Alternative mechanisms for DNA engagement by BET bromodomain-containing proteins

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Methods:

Protein Purification

To the cell pellet was added 40 mL of lysis buffer (50 mM phosphate, 300 mM NaCl, pH 7.4) and 40 mg phenylmethanesulfonyl fluoride (PMSF) and the mixture was allowed to thaw at room temperature for 30 min. Cells were put on ice and sonicated in 30 second intervals followed by 60 s of cooling for a total of 12 min sonication time. The lysed cells were centrifuged at 100000 *x* g for 30 min. The supernatant was decanted from the pelleted cell debris and filtered using Whatman filter paper. Ni affinity purification was done using a Ni HisTrap FF 5 mL column (GE Healthcare) on an AKTA Fast Protein Liquid Chromatography (FPLC) system by monitoring the absorbance at 280 nm. Proteins were eluted with a 0-100% gradient of wash buffer (50 mM phosphate, 100 mM NaCl, 40 mM imidazole, pH 7.4) and elution buffer (50 mM phosphate, 100 mM NaCl, 400 mM imidazole, pH 7.4) across 20 column volumes. Purified protein was then buffer exchanged into storage buffer (50 mM HEPES, 100 mM NaCl, pH 7.4) using a HiPrep desalting column (GE Healthcare) equilibrated with 1 column volume of buffer. The hexahistidine (His_6) tag was removed by adding His_6-Tobacco Etch Virus (TEV) protease and incubating for 4-16 h at 4°C. Nickel nitriloacetic acid (Ni-NTA) affinity resin was added, incubated for 2-24 h at 4°C, then filtered to remove the TEV. Protein purity was assessed using SDS-polyacrylamide gel electrophoresis (12% Bis-Tris, 1.0 mM gels; running conditions: 120 V, 90 min in MES buffer). Protein was concentrated to ~25-35 μM using Amicon Ultra-15 (Millipore) centrifugal filters with

either a 3 kDa or a 10 kDa molecular weight cut off (MWCO = 3000 Da or 10000 Da), flash frozen and stored at -20°C. Quadrupole Time-of-Flight (Q-TOF) LC/MS was used to confirm the identity of the protein and determine percent fluorine incorporation using the following equation.

$$\%\ Incorporation = \frac{(0\ F\ protein*0)+(1F\ protein*1)+\cdots(nF\ protein*n)}{(0F\ protein*n)+(1F\ protein*n)+\cdots(nF\ protein*n)} * 100$$

where (0F protein) denotes the integration value of the mass peak with 0 fluorine and so on. n is the number of fluorine nuclei incorporated.
Protein masses and fluorine incorporation are shown in table S1.

Table S1: Masses and percent fluorine incorporation determined by LC/MS for bromodomains expressed in this study.

Protein	Calculated m/z (Da)	Observed m/z (Da)	% Fluorine Incorporation
5FW BRD4-BD1	15137	15136	98
5FW BRDT-BD1	14185	14184	95
His_6 3FY BRD4-BD1	17693	17694	98
^{15}N BRD4-BD1	15253	15253	N.A.
5FW N140A BRD4-BD1	15093	15094	98
^{15}N N140A BRD4-BD1	15210	15210	N.A.
5FW R68S K72S K76S BRD4-BD1	14986	14984	98
3FY R68S K72S K76S BRD4-BD1	15058	15057	98
^{15}N R68S K72S K76S BRD4-BD1	15092	15093	N.A.
^{15}N BRDT-BD1	14309	14309	N.A.
5FW K37S K41S K45S BRDT-BD1	14025	14023	98
Unlabeled BRD4-BD1	15083	15084	N.A.
5FW BPTF BD	16922	16921	99
5FW BRD4-BD2	15054	15054	95
Unlabeled BRD4-BD2	13182	13183	N.A.
His_{10} 5FW BRD3-BD1	17639	17638	98

His_{10} 5FW BRD3-BD2	16317	16313	98
His_{10} 5FW BRD2-BD1	17891	17888	98
His_6 5FW BRD2-BD2	15835	15831	98
His_6 5FW CREBBP BD	16699	16699	99
5FW BRD4-T	47938	47936	94
Unlabeled BRDT-T	47418	47418	N.A.
His_6-5FW BRDT-T	49466	49458	99

Table S2: Peptide theoretical and observed mass using MALDI-TOF MS.

Peptide	**Sequence**	**Calculated $[M+H]^+$**	**Observed $[M+H]^+$**
H4 K5ac,K8ac,K12ac,K16ac	H_2N-YSGRGKacGGKacGLGKacGGAKacRHRK-C(O)NH_2	2322.5	2322.36

Table S3: DNA oligonucleotides used in NMR studies.

DNA oligonucleotides	**DNA sequences**
25 bp dsDNA	Forward-5′-CGAAGTGGCCGAGTGGTCTATGGCG-3′ Reverse- 5′-CGCCATAGACCACTCGGCCACTTCG-3′
40 bp dsDNA	Forward- 5′-CAGGCTGGTCTTGA ACTCCTGACCTCAGATGATCCATGTG-3′ Reverse- 5′-C ACATGGATCATCTGAGGTCAGGAGTTCAAGAC CAGCCTG-3′
41 bp dsDNA	Forward-5′-GTTTG GTGAA CCAAC ACTAC GGAAT AAAAC AGGCC TCAAGG-3′ Reverse-5′-CCTTG AGGCC TGTTT TATTC CGTAG TGTTG GTTCA CCAAAC-3′
66 bp dsDNA	Forward-5′-CGATATAGTGTAACGGCTATCACATCACGCTTTCACCGTGGAGA CCGGGGTTCGACTCCCCGTATC-3′ Reverse-5′-GATACGGGGAGTCGAACCCCGGTCTCCACGGTGAAAGCGTGAT GTGATAGCCGTTACACTATATCG-3′

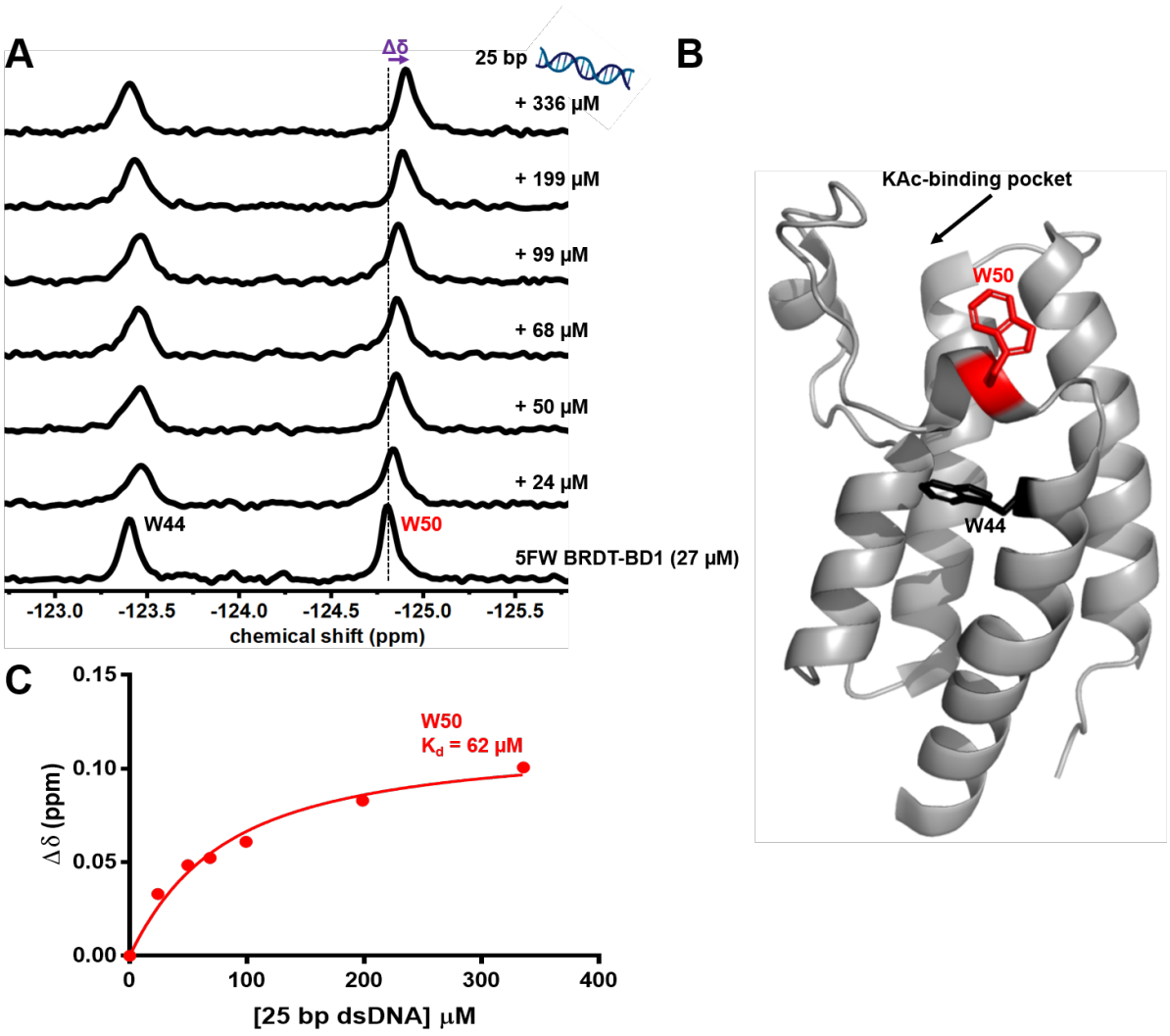


Figure S1: PrOF NMR experiments with 25 bp dsDNA binding to 5FW BRDT-BD1. (A) Stacked ^{19}F NMR spectra, (B) X-ray crystal structure of BRDT-BD1 (PDB ID: 7L73) showing W50 around KAc-binding pocket, and W44, colored red and black respectively (C) Binding isotherm of 25 bp dsDNA (24-336 μM) with 27 μM 5FW BRDT-BD1. W50 is the WPF shelf tryptophan in BD1 of BRDT, colored red. Change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

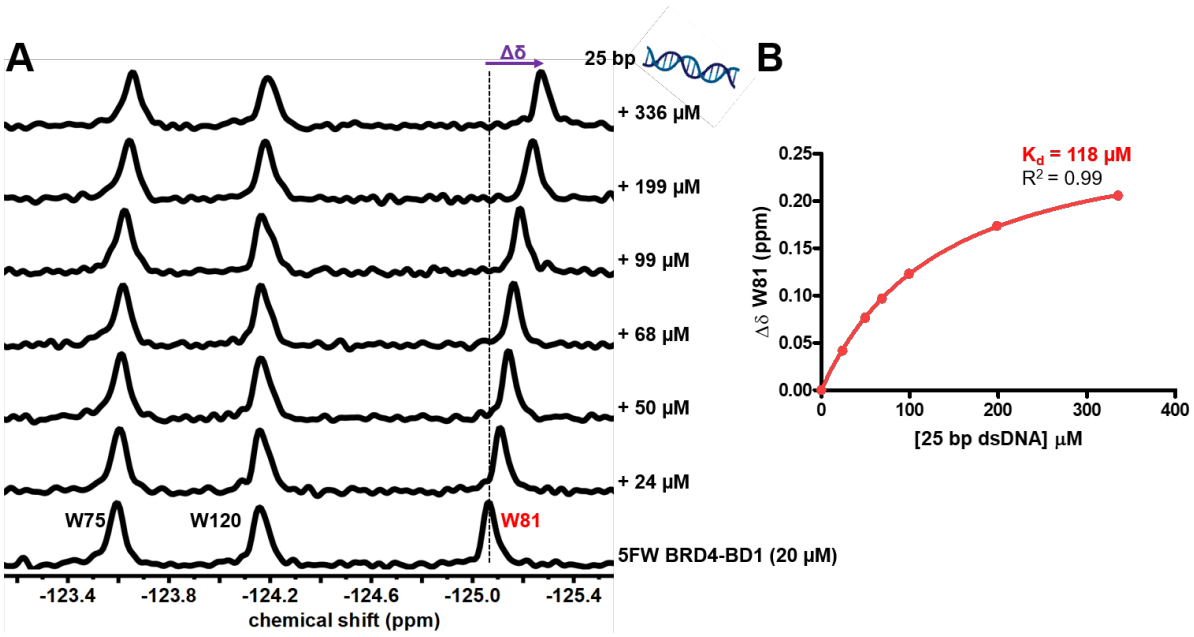


Figure S2: PrOF NMR experiments with 25 bp dsDNA binding to 5FW BRD4-BD1. (A) Stacked ^{19}F NMR spectra and (B) binding isotherm of 25 bp dsDNA (24 -336 µM) with 20 µM 5FW BRDT-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. Change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

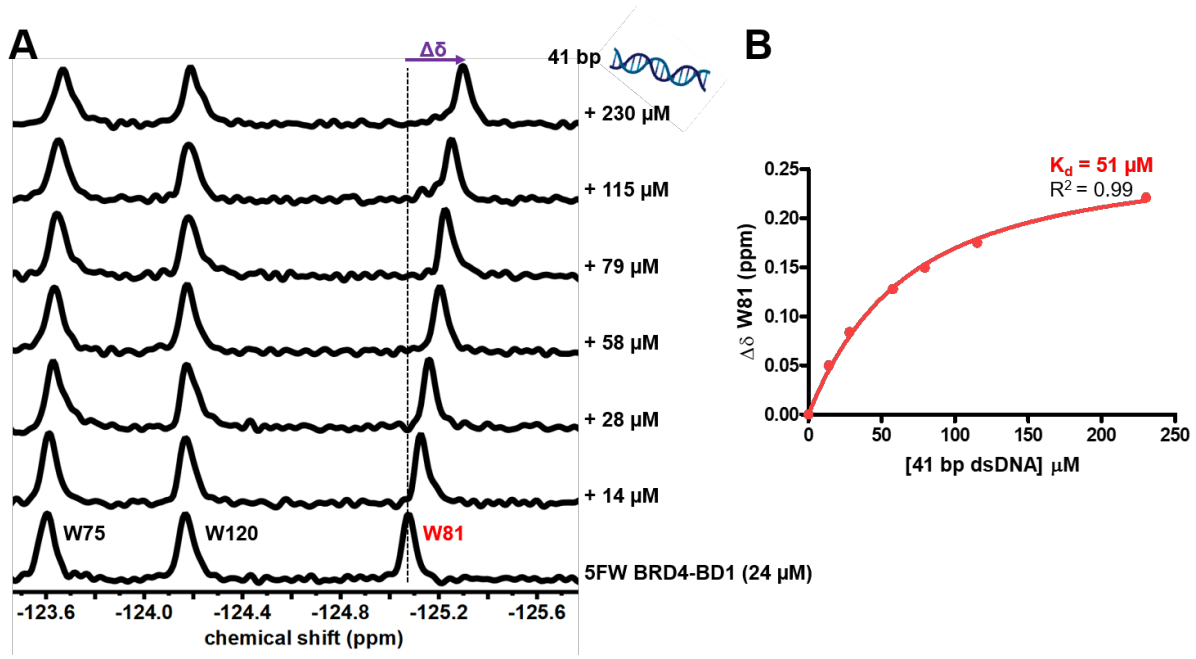


Figure S3: PrOF NMR experiments with 41 bp dsDNA binding to 5FW BRD4-BD1. (A) Stacked ^{19}F NMR spectra and (B) binding isotherm of 41 bp dsDNA (14-230 µM) with 24 µM 5FW BRD4-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. Change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

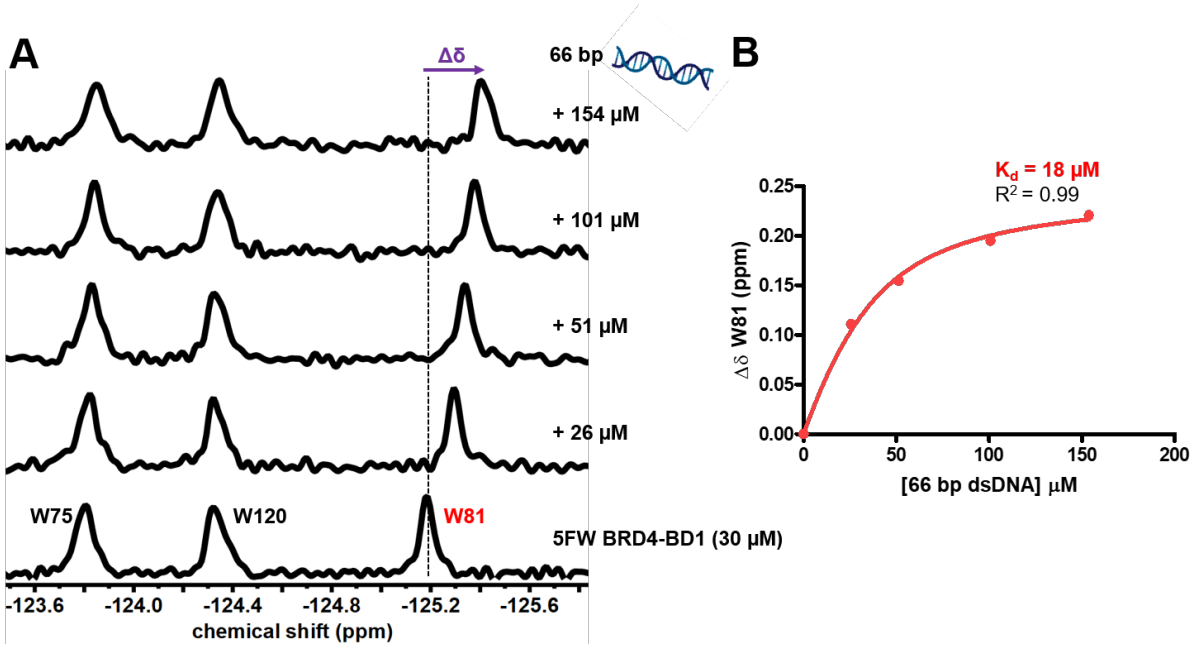


Figure S4: : PrOF NMR experiments with 66 bp dsDNA binding to 5FW BRD4-BD1. (A) Stacked ^{19}F NMR spectra and (B) binding isotherm of 66 bp dsDNA (26-154 μM) with 30 μM 5FW BRD4-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. Change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

