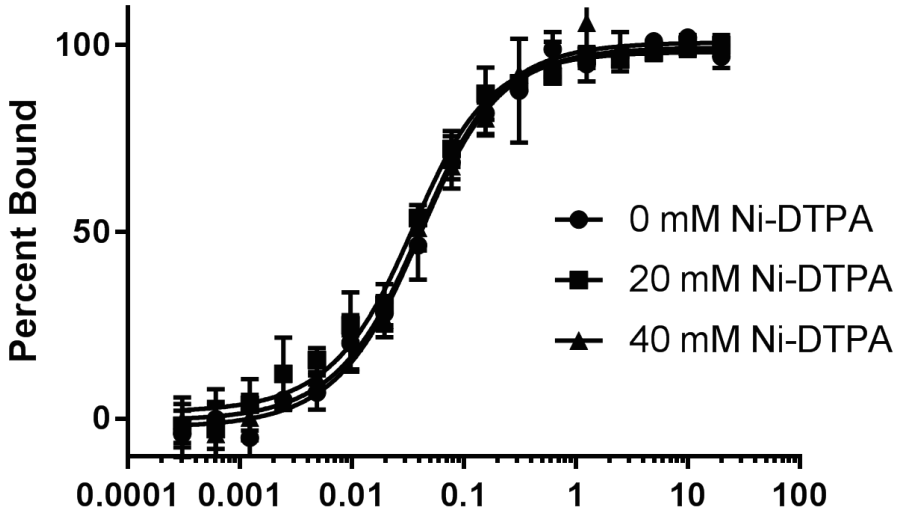


Figure S7. Fluorescence anisotropy measurement of binding of Brd4 to BI-BODIPY in the presence of 0, 20, and 40 mM and of chelated nickel. The K_d values are not significantly perturbed.

Table S1. Dissociation constants from binding of BI-BODIPY to Brd4 with increasing concentrations of Ni-DTPA.