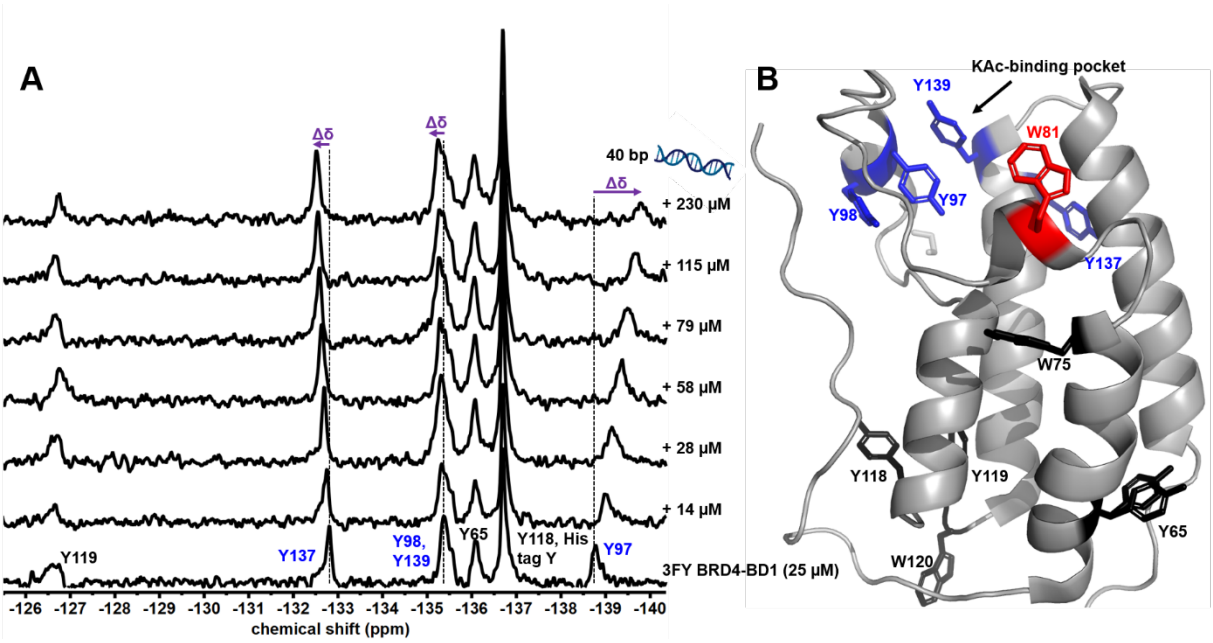


Figure S5: PrOF NMR experiments with (A) 40 bp dsDNA (14-230 μM) binding to 25 μM 3FY BRD4-BD1. (B) X-ray crystal structure of BRD4-BD1 (PDB ID: 3UVW) showing Y97, Y98, Y137, Y139, and W81 are around the KAc-binding site in BD1 of BRD4, colored blue and red respectively. Y65, Y118, Y119, W75, and W120 are shown in black. Change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

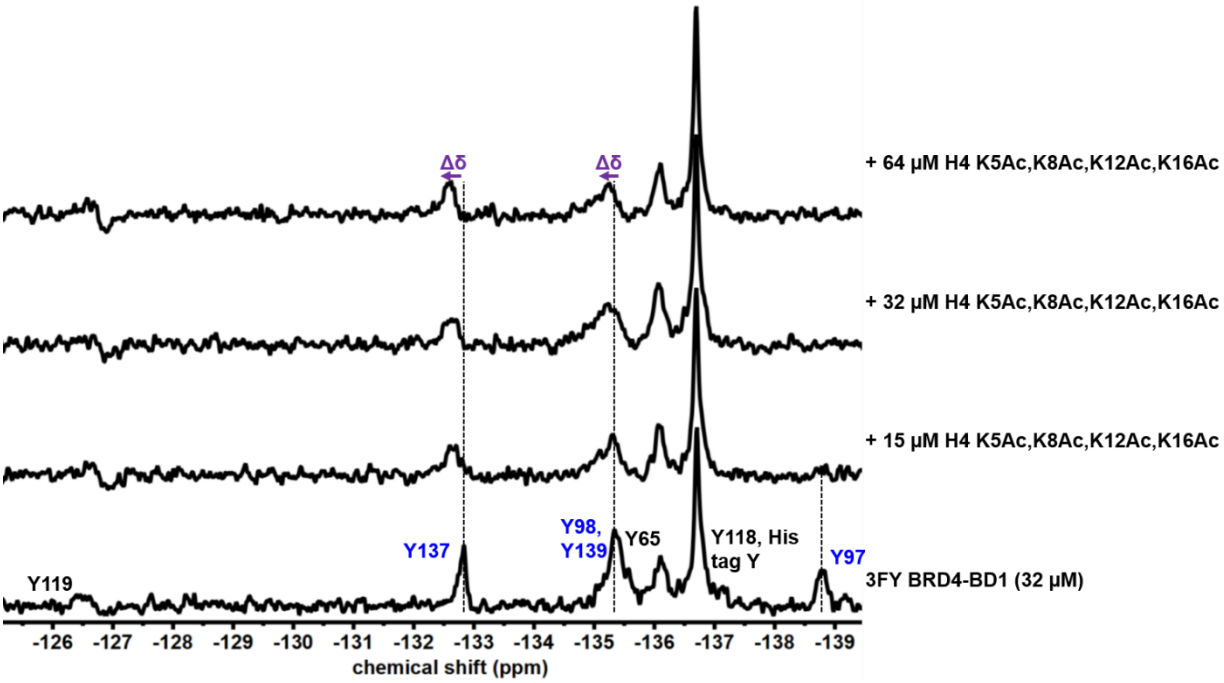


Figure S6: PrOF NMR experiments with H4 K5Ac,K8Ac,K12Ac,K16Ac binding to 32 μM 3FY BRD4-BD1. Y97, Y98, Y137, and Y139 are around the KAc-binding site in BD1 of BRD4, colored blue. Change in the chemical shift (Δδ) is shown in purple. Dashed line is indicating either Δδ (Y137, Y98, Y139) or broadening due to intermediate chemical exchange (Y97).

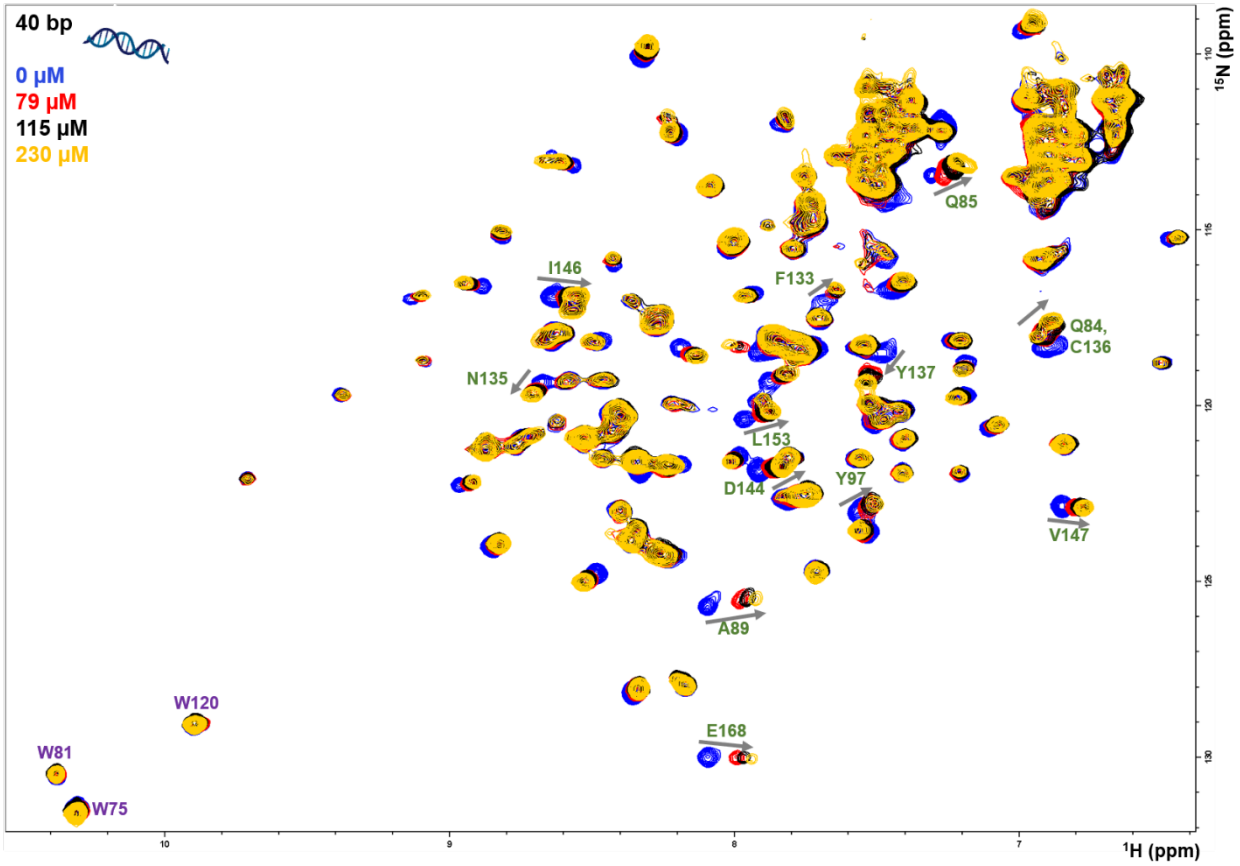
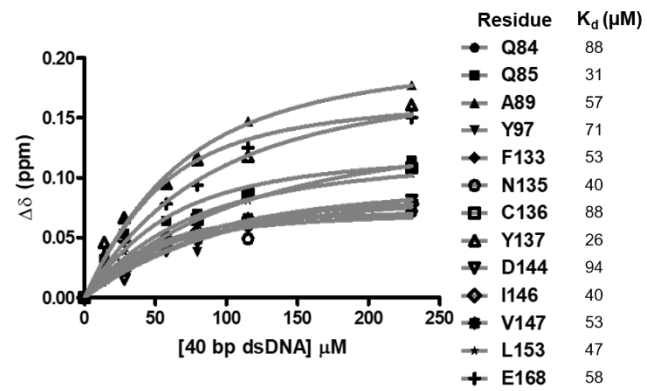


Figure S7: ^{1}H-^{15}N HSQC NMR experiments with 40 bp dsDNA binding to 44 µM of **^{15}N BRD4-BD1**. Spectra are color coded according to DNA concentrations as shown in the legend. Titrations were performed at DNA concentrations of 0 µM, 14 µM, 28 µM, 58 µM, 79 µM, 115 µM, and 230 µM. For clarity, only 4 points are displayed, and spectra are color coded accordingly as shown in the legend. Residues that are perturbed greater than the average plus one standard deviation are labelled in green, and the chemical shift perturbation (Δδ) is shown in grey. Cross-peaks from the indole NH of tryptophan sidechains are shown in purple.

A



Residue-averaged K_d = 57 ± 6 μM

B

Ligand	Chemical Shift Perturbation (Δδ) (ppm)												
[40 bp dsDNA] μM	Q84	Q85	A89	Y97	F133	N135	C136	Y137	D144	I146	V147	L153	E168
0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0.0216	0.0319	0.0380	0.0267	0.0230	0.0230	0.0216	0.0459	0.0156	0.0156	0.0156	0.0267	0.0235
28	0.0267	0.0533	0.0662	0.0267	0.0230	0.0267	0.0267	0.0667	0.0156	0.0235	0.0235	0.0380	0.0469
58	0.0459	0.0638	0.0963	0.0380	0.0533	0.0492	0.0459	0.0948	0.0391	0.0469	0.0447	0.0516	0.0782
79	0.0653	0.0697	0.1192	0.0380	0.0533	0.0492	0.0653	0.1149	0.0469	0.0547	0.0516	0.0662	0.0938
115	0.0868	0.0893	0.1472	0.0638	0.0583	0.0492	0.0868	0.1178	0.0625	0.0625	0.0662	0.0811	0.1251
230	0.1083	0.1140	0.1773	0.0760	0.0720	0.0720	0.1083	0.1608	0.0811	0.0782	0.0811	0.1033	0.1501

Figure S8: (A) Binding isotherms of ^{1}H-^{15}N HSQC NMR titration of 40 bp dsDNA binding to 44 μM of **^{15}N BRD4-BD1**. (B) Chemical shift perturbations of significant residues to plot residue-averaged K_d.

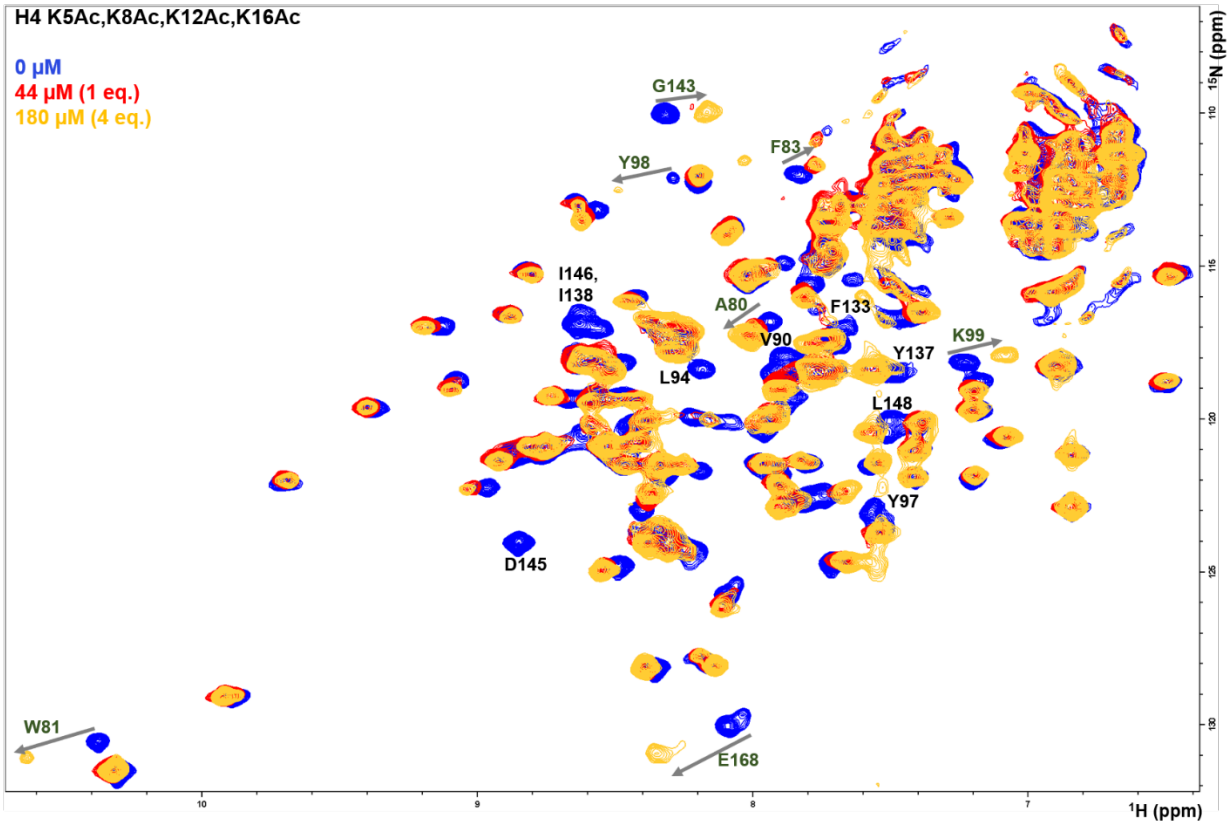


Figure S9: ^{1}H-^{15}N HSQC NMR experiments with H4 K5Ac,K8Ac,K12Ac,K16Ac binding to 44 µM of **^{15}N BRD4-BD1**. Spectra are color coded according to H4 peptide concentrations as shown in the legend. Titrations were performed at H4 peptide concentrations of 0 µM, 44 µM, and 180 µM. Residues that are perturbed greater than the average plus one standard deviation are labelled in green and the residues that are broadened to baseline in the ligand-bound state are labelled in black. The chemical shift perturbation (Δδ) is shown in grey.

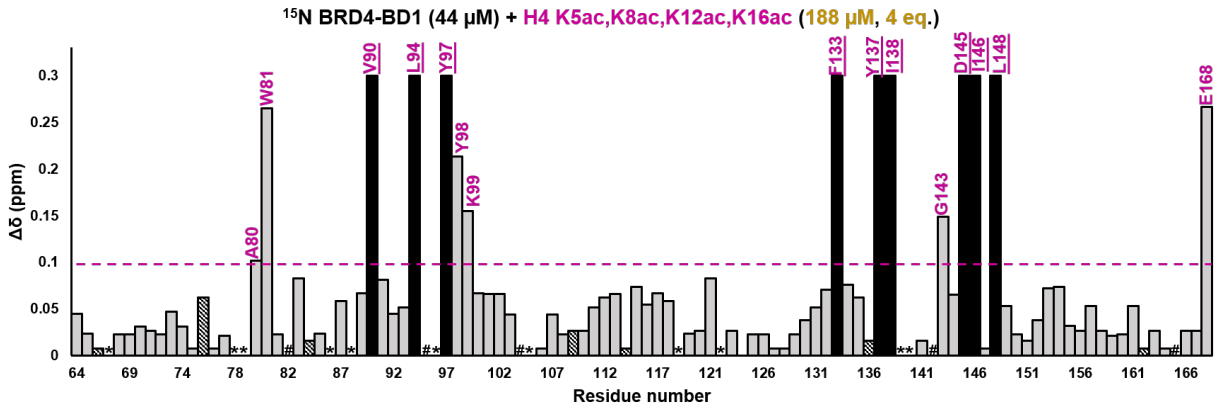


Figure S10: ^{1}H-^{15}N HSQC NMR experiments with H4 K5Ac,K8Ac,K12Ac,K16Ac binding to 44 µM of **^{15}N BRD4-BD1**. Normalized CSPs are plotted as a function of residue upon titration H4 K5Ac,K8Ac,K12Ac,K16Ac for the peptide concentration of 188 µM (4 eq.). Residues that are perturbed greater than the average plus one standard deviation are labeled in pink. A pink line marks this level of significance. Underlined residues are broadened to baseline in the ligand-bound state and are arbitrarily assigned CSPs of 0.3 ppm and are shown as black bars. Dashed bars (L66, W75, Q84, T109, L114, C136, and M162) indicate CSPs at 44 µM (1 eq.) of peptide concentration as the chemical shift at 188 µM could not be assigned. * indicates missing resonance and # indicates proline residue.

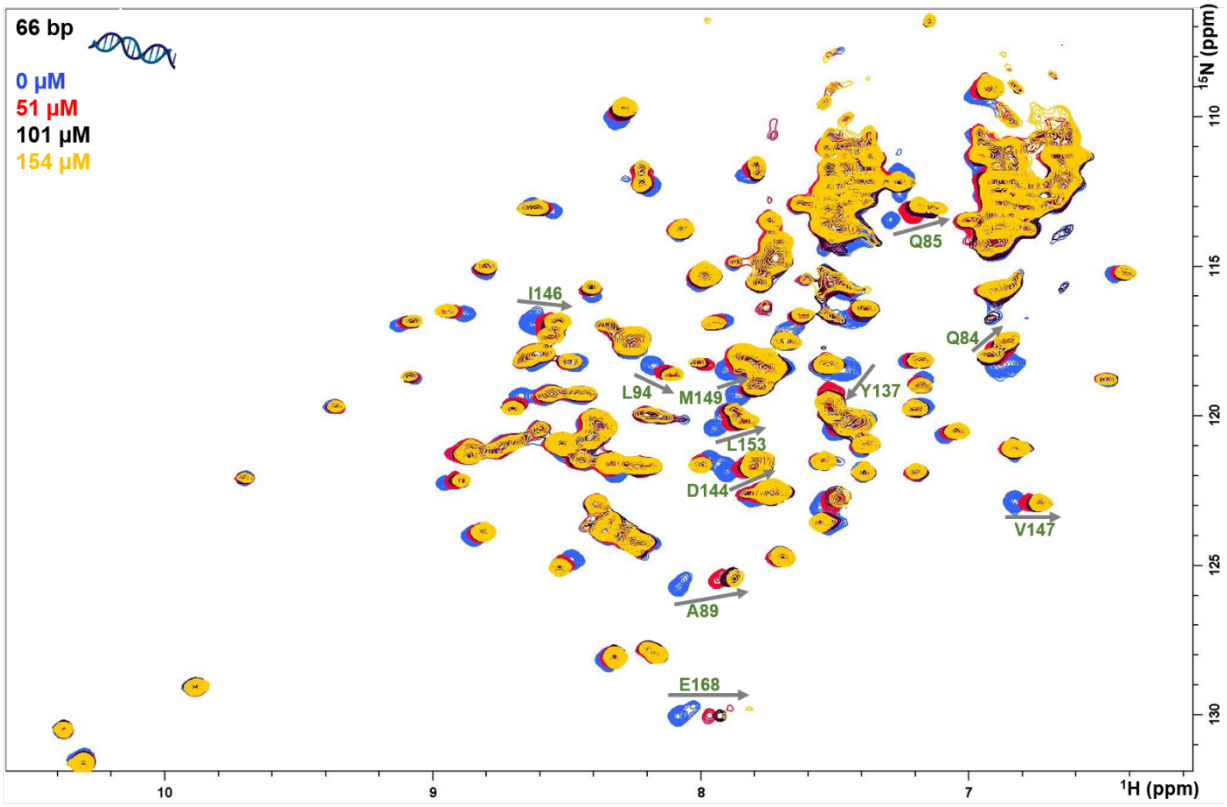


Figure S11: ^{1}H-^{15}N HSQC NMR experiments with 66 bp dsDNA binding to 44 µM of **^{15}N BRD4-BD1**. Spectra are color coded according to DNA concentrations as shown in the legend. Titrations were performed at DNA concentrations of 0 µM, 14 µM, 26 µM, 51 µM, 101 µM, and 154 µM. For clarity, only 4 points are displayed, and spectra are color coded accordingly as shown in the legend. Residues that are perturbed greater than the average plus one standard deviation are labelled in green, and the chemical shift perturbation (Δδ) is shown in grey.

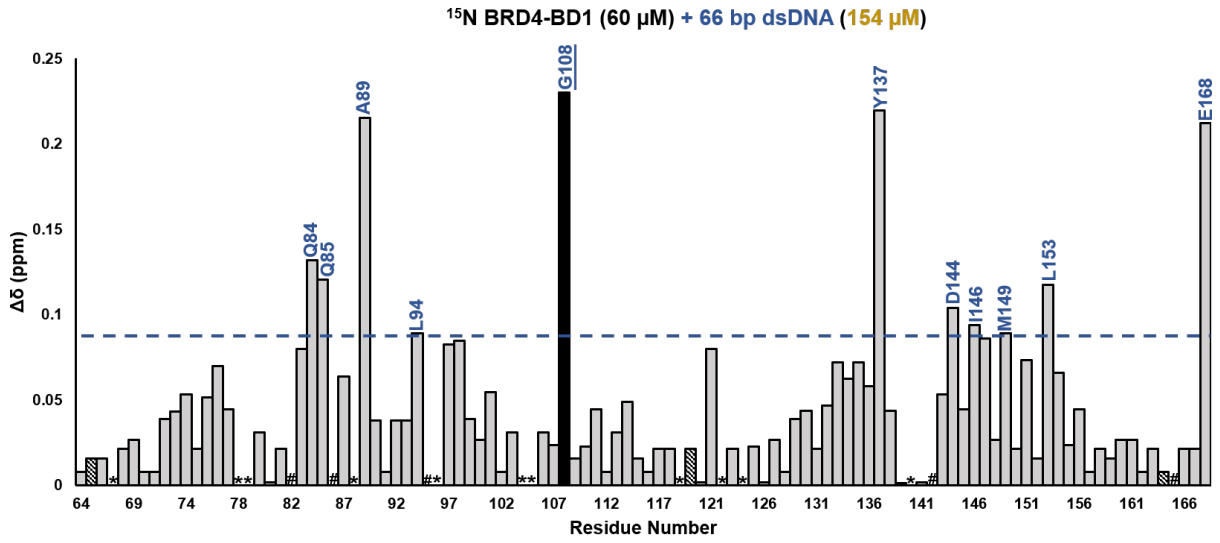
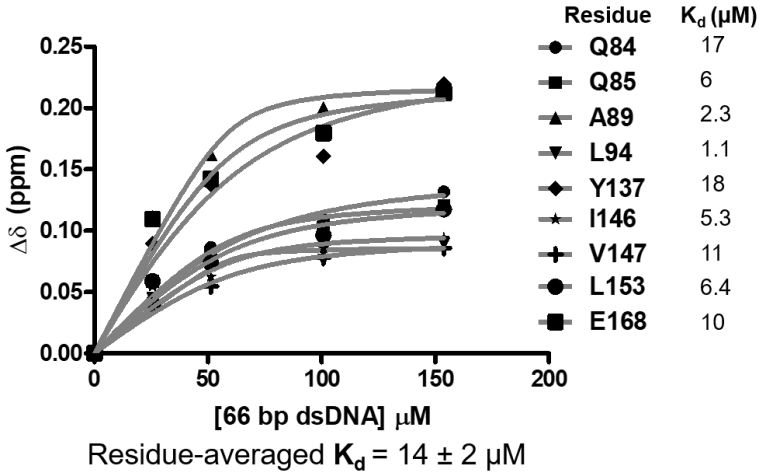


Figure S12: ^{1}H-^{15}N HSQC NMR experiment with 66 bp dsDNA binding to **^{15}N BRD4-BD1.** Normalized CSPs are plotted as a function of residue upon titration of 66 bp dsDNA for the DNA concentration of 154 µM and residues that are perturbed greater than the average plus one standard deviation are labeled in blue. A blue dashed line marks this level of significance. Dashed bars indicate a CSP at a lower concentration of DNA (26 µM for L164, 51 µM for Y65 and 101 µM for W120 side-chain indole NH resonance) as the chemical shift at 154 µM could not be assigned. These CSPs were also accounted for average and standard deviation calculations. Underlined residue (G108) is broadened to baseline in the ligand-bound state and is arbitrarily assigned a CSP of 0.23 ppm and is shown as a black bar. * indicates missing resonance and # indicates proline residue.

A



B

Ligand	Chemical Shift Perturbation (Δδ) (ppm)								
[66 bp dsDNA] μM	Q84	Q85	A89	L94	Y137	I146	V147	L153	E168
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
26	0.0432	0.0588	0.1115	0.0447	0.0895	0.0547	0.0391	0.0588	0.1094
51	0.0864	0.0826	0.1622	0.0697	0.1378	0.0625	0.0547	0.0736	0.1423
101	0.1091	0.1033	0.2001	0.0760	0.1608	0.0860	0.0782	0.0963	0.1798
154	0.1317	0.1205	0.2154	0.0893	0.2195	0.0938	0.0860	0.1177	0.2122

Figure S13: (A) Binding isotherms of ^{1}H-^{15}N HSQC NMR titration of 66 bp dsDNA binding to 60 μM of **^{15}N BRD4-BD1**. (B) Chemical shift perturbations of significant residues to plot residue-averaged K_d. Though a one-site ligand-binding model was used for K_d calculations, additional binding models might be needed to fit the CSPs for 66 bp dsDNA as this sequence was previously found to bind bivalently to BRDT-BD1.[1]

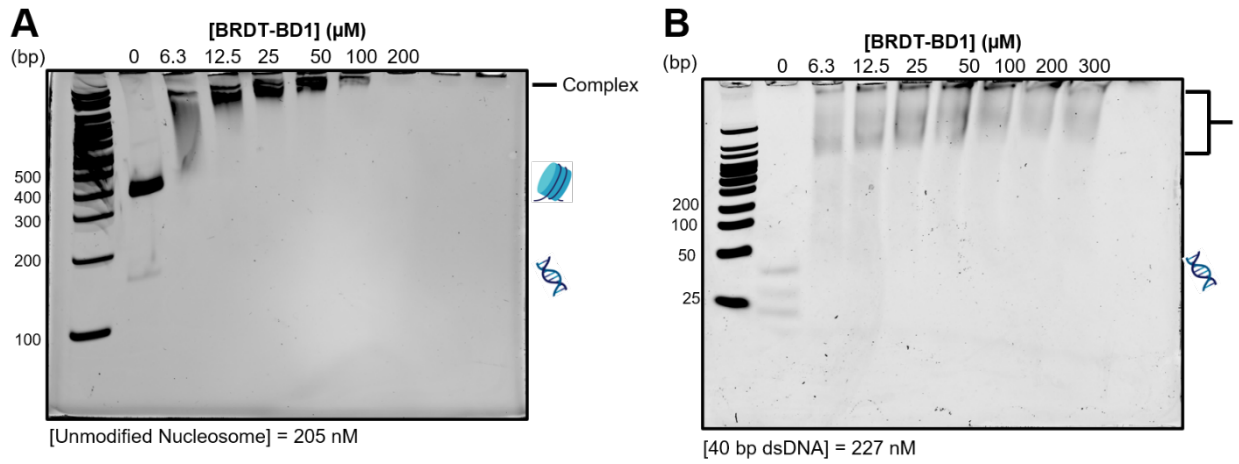


Figure S14: EMSA titrations of BRDT-BD1 interacting with (A) unmodified nucleosomes and (B) 40 bp dsDNA. Shifted bands containing BRDT-BD1 complexes with either nucleosomes or DNA are indicated.

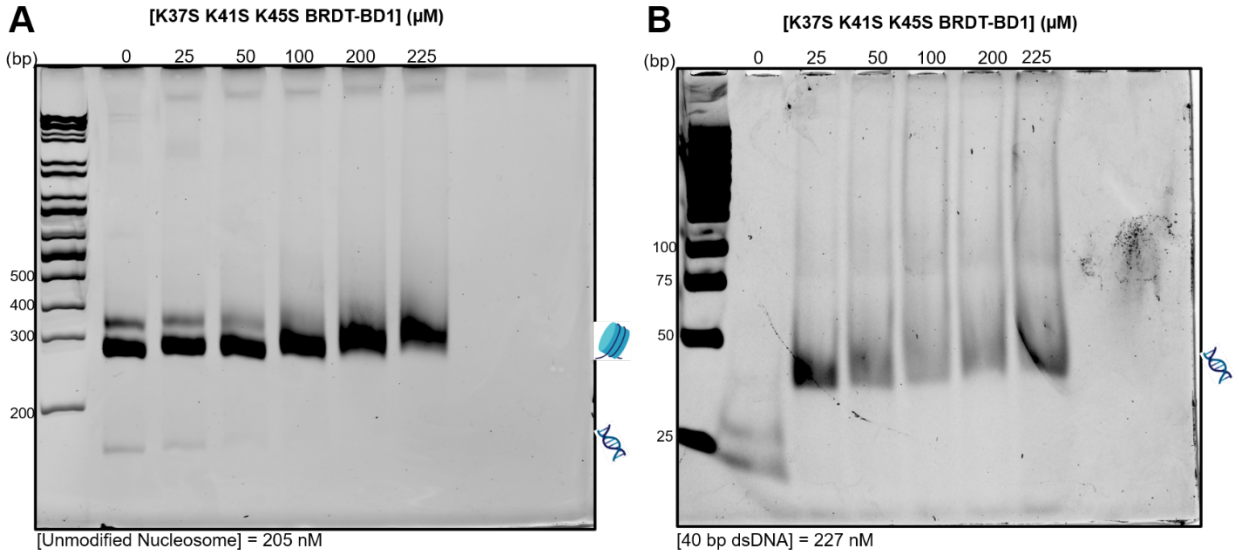


Figure S15: EMSA titrations of K37S K41S K45S BRDT-BD1 interacting with (A) unmodified nucleosomes and (B) 40 bp dsDNA.

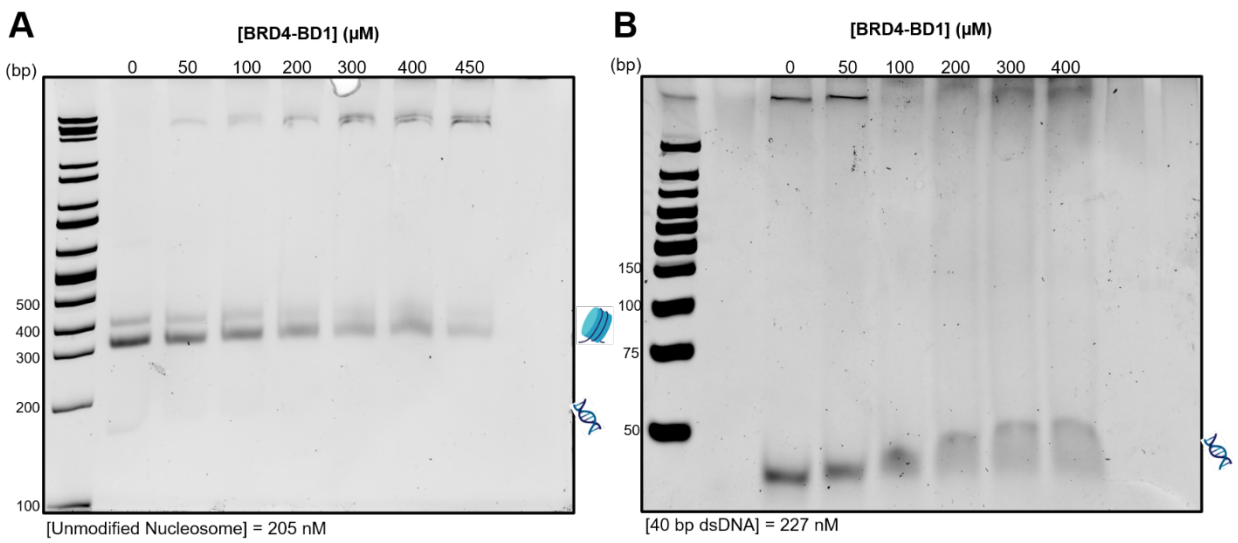


Figure S16: EMSA titrations of BRD4-BD1 interacting with (A) unmodified nucleosomes and (B) 40 bp dsDNA.

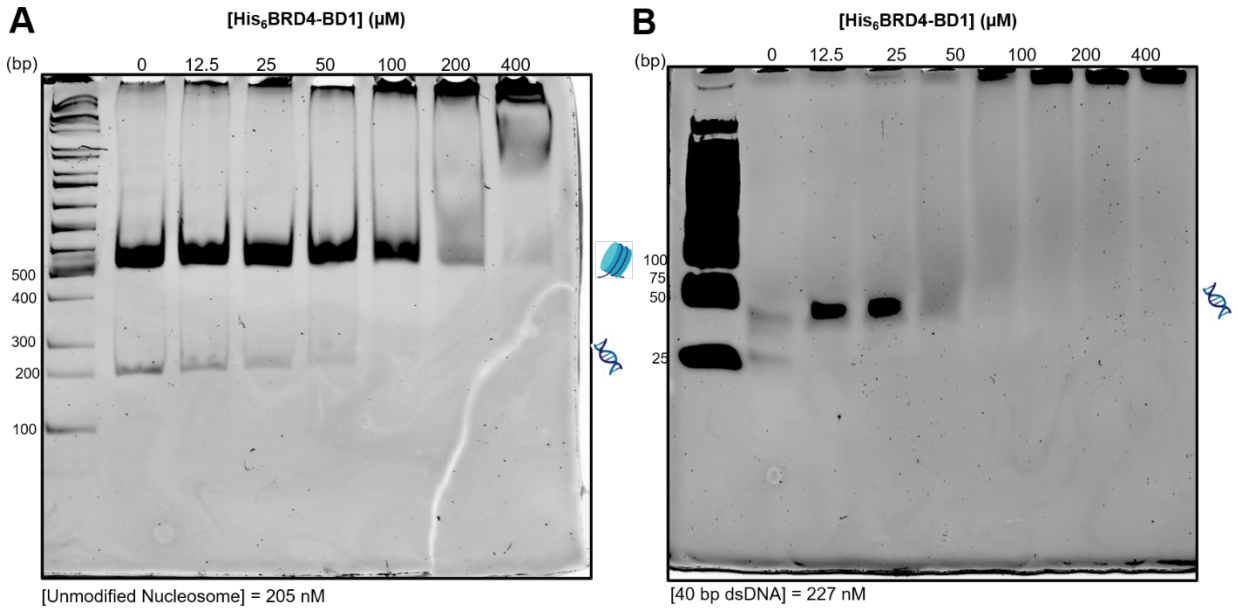


Figure S17: EMSA titrations of His_6 BRD4-BD1 interacting with (A) unmodified nucleosomes and (B) 40 bp dsDNA. Here, smearing and consequent shift is evident for both the free nucleosome band and the dsDNA band with increasing protein concentration, supporting a robust protein-DNA

interaction. However, upon the removal of the His_6-tag, as shown in Figure S16, only a weaker loss in intensity of the free nucleosome or dsDNA band is observed with similar protein concentration. This informs us that the His_6-tag might be interacting with dsDNA non-specifically to give an apparent higher-affinity to His_6BRD4-BD1 for both dsDNA and nucleosomes. Thus, the mobility-shift seen with His_6-tagged protein is only an artifact of the polyhistidine tag as reported by others.[2]

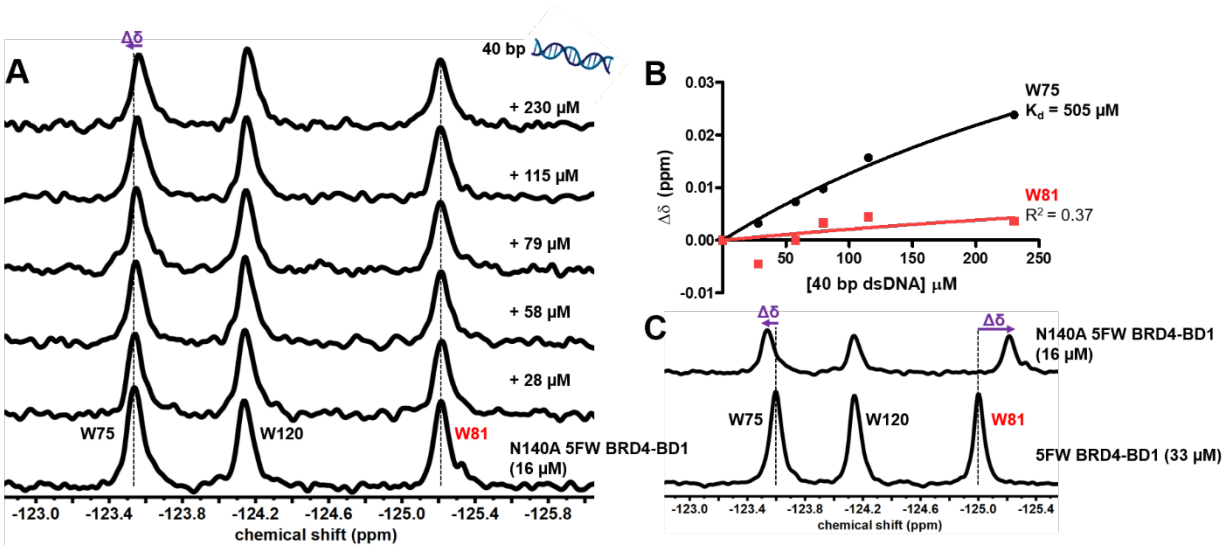


Figure S18: PrOF NMR experiments with 40 bp dsDNA binding to N140A 5FW BRD4-BD1. (A) Stacked ^{19}F NMR spectra and (B) binding isotherm of 40 bp dsDNA (28-230 μM) with 16 μM N140A 5FW BRD4-BD1. Due to poor fitting of the binding isotherm (R^2 = 0.37), K_d could not be determined. (C) Stacked ^{19}F NMR spectra of N140A and unmutated 5FW BRD4-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. Changes in the chemical shift (Δδ) are indicated in purple. W81 moves upfield by 0.21 ppm and W75 moves downfield by 0.06 ppm in N140 5FW BRD4-BD1 when aligned with ^{19}F NMR spectra of unmutated 5FW BRD4-BD1.