Ni-DTPA (mM)	K_d (nM)
0	33 ± 11
20	38 ± 19
40	32 ± 5

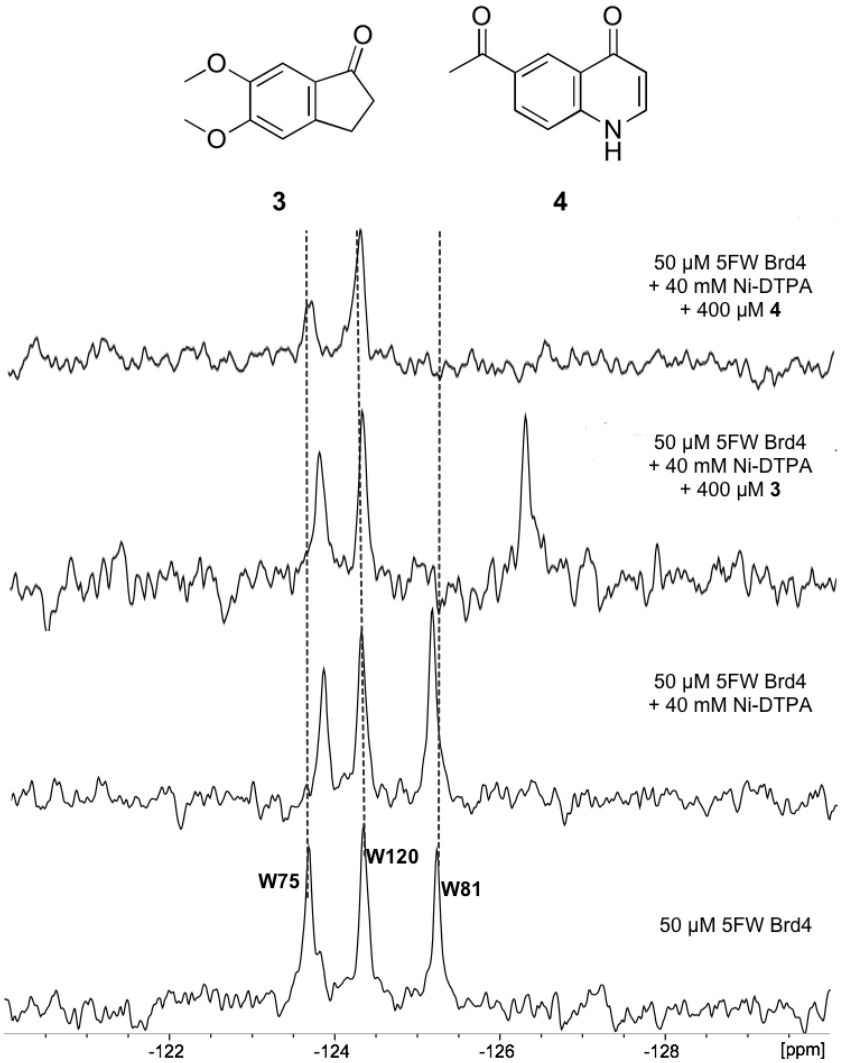


Figure S8. PrOF NMR spectra of 50 µM 5FW Brd4 in the presence of various fragments. Binding is evident in the presence of 20 mM Ni-DTPA for compounds 3 and 4, as shown by perturbations to the chemical shift or peakwidth of W81. Compounds **3** and **4** also bind to 5FW Brd4 in the absence of Ni-DTPA. Spectra were taken on a 600 MHz Bruker Avance III spectrometer equipped with a quadruple resonance HFCN CryoProbe without proton decoupling.

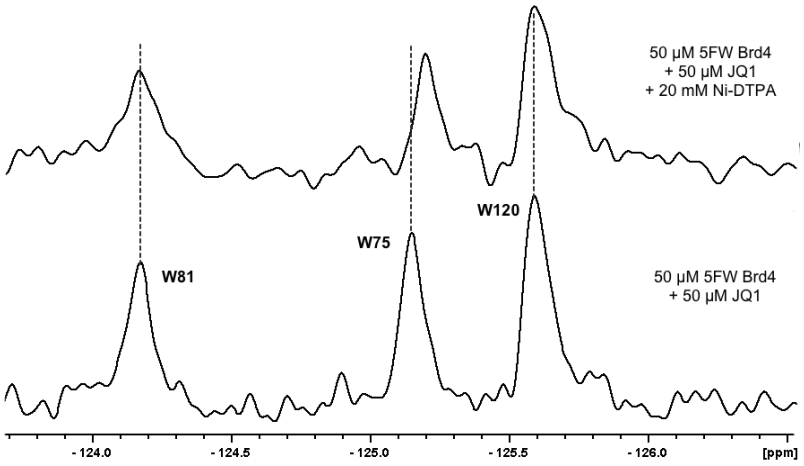


Figure S9. PrOF NMR spectra of 5FW Brd4 with (+)-JQ1 with and without Ni-DTPA. The addition of (+)-JQ1 causes W81 to move downfield of W75 and W120. The new resonance appears at the same chemical shift in the presence and absence of Ni-DTPA.

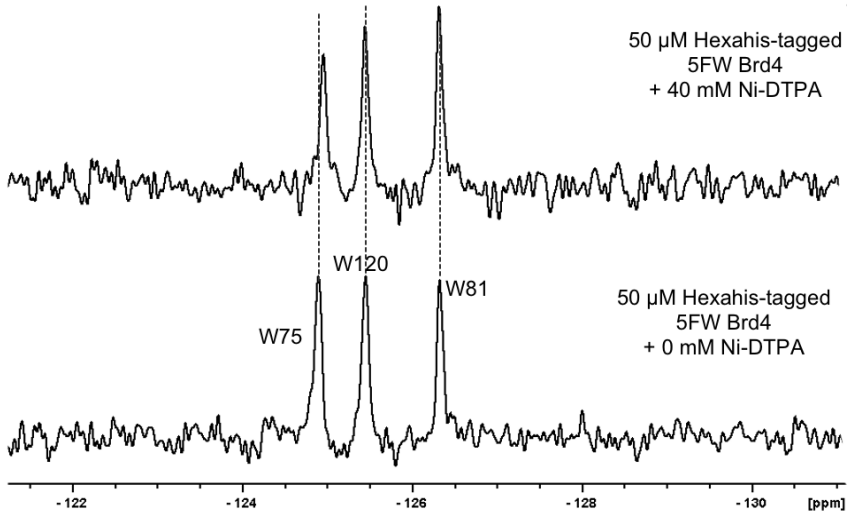


Figure S10. PrOF NMR spectra of hexahis-tagged 5FW Brd4 with 0 and 20 mM Ni-DTPA. Despite its capacity to tightly bind Ni(II), the presence of a hexahistidine tag on 5FW Brd4 is compatible with Ni-DTPA. No significant perturbation or broadening of resonances is observed upon titration of Ni-DTPA.