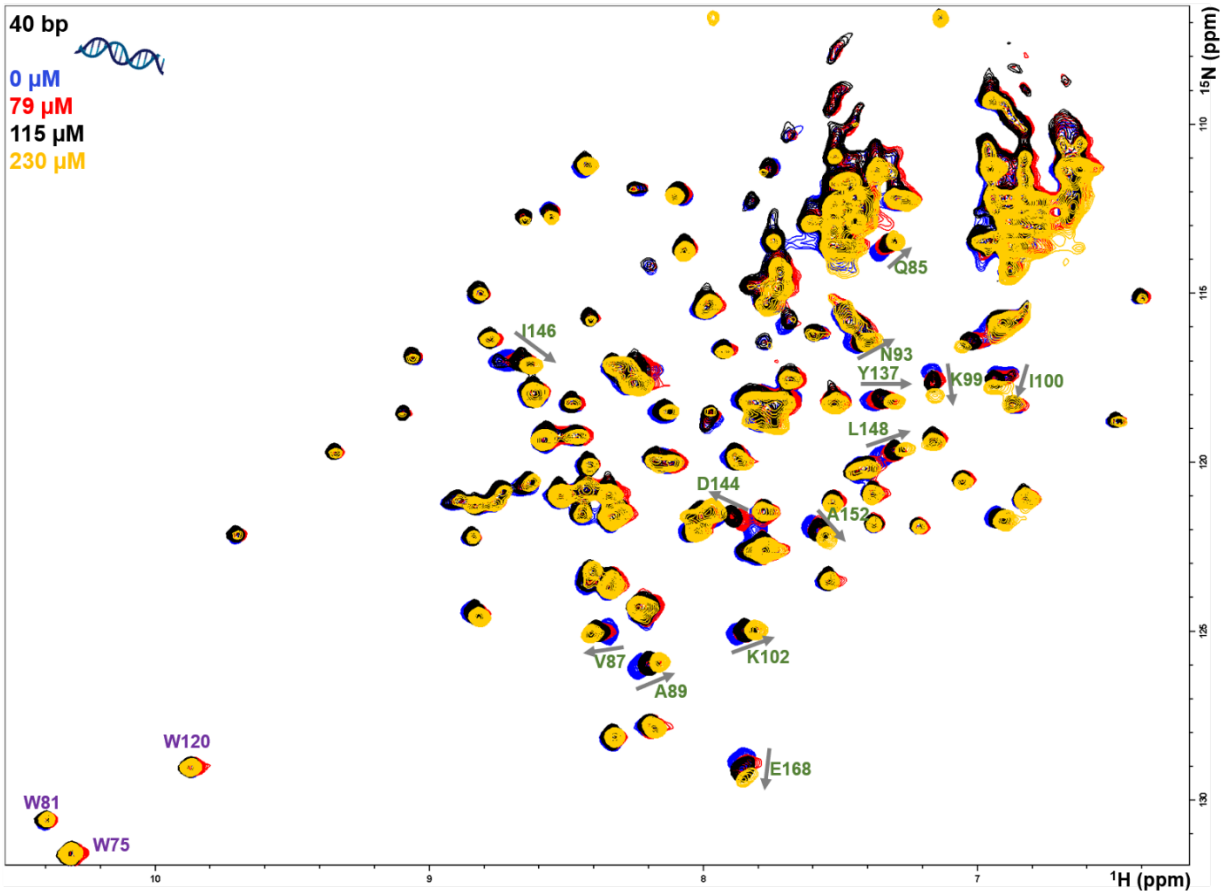


Figure S19: ^{1}H-^{15}N HSQC NMR experiments with 40 bp dsDNA binding to 35 µM of **^{15}N N140A BRD4-BD1**. Spectra are color coded according to DNA concentrations as shown in the legend. Titrations were performed at DNA concentrations of 0 µM, 28 µM, 79 µM, 115 µM, and 230 µM. For clarity, only 4 points are displayed, and spectra are color coded accordingly as shown in the legend. Residues that are perturbed greater than the average plus one standard deviation are labelled in green, and the chemical shift perturbation (Δδ) is shown in grey.

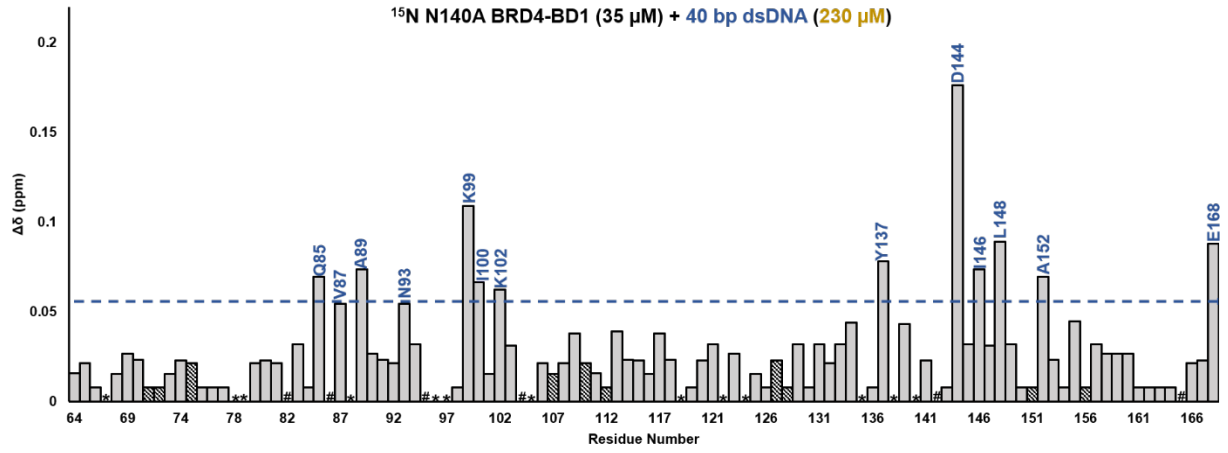
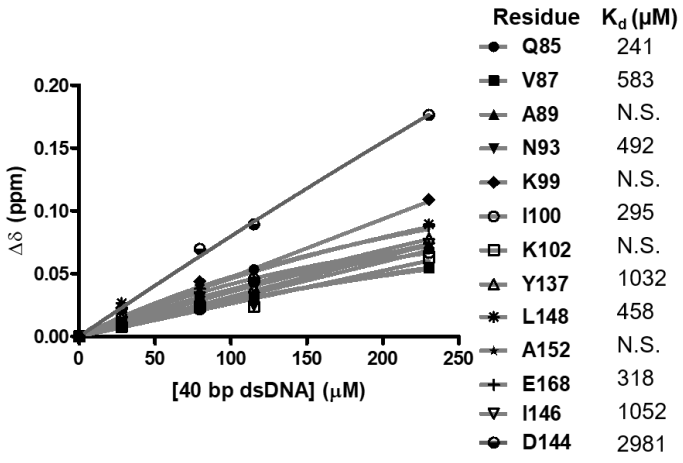


Figure S20: ^{1}H-^{15}N HSQC NMR experiment with 40 bp dsDNA binding to **^{15}N N140 BRD4-BD1.** Normalized CSPs are plotted as a function of residue upon titration of 40 bp dsDNA for the DNA concentration of 230 µM and residues that are perturbed greater than the average plus one standard deviation are labeled in blue. A blue dashed line marks this level of significance. Dashed bars indicate a CSP at a lower concentration of DNA (79 µM for W75, M107, D128, and 115 µM for L71, K72, I110, K112, Q127, E151, L156) as the chemical shift at 230 µM could not be assigned. These CSPs were also accounted for average and standard deviation calculations. * indicates missing resonance and # indicates proline residue.

A



Residue-averaged K_d = 780 ± 94 μM

B

Ligand [40 bp dsDNA]	Chemical Shift Perturbation (Δδ) (ppm)										
μM	Q85	A89	N93	I100	K102	Y137	D144	I146	L148	A152	E168
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
26	0.0078	0.0078	0.0078	0.0216	0.0078	0.0156	0.0156	0.0078	0.0266	0.0078	0.0230
51	0.0319	0.0312	0.0313	0.0216	0.0235	0.0312	0.0697	0.0312	0.0379	0.0234	0.0439
101	0.0533	0.0312	0.0235	0.0459	0.0235	0.0391	0.0892	0.0390	0.0446	0.0318	0.0439
154	0.0697	0.0735	0.0547	0.0666	0.0626	0.0781	0.1764	0.0735	0.0892	0.0697	0.0878

Figure S21: (A) Binding isotherms of ^{1}H-^{15}N HSQC NMR titration of 40 bp dsDNA binding to 35 μM of **^{15}N N140A BRD4-BD1**. (B) Chemical shift perturbations of significant residues to plot residue- averaged K_d.

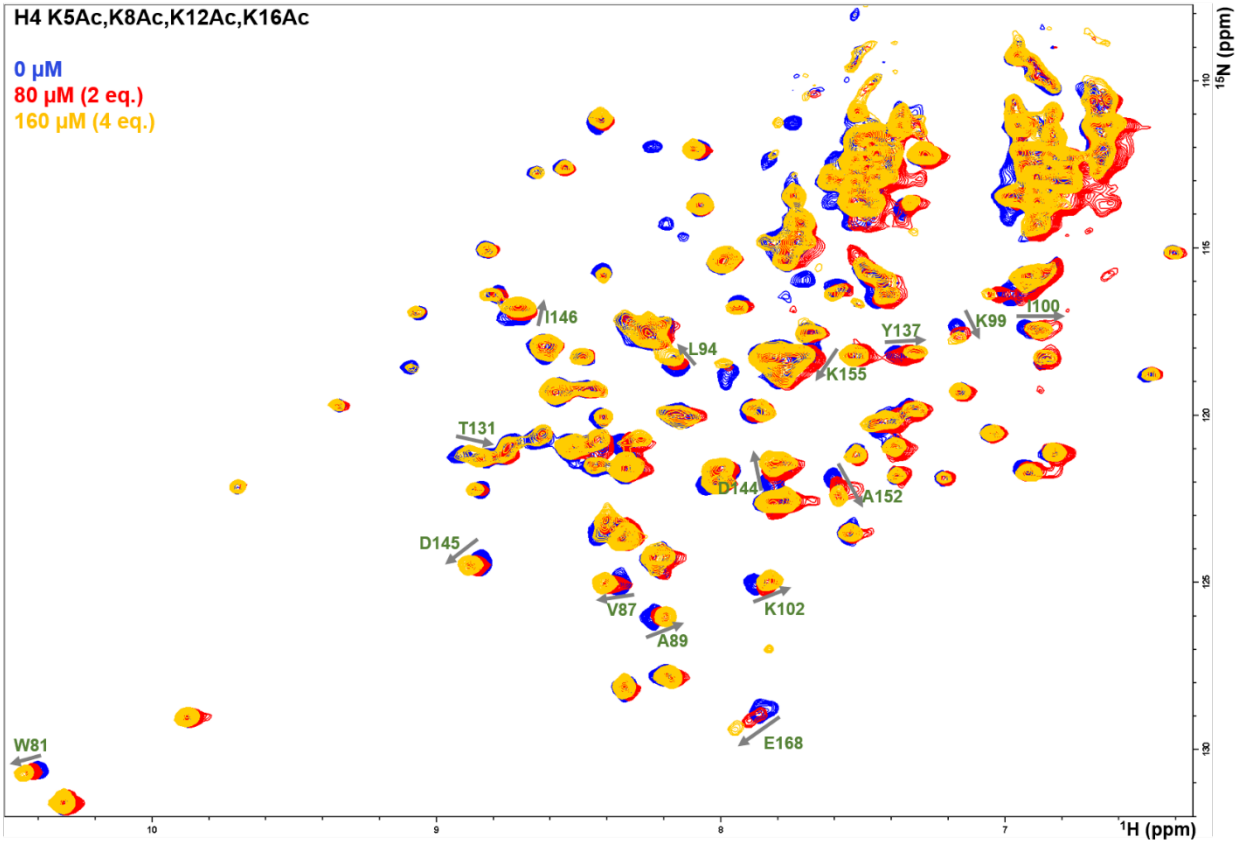


Figure S22: ^{1}H-^{15}N HSQC NMR experiments with 40 bp dsDNA binding to 40 µM of **^{15}N N140A BRD4-BD1**. Spectra are color coded according to peptide concentrations as shown in the legend. Titration was performed at peptide concentrations of 0 µM, 80 µM, and 160 µM. Spectra are color coded accordingly as shown in the legend. Residues that are perturbed greater than the average plus one standard deviation are labelled in green, and the chemical shift perturbation (Δδ) is shown in grey.

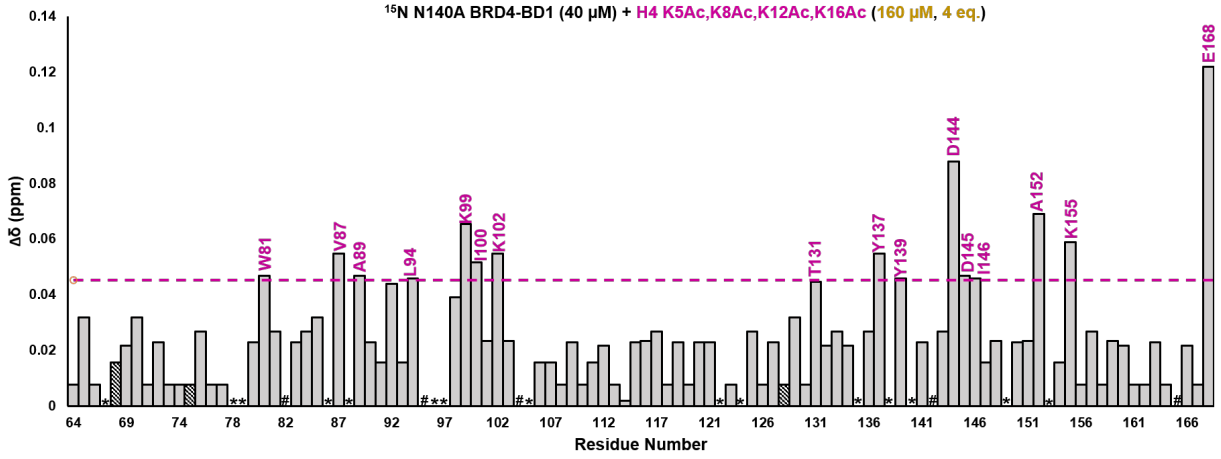


Figure S23: ^{1}H-^{15}N HSQC NMR experiments with H4 K5Ac,K8Ac,K12Ac,K16Ac binding to 40 µM of **^{15}N N140A BRD4-BD1**. Normalized CSPs are plotted as a function of residue upon titration H4 K5Ac,K8Ac,K12Ac,K16Ac for the peptide concentration of 160 µM (4 eq.). Residues that are perturbed greater than the average plus one standard deviation are labeled in pink. A pink line marks this level of significance. Dashed bars (R68, W75 indole side-chain NH resonance, D128) indicate CSPs at 80 µM (2 eq.) of peptide concentration as the chemical shift at 188 µM could not be assigned. * indicates missing resonance and # indicates proline residue.

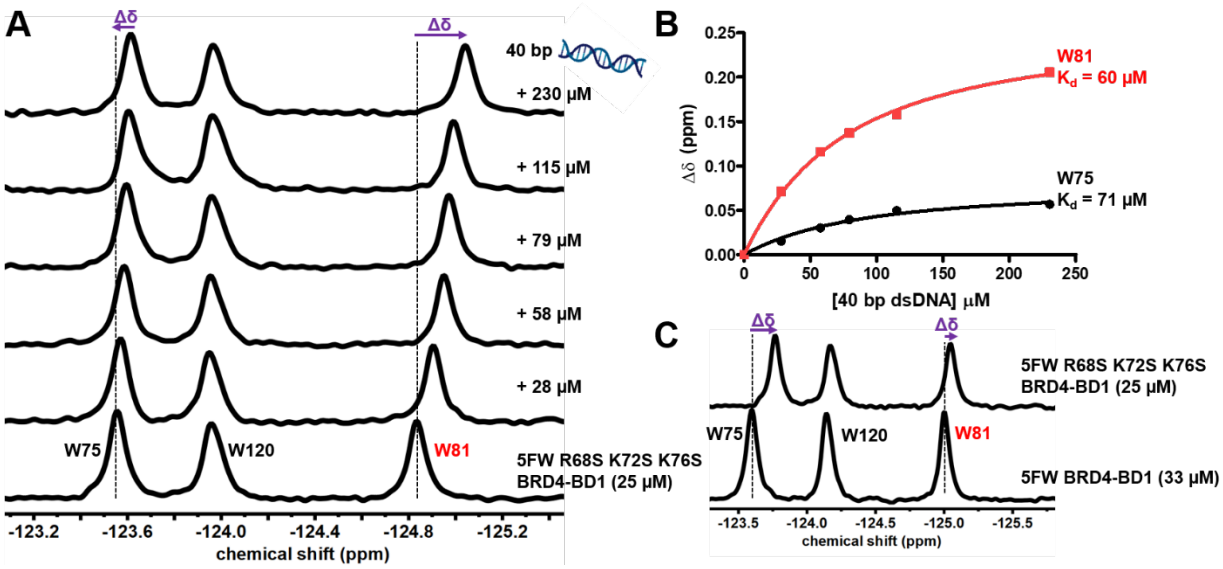


Figure S24: PrOF NMR experiments with 40 bp dsDNA binding to 5FW R68S K72S K76S BRD4-BD1. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 25 µM 5FW R68S K72S K76S BRD4-BD1. (C) Stacked ^{19}F NMR spectra of R68S K72S K76S and unmutated 5FW BRD4-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line. W81 and W75 move upfield by 0.04 ppm and 0.17 ppm respectively, in 5FW R68S K72S K76S BRD4-BD1, when aligned with ^{19}F NMR spectra of unmutated 5FW BRD4-BD1.

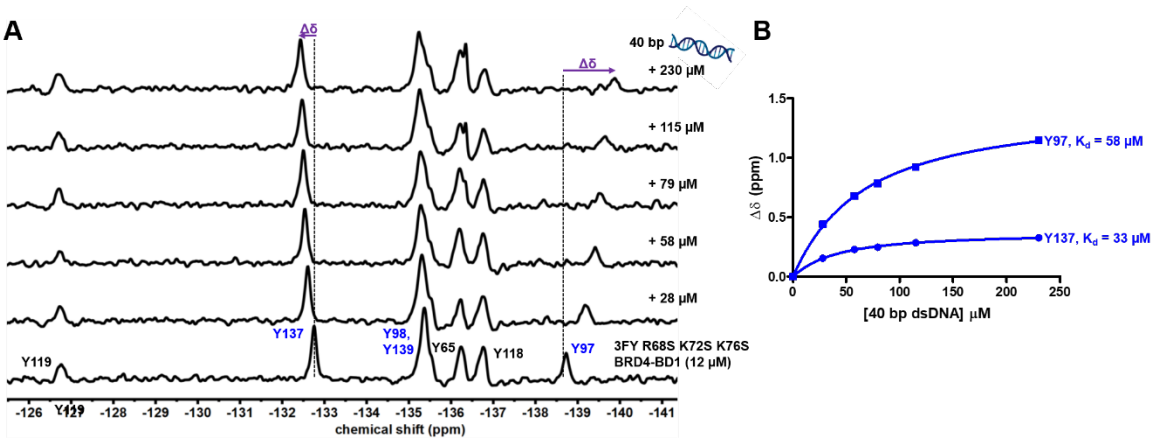


Figure S25: PrOF NMR experiments with 40 bp dsDNA binding to 3FY R68S K72S K76S BRD4-BD1. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 μM) with 12 μM 3FY R68S K72 SK76S BRD4-BD1. Y97, Y98, Y137, and Y139 are around the KAc-binding site in BD1 of BRD4, colored blue. Y65, Y118, and Y119 are shown in black. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

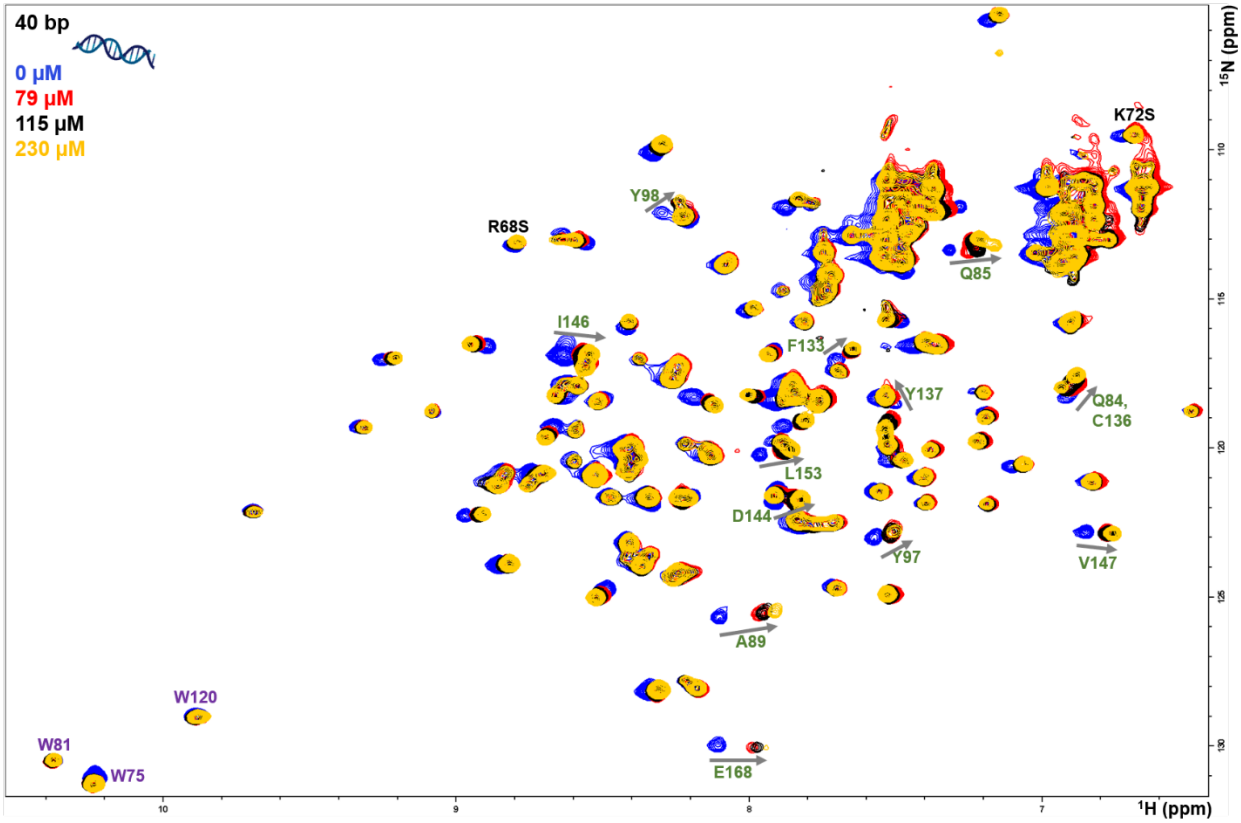


Figure S26: ^{1}H-^{15}N HSQC NMR experiments with 40 bp dsDNA binding to 40 µM of **^{15}N R68S K72S K76S BRD4-BD1**. Spectra are color coded according to DNA concentrations as shown in the legend. Titrations were performed at DNA concentrations of 0 µM, 28 µM, 58 µM, 79 µM, 115 µM, and 230 µM. For clarity, only 4 points are displayed, and spectra are color coded accordingly as shown in the legend. Residues that are perturbed greater than the average plus one standard deviation are labelled in green, and the chemical shift perturbation (Δδ) is shown in grey. Cross-peaks from the indole NH of tryptophan sidechains are shown in purple. Mutated residues R68S and K72S are labeled in black.

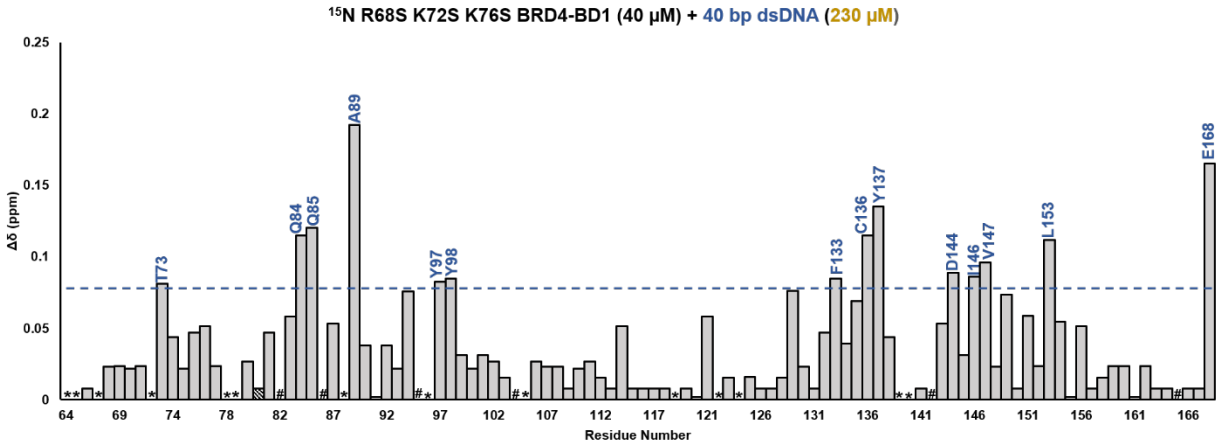
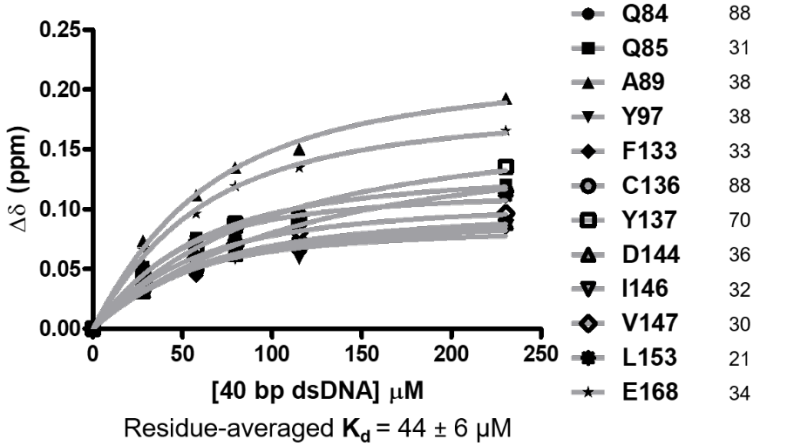


Figure S27: ^{1}H-^{15}N HSQC NMR experiment with 40 bp dsDNA binding to **^{15}N R68S K72S K76S BRD4-BD1.** Normalized CSPs are plotted as a function of residue upon titration of 40 bp dsDNA for the DNA concentration of 230 µM and residues that are perturbed greater than the average plus one standard deviation are labeled in blue. A blue dashed line marks this level of significance. Dashed bar indicates the CSP of W81 indole side-chain NH resonance at 115 µM of DNA as the chemical shift at 230 µM could not be assigned. This CSP was also accounted for average and standard deviation calculations. * indicates missing resonance and # indicates proline residue.

A



B

Ligand	Chemical Shift Perturbation (Δδ) (ppm)											
[40 bp dsDNA] μM	Q84	Q85	A89	Y97	F133	C136	Y137	D144	I146	V147	L153	E168
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
28	0.0318	0.0447	0.0736	0.0379	0.0380	0.0318	0.0439	0.0313	0.0313	0.0391	0.0516	0.0662
58	0.0491	0.0760	0.1116	0.0516	0.0447	0.0491	0.0667	0.0548	0.0547	0.0588	0.0736	0.0963
79	0.0719	0.0893	0.1346	0.0588	0.0638	0.0719	0.0878	0.0626	0.0625	0.0704	0.0811	0.1193
115	0.0919	0.0962	0.1502	0.0588	0.0638	0.0919	0.0919	0.0704	0.0704	0.0811	0.0887	0.1346
230	0.1148	0.1205	0.1925	0.0825	0.0848	0.1148	0.1354	0.0887	0.0860	0.0963	0.1116	0.1656

Figure S28: (A) Binding isotherms of ^{1}H-^{15}N HSQC NMR titration of 40 bp dsDNA binding to 40 μM of **^{15}N R68S K72S K76S BRD4-BD1**. (B) Chemical shift perturbations of significant residues to plot residue- averaged K_d.

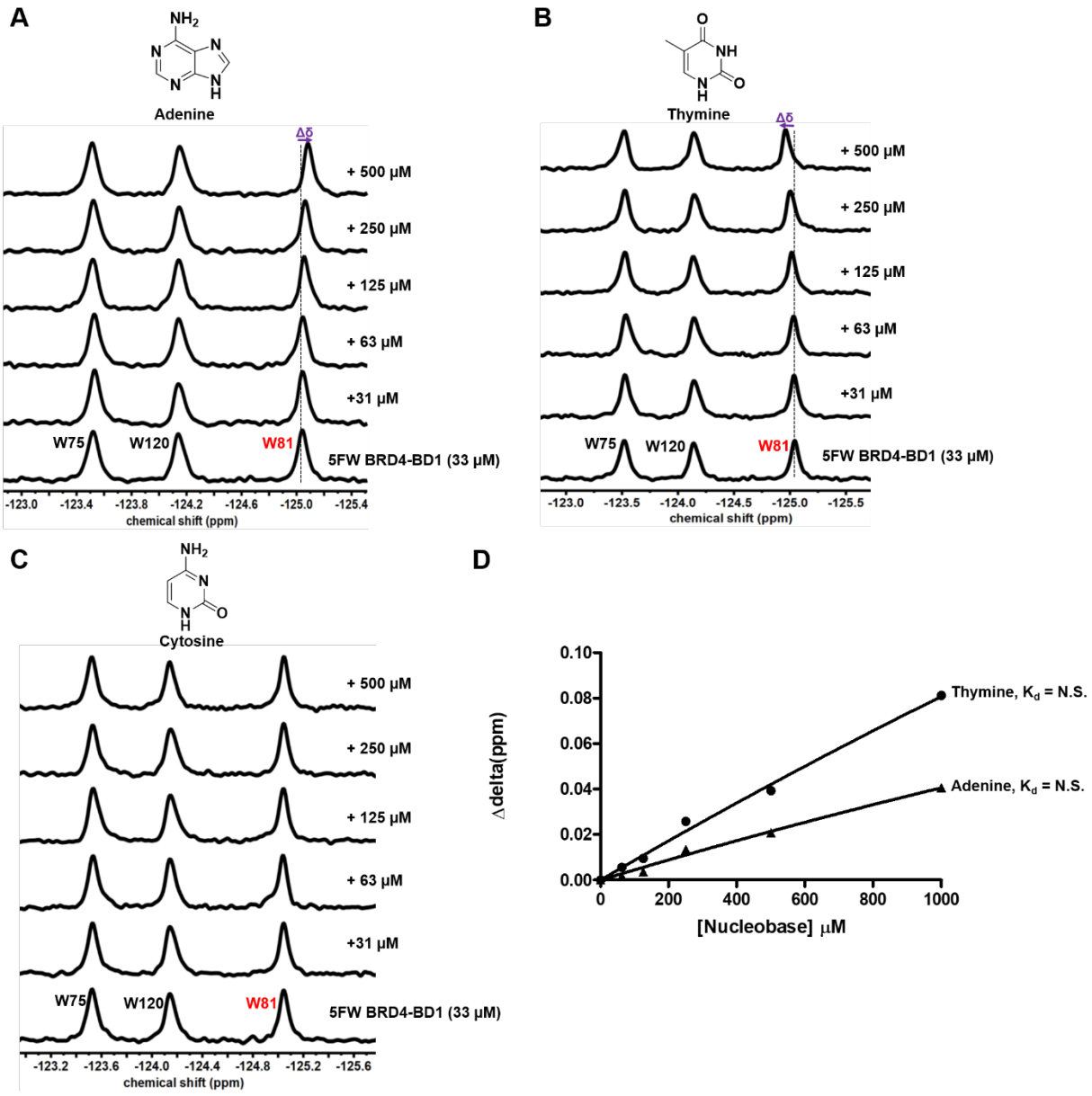


Figure S29: PrOF NMR experiments with nucleobases binding to 5FW BRD4-BD1. Stacked ^{19}F NMR spectra of (A) adenine (B) thymine (C) cytosine and (D) Binding isotherm of thymine and adenine with 33 µM 5FW BRD4-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

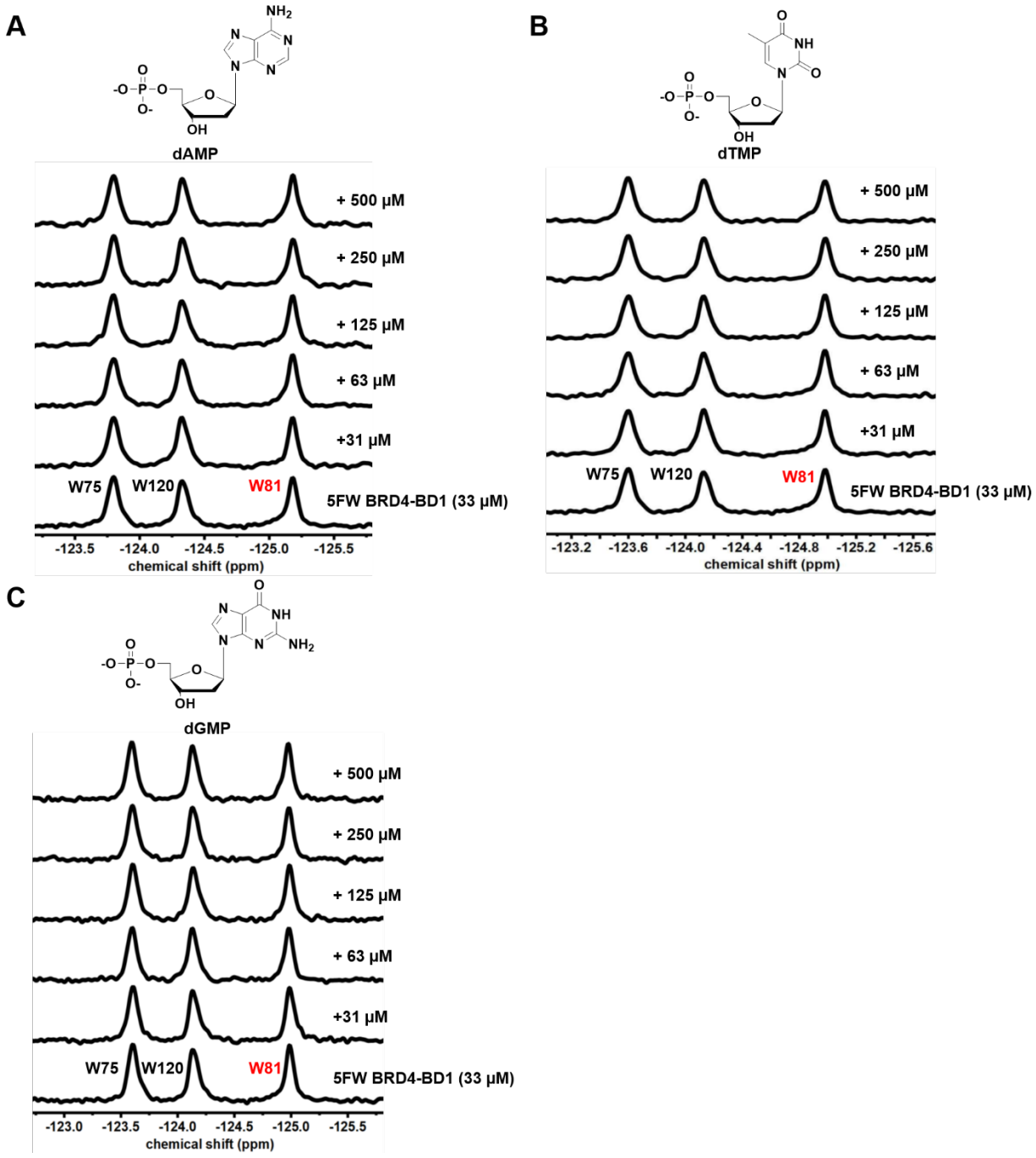


Figure S30: PrOF NMR experiments with deoxyribonucleotides binding to 5FW BRD4-BD1. Stacked ^{19}F NMR spectra of (A) dAMP (B) dTMP and (C) dGMP with 33 µM 5FW BRD4-BD1. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red.