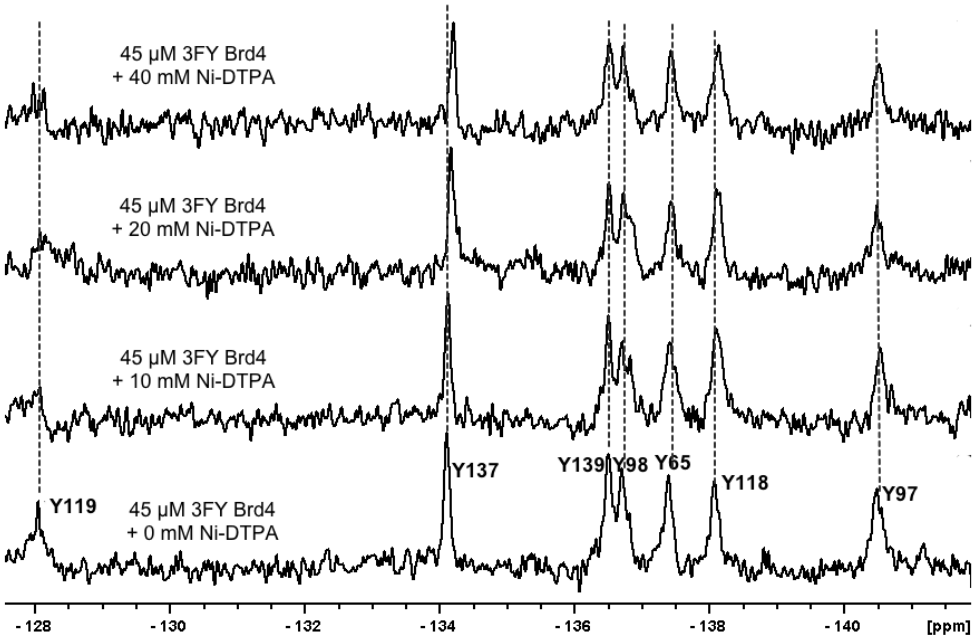


Figure S11. 3FY Brd4 with various concentrations of Ni-DTPA. With the exception of Y119, which is broad even in the absence of Ni-DTPA, resonances show very little perturbation upon titration of Ni-DTPA up to 40 mM.

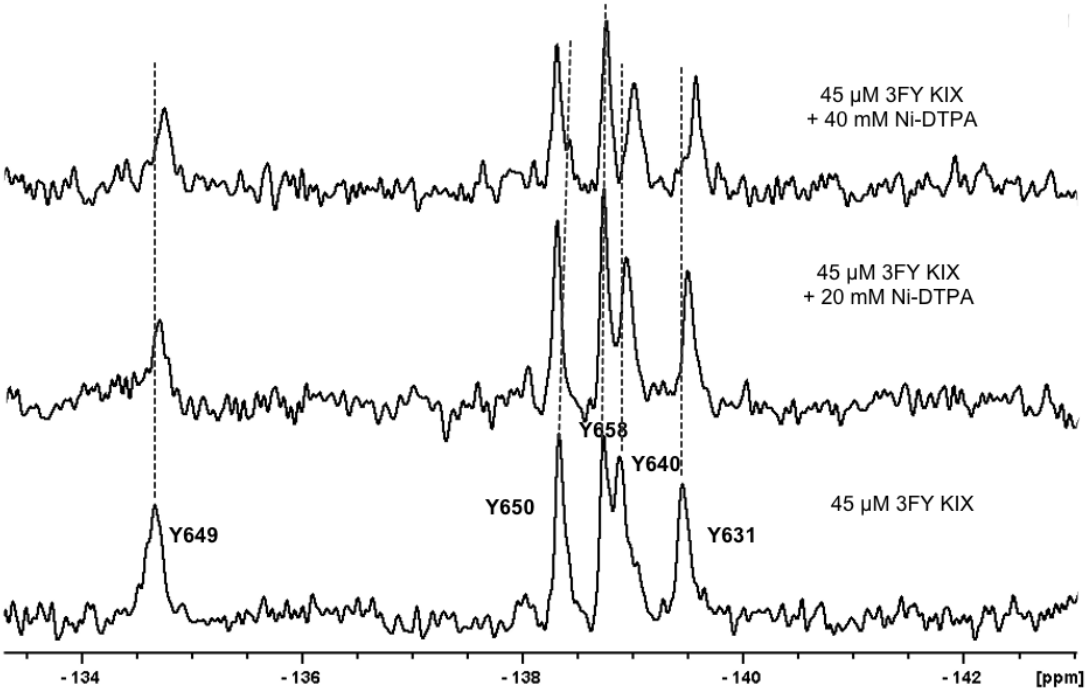


Figure S12. 3FY KIX with increasing concentrations of Ni-DTPA. The spectrum of 3FY KIX shows slight perturbation in the presence of 20 mM Ni-DTPA. More significant perturbations are seen in the presence of 40 mM Ni-DTPA.