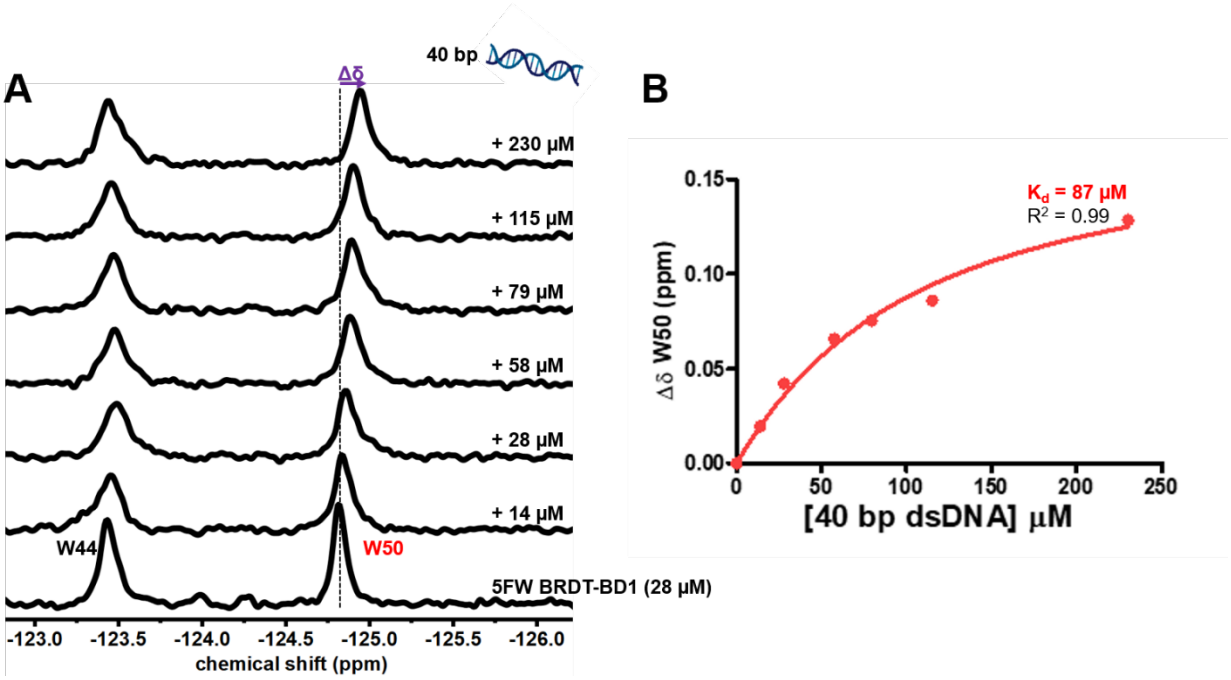


Figure S31: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRDT-BD1. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 28 µM 5FW BRDT-BD1. W50 is the WPF shelf tryptophan in BD1 of BRDT, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

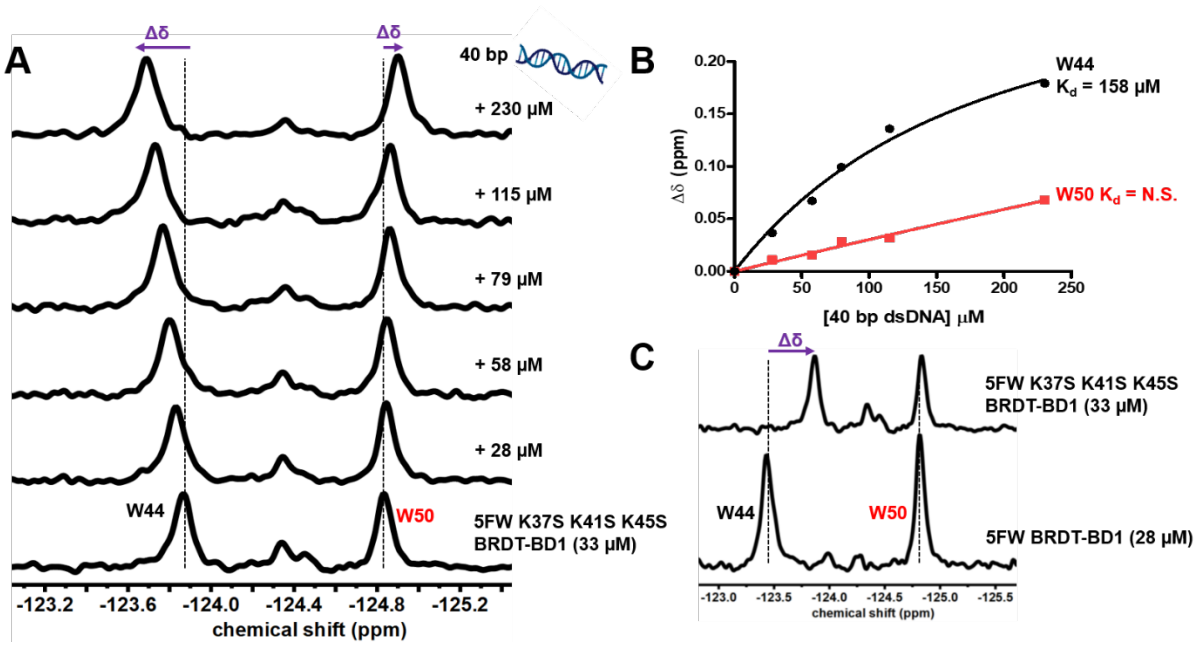


Figure S32: PrOF NMR experiments with 40 bp dsDNA binding to 5FW K37S K41S K45S BRDT-BD1. (A) Stacked ^{19}F NMR spectra and (B) binding isotherm of 40 bp dsDNA (28-230 μM) with 33 μM 5FW K37S K41S K45S BRDT-BD1. (C) Stacked ^{19}F NMR spectra of 5FW K37S K41S K45S and unmutated BRDT-BD1. W50 is the WPF shelf tryptophan in BD1 of BRDT, colored red. The change in the chemical shift (Δδ) is shownin purple and is indicated by the dashed line. W44 moves upfield by 0.44 ppm in 5FW K37S K41S K45S BRDT-BD1when aligned with ^{19}F NMR spectra of unmutated 5FW BRDT-BD1.

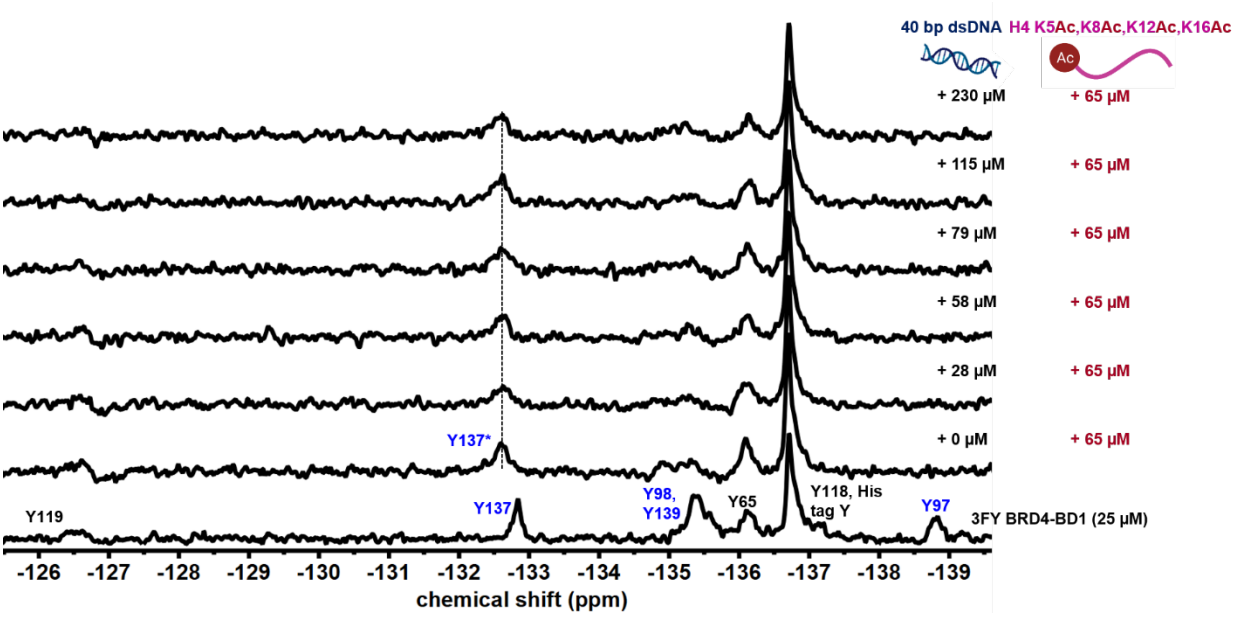


Figure S33: PrOF NMR experiments with 40 bp dsDNA (14-230 µM) binding to 25 µM 3FY BRD4-BD1 pre-bound to 65 µM H4 K5Ac,K8Ac,K12Ac,K16Ac. Y97, Y98, Y137, and Y139 are around the KAc-binding site in BD1 of BRD4, colored blue. Y65, Y118, and Y119 are shown in black. The dashed line indicates minimal CSP (0.03 ppm) of Y137. ^{19}F resonance for the ligand-bound protein is indicated with an asterisk.

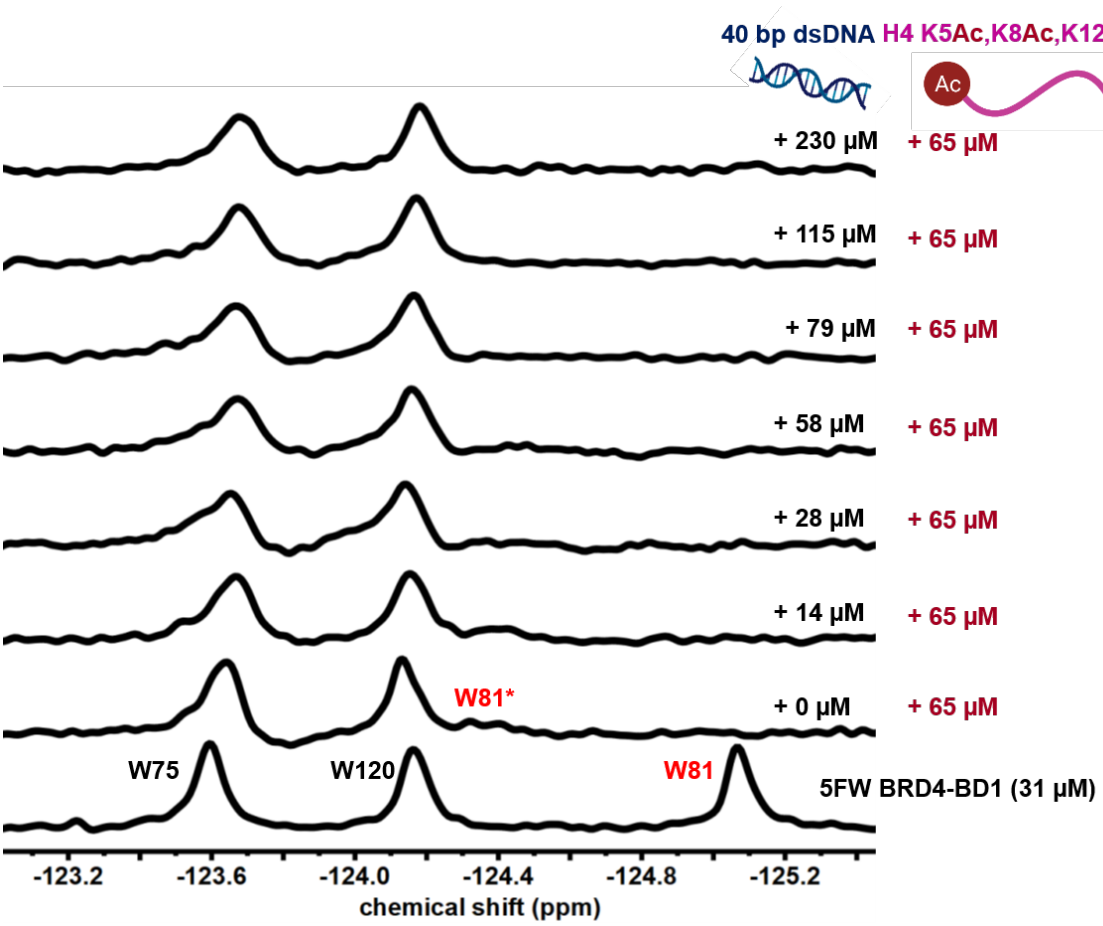


Figure S34: PrOF NMR experiments with 40 bp dsDNA (14-230 µM) binding to 31 µM 5FW BRD4-BD1 pre-bound to 65 µM H4 K5Ac,K8Ac,K12Ac,K16Ac. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. ^{19}F resonance for the ligand-bound protein is indicated with an asterisk.

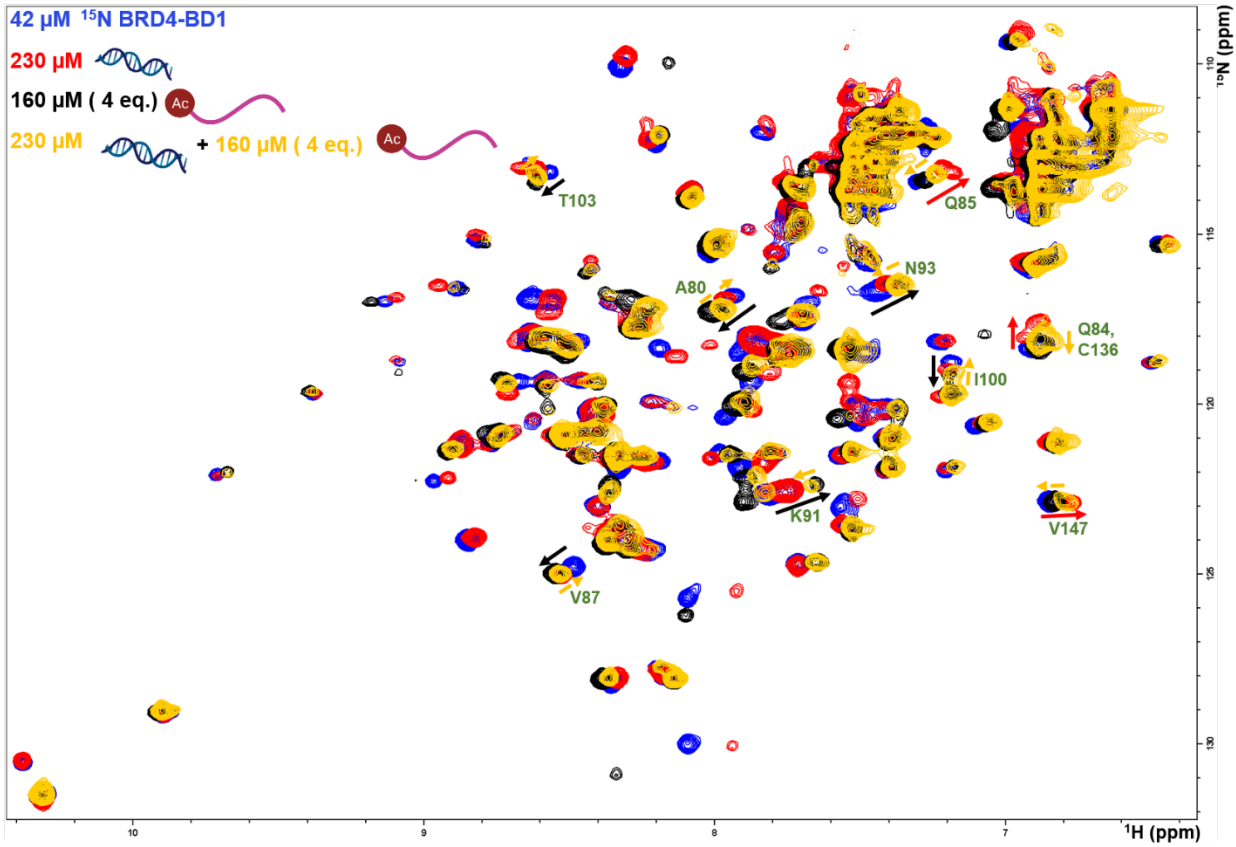


Figure S35: Overlay of ^{1}H-^{15}N HSQC NMR spectra of 42 µM of ^{15}N BRD4-BD1 (blue) and in the presence of 230 µM 40 bp dsDNA (red), 160 µM H4 K5Ac,K8Ac,K12Ac,K16Ac (black), or both 230 µM 40 bp dsDNA and 160 µM H4 K5Ac,K8Ac,K12Ac,K16Ac (yellow). Arrows trace the linear trajectory between apo and peptide-bound BRD4-BD1 (black) or apo and DNA-bound BRD4-BD1 (red) and upon titration of dsDNA into peptide-bound BRD4-BD1 (yellow) and vice versa.

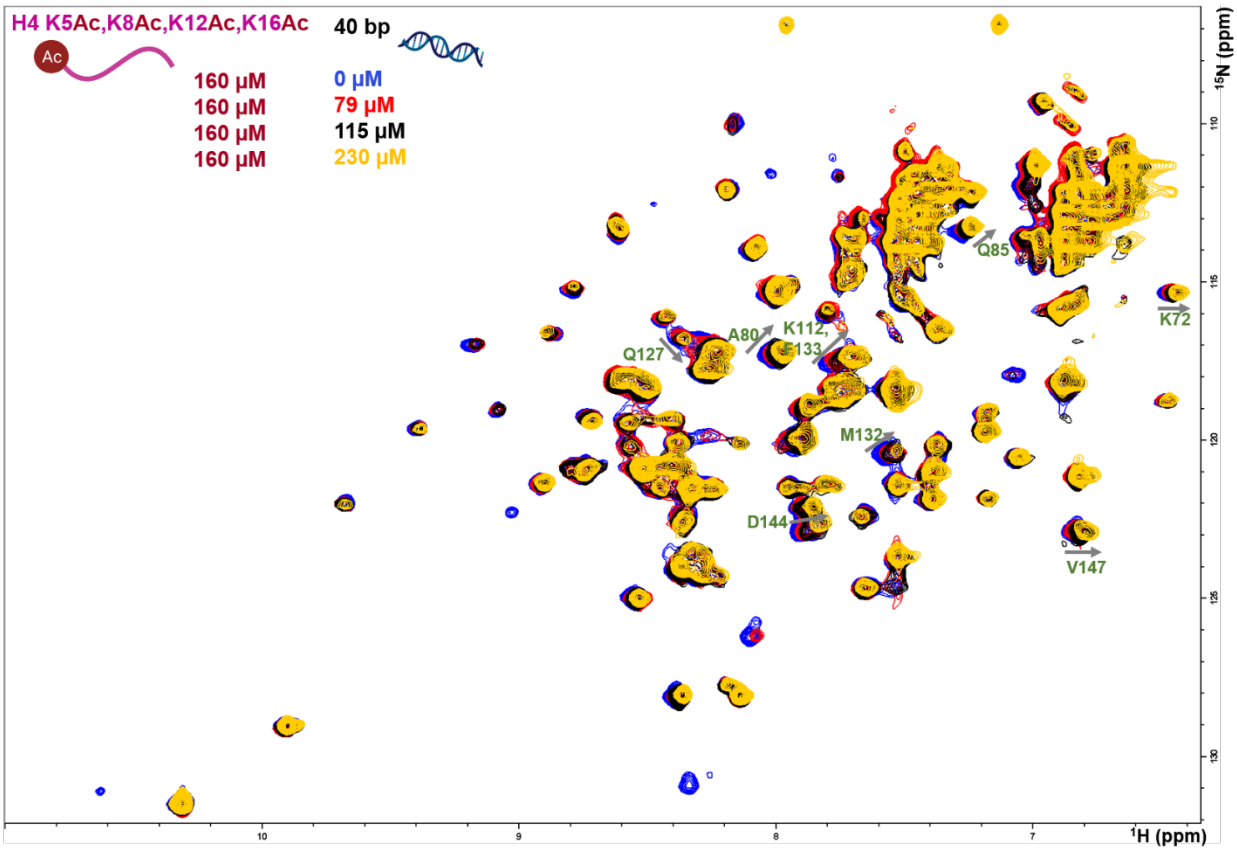


Figure S36: ^{1}H-^{15}N HSQC NMR experiments with 40 bp dsDNA binding to 42 µM of **^{15}N BRD4-BD1** pre-bound to 160 µM H4 K5Ac,K8Ac,K12Ac,K16Ac. Spectra are color coded according to DNA concentrations as shown in the legend. Titrations were performed at DNA concentrations of 0 µM, 28 µM, 58 µM, 79 µM, 115 µM, and 230 µM. For clarity, only 4 points are displayed, and spectra are color coded accordingly as shown in the legend. Residues that are perturbed greater than the average plus one standard deviation are labelled in green, and the chemical shift perturbation (Δδ) is shown in grey.

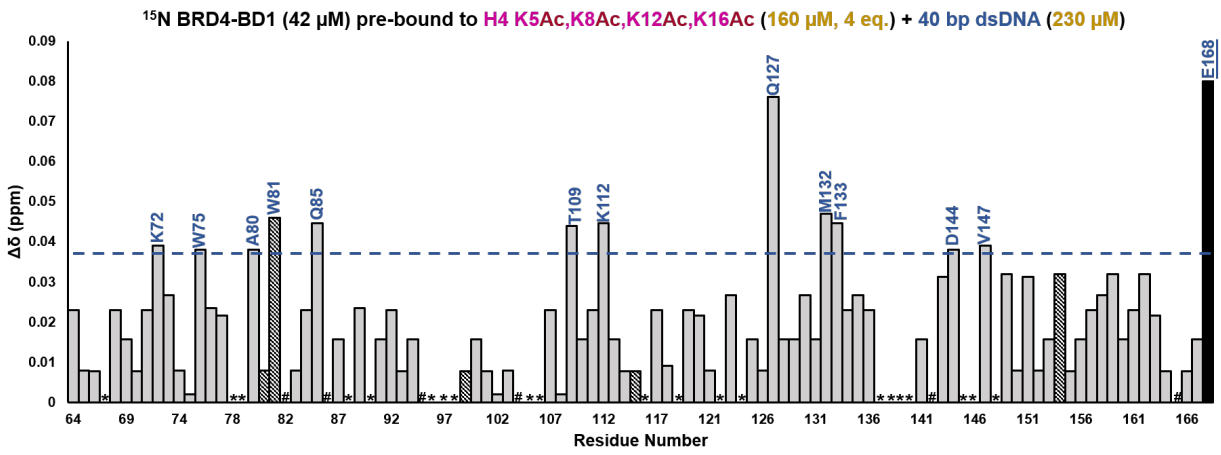
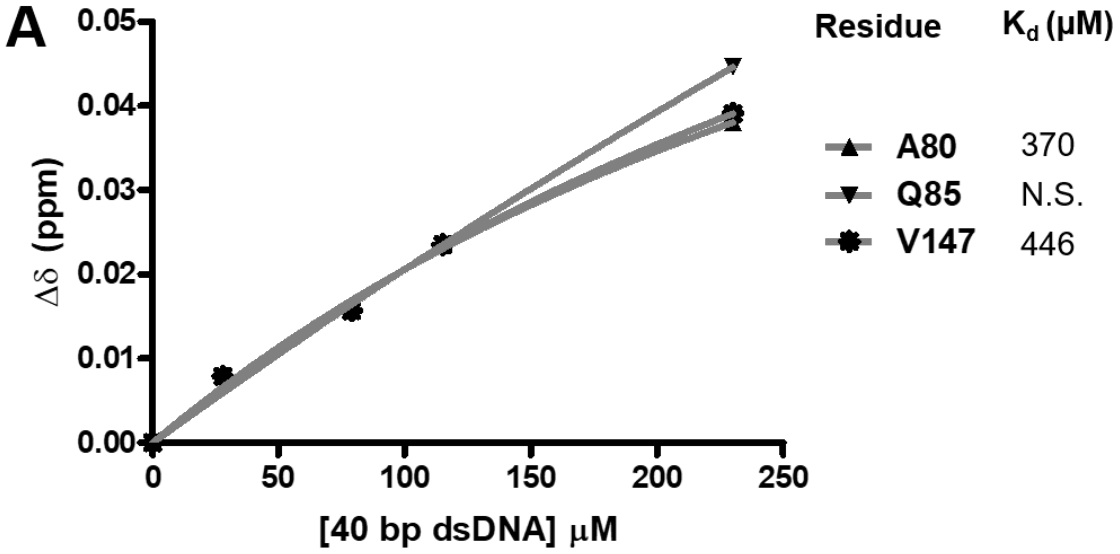


Figure S37: ^{1}H-^{15}N HSQC NMR experiment with 40 bp dsDNA binding to 42 µM of **^{15}N BRD4-BD1** pre-bound to 160 µM H4 K5Ac,K8Ac,K12Ac,K16Ac**.** Normalized CSPs are plotted as a function of residue upon titration of 40 bp dsDNA for the DNA concentration of 230 µM and residues that are perturbed greater than the average plus one standard deviation are labeled in blue. A blue dashed line marks this level of significance. Dashed bars indicate a CSP at a different concentration of DNA (79 µM for W81 amide NH resonance and 115 µM for W81 indole side-chain NH resonance, K99, E154) as the chemical shift at 230 µM could not be assigned. These CSPs were also accounted for average and standard deviation calculations. E168 broadened to baseline in the DNA-bound state and is arbitrarily assigned a CSP of 0.08 ppm and is indicated by a black bar.* indicates missing resonance and # indicates proline residue.

A



B

Ligand [40 bp dsDNA] μM	Chemical Shift Perturbation (Δδ) (ppm) A80	Q85	V147
0	0.0000	0.0000	0
28	0.0079	0.0078	0.0079
79	0.0157	0.0156	0.0157
115	0.0235	0.0234	0.0235
230	0.0380	0.0447	0.0391

Figure S38: (A) Binding isotherms of ^{1}H-^{15}N HSQC NMR titration of 40 bp dsDNA binding to 42 μM of **^{15}N BRD4-BD1** pre-bound to 160 μM H4 K5Ac,K8Ac,K12Ac,K16Ac. (B) Chemical shift perturbations of significant residues to plot K_d values. Only residues giving $R^2 \geq 0.99$ are shown.

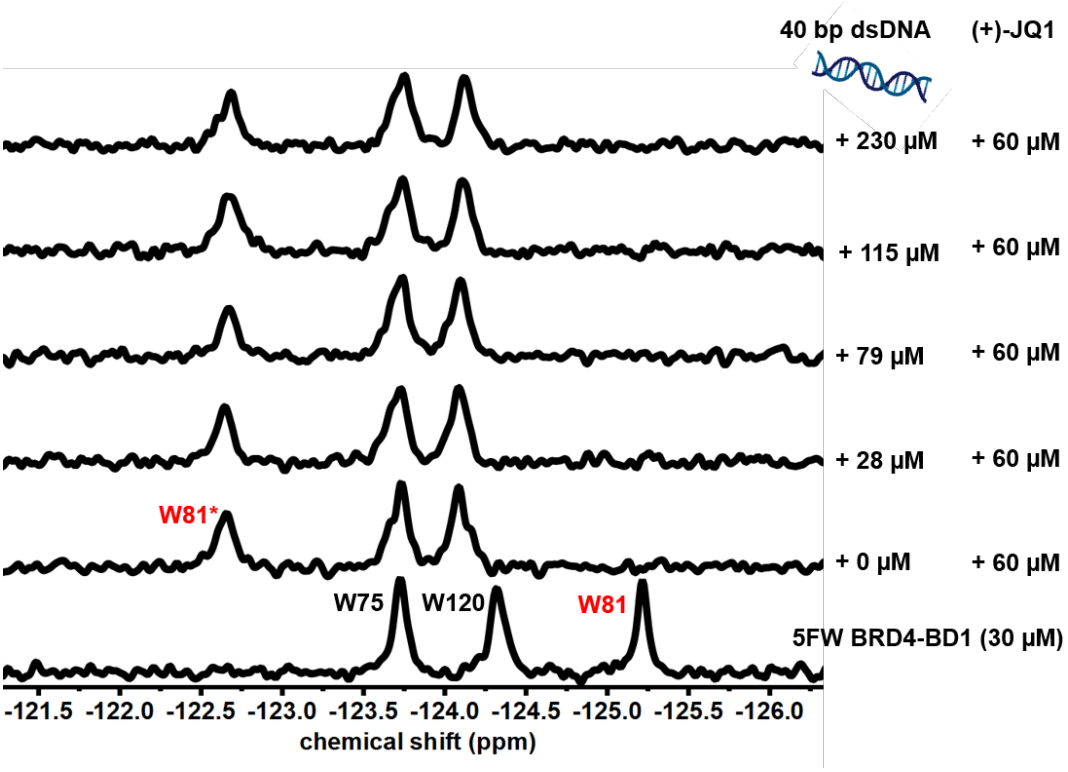


Figure S39: PrOF NMR experiments with 40 bp dsDNA (14-230 µM) binding to 31 µM 5FW BRD4-BD1 pre-bound to 65 µM H4 K5Ac,K8Ac,K12Ac,K16Ac. W81 is the WPF shelf tryptophan in BD1 of BRD4, colored red. ^{19}F resonance for the ligand-bound protein is indicated with an asterisk.

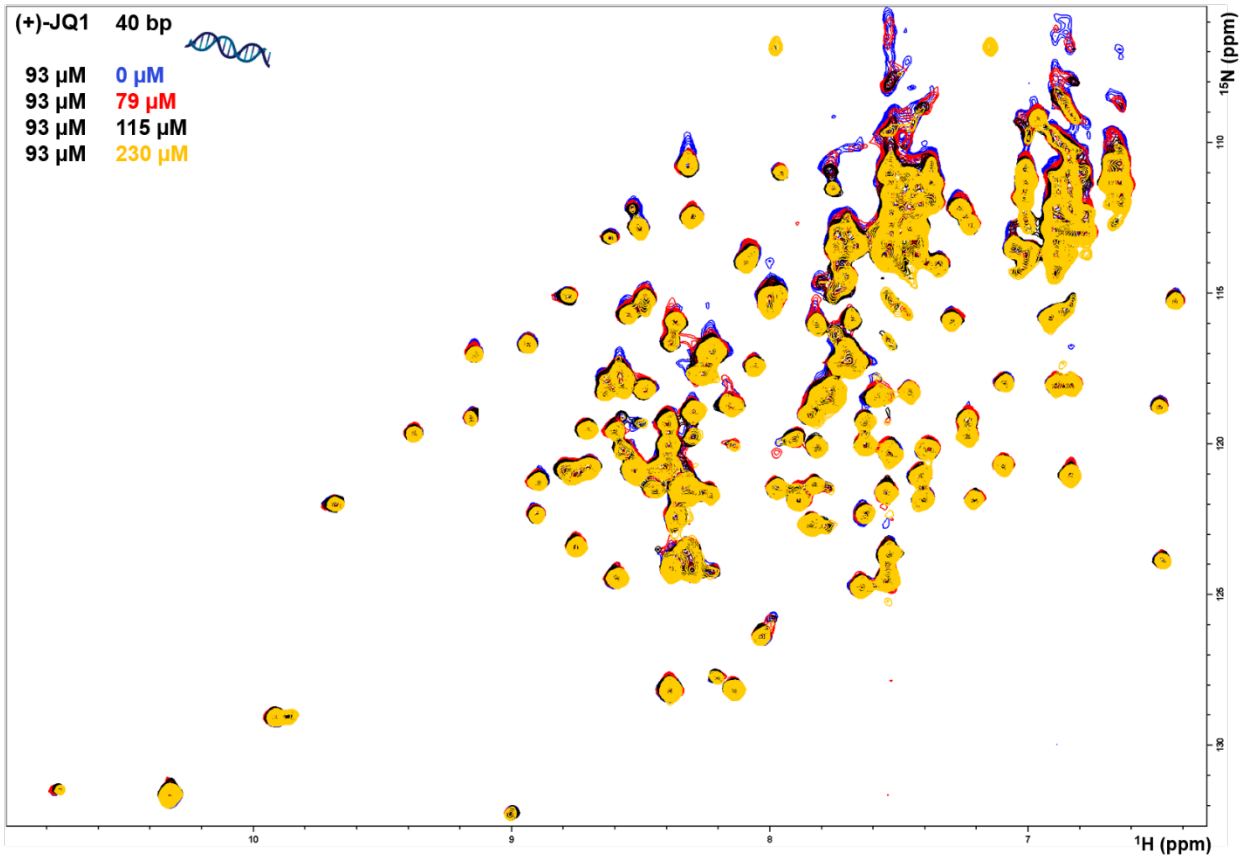


Figure S40: ^{1}H-^{15}N HSQC NMR experiments with 40 bp dsDNA binding to 42 µM of **^{15}N BRD4-BD1** pre-bound to 93 µM (+)-JQ1. Spectra are color coded according to DNA concentrations as shown in the legend. Titrations were performed at DNA concentrations of 0 µM, 28 µM, 58 µM, 79 µM, 115 µM, and 230 µM. For clarity, only 4 points are displayed, and spectra are color coded accordingly as shown in the legend.

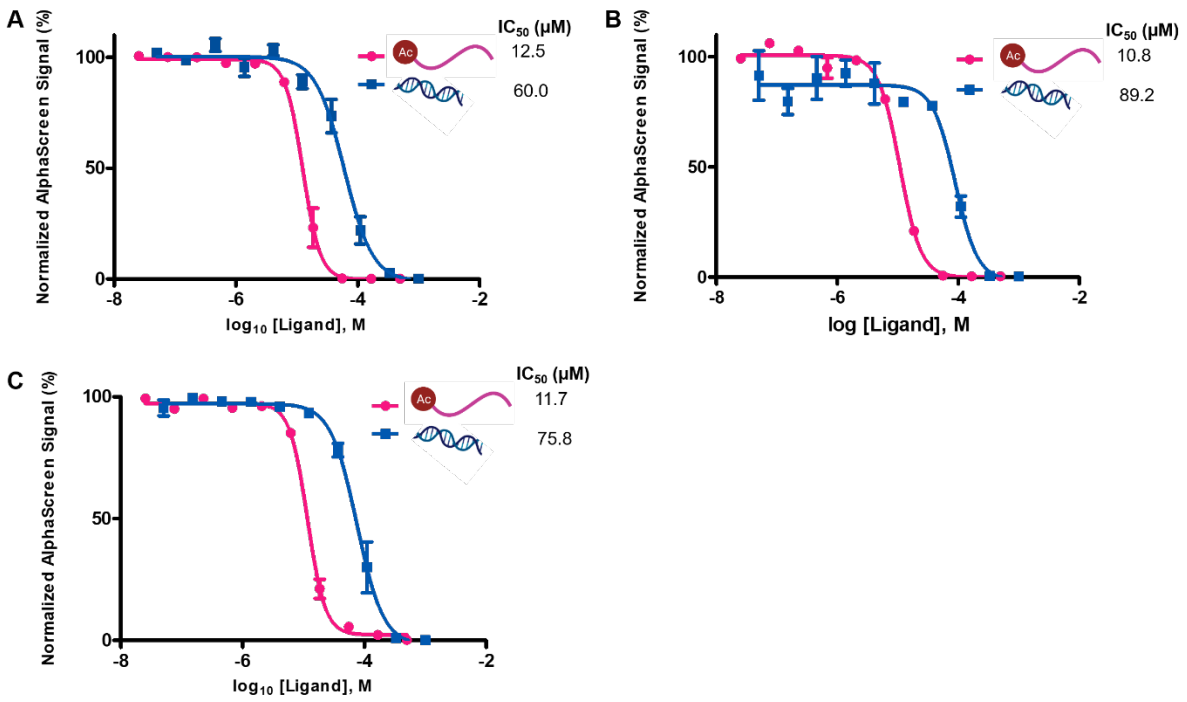


Figure S41: AlphaScreen competition experiments with 9xHis-BRD4-BD1 using a biotinylated histone peptide, reported as mean ± SD of three experimental replicates (A, B, C) performed in duplicate giving an average IC_{50} of 75 ± 12 μM.