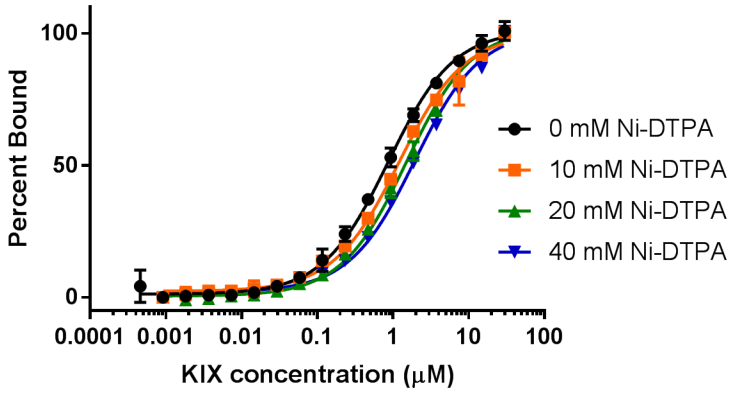


Figure S13. Fluorescence anisotropy shows the binding of Fl-MLL to unlabelled KIX in the presence of increasing concentrations of Ni-DTPA. The measured dissociation constants increase in a dose-dependent manner.

Table S2. Dissociation constants from binding of Fl-MLL to unlabelled KIX with increasing concentrations of Ni-DTPA.

Ni-DTPA Concentration (mM)	K_d (μM)
0	0.85 ± 0.07
10	1.3 ± 0.2
20	1.6 ± 0.2
40	2.1 ± 0.3

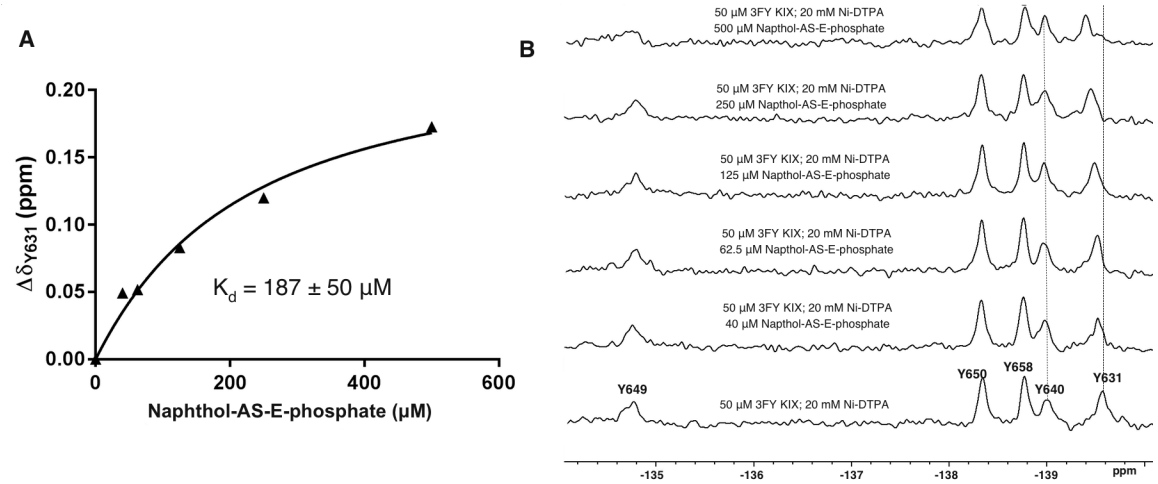


Figure S14. The binding of Naphthol-AS-E-phosphate to 3FY KIX in the presence of 20 mM Ni-DTPA. A) Binding isotherm showing changes in chemical shift of Y631 upon titration of Naphthol-AS-E-phosphate. B) PrOF NMR spectra showing perturbations to NMR spectra upon titration of Naphthol-AS-E-phosophate.