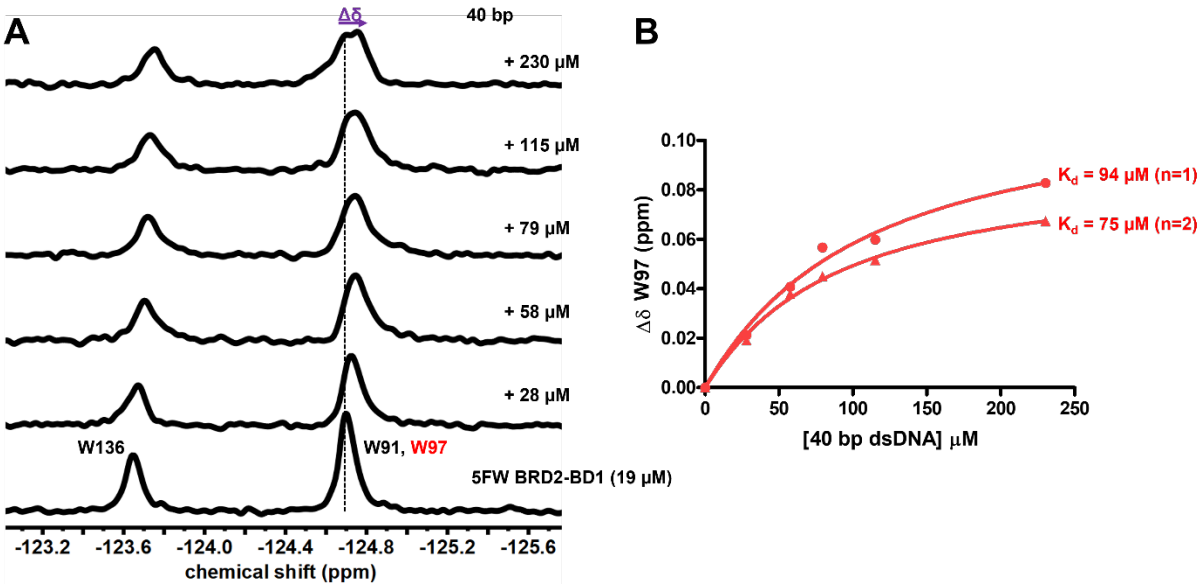


Figure S42: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRD2-BD1. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherms of 40 bp dsDNA (28-230 µM) with 19 µM 5FW BRD2-BD1. W91 is the WPF shelf tryptophan in BD1 of BRD2, colored red.[3] The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

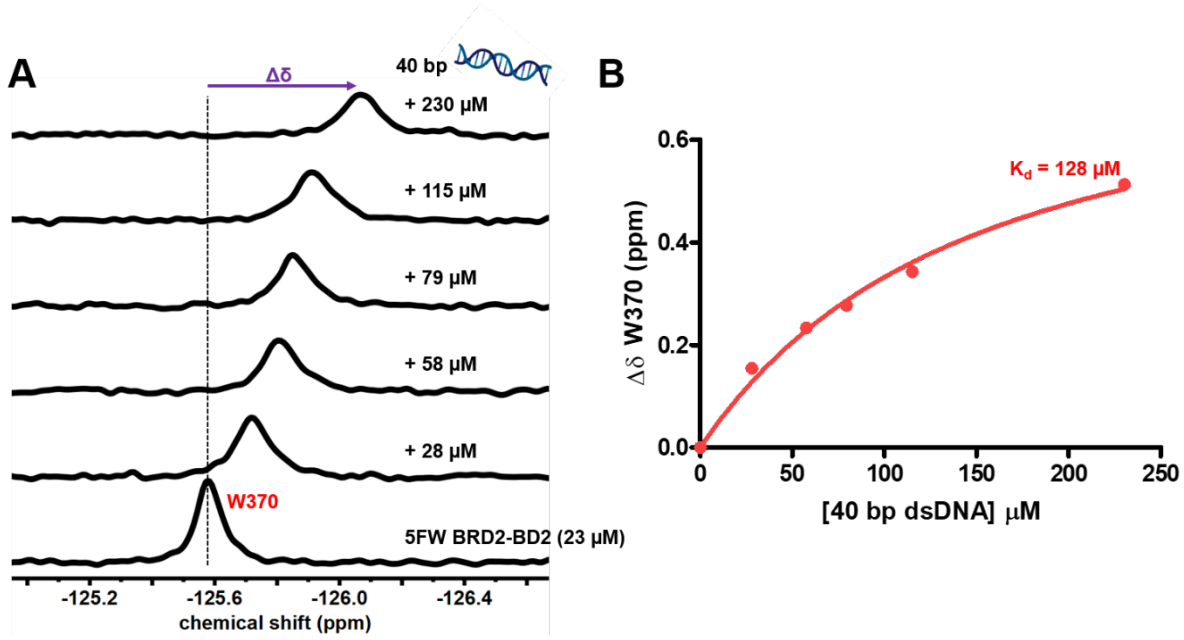


Figure S43: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRD2-BD2. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 23 µM 5FW BRD2-BD2. W370 is the WPF shelf tryptophan in BD2 of BRD2, colored red.[3] The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

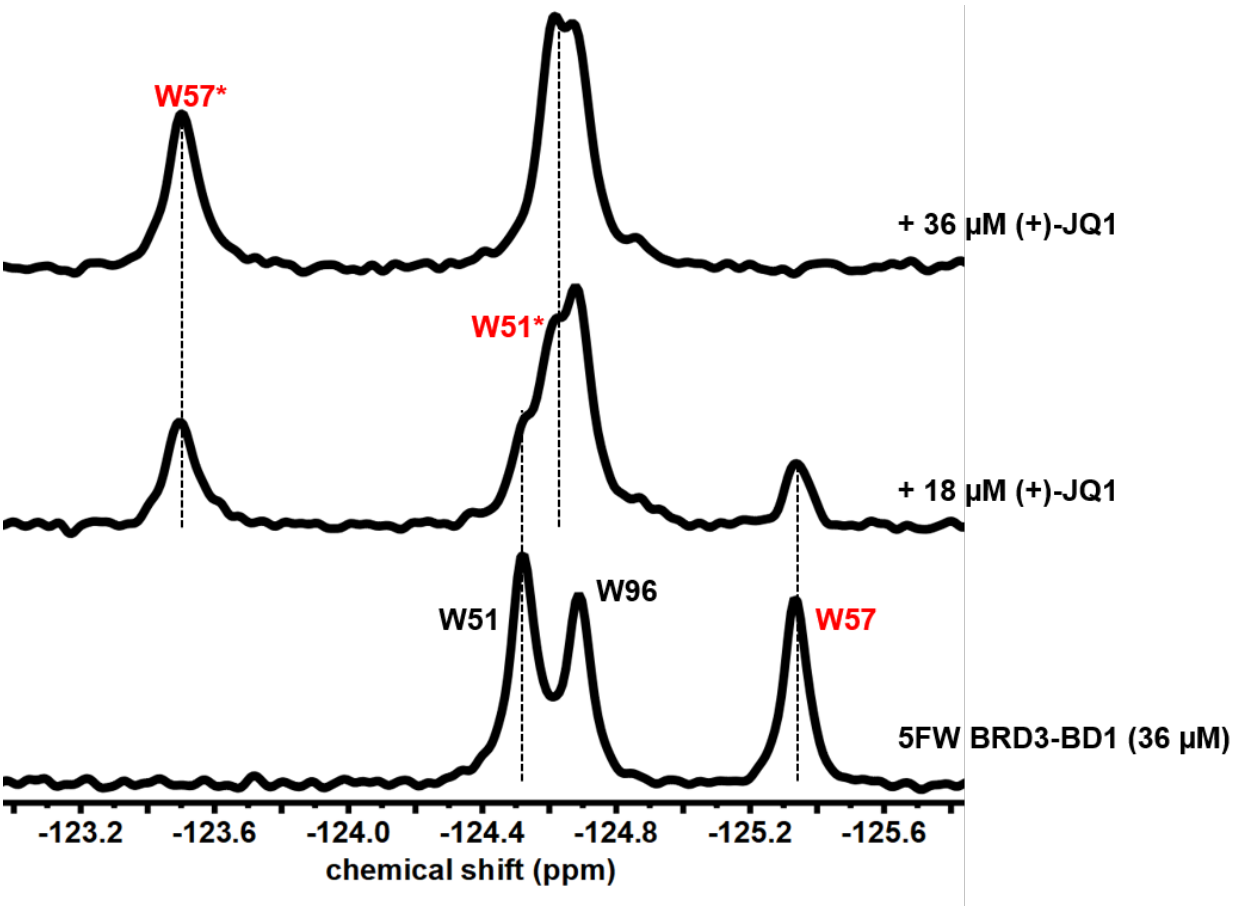


Figure S44: PrOF NMR experiment of 5FW BRD3-BD1 with (+)-JQ1 to assign ^{19}F resonances. W57 is the WPF shelf tryptophan in BD2 of BRD2, colored red. Dashed line indicates change in the appearance of a resonance due to interaction of the protein with (+)-JQ1.

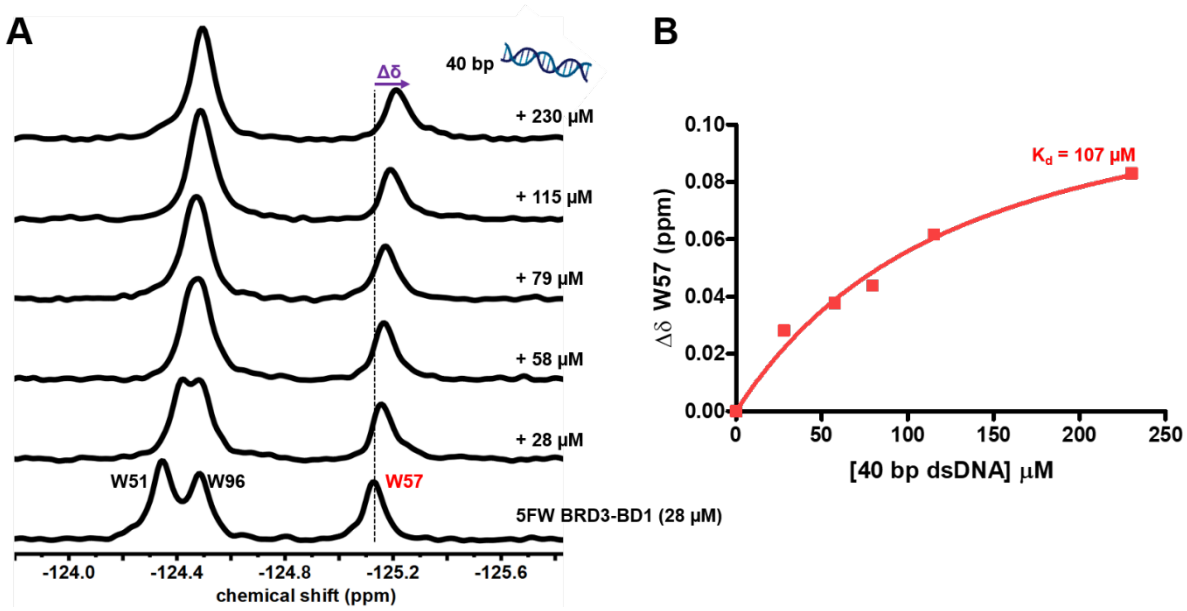


Figure S45: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRD3-BD1. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 28 µM 5FW BRD3-BD1. W57 is the WPF shelf tryptophan in BD1 of BRD3, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

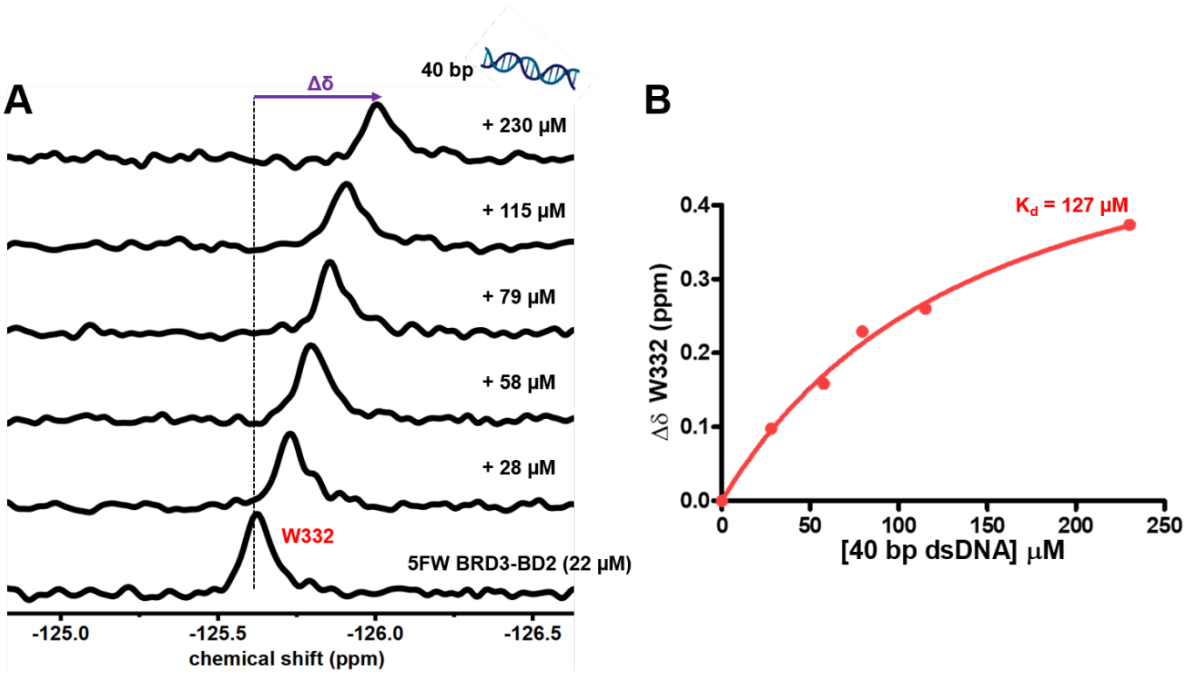


Figure S46: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRD3-BD2. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 22 µM 5FW BRD3-BD1. W332 is the WPF shelf tryptophan in BD2 of BRD3, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

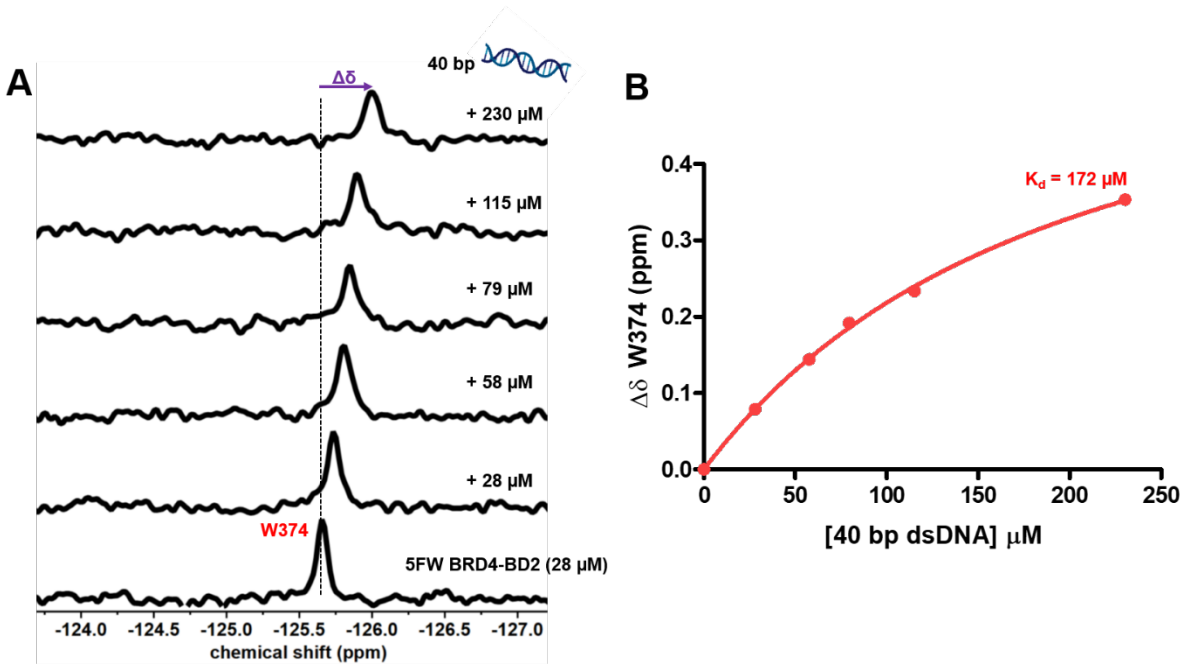


Figure S47: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRD4-BD2. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 μM) with 28 μM 5FW BRD4-BD2. W374 is the WPF shelf tryptophan in BD2 of BRD4, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

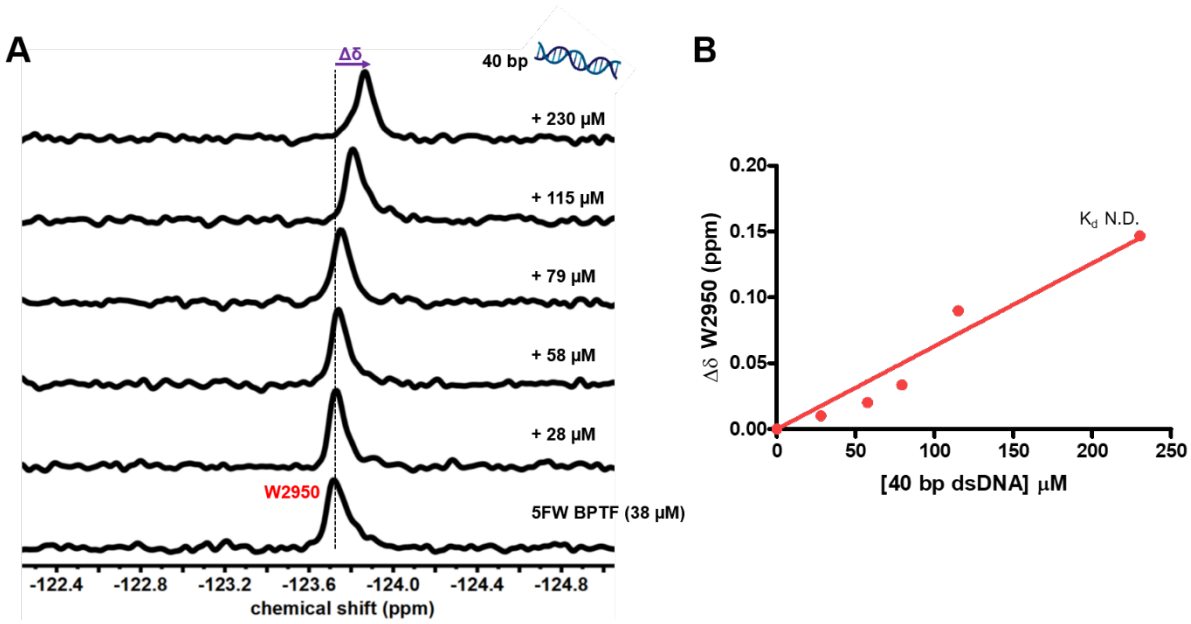


Figure S48: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BPTF. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 38 µM 5FW BPTF. W2950 is the WPF shelf tryptophan in BPTF, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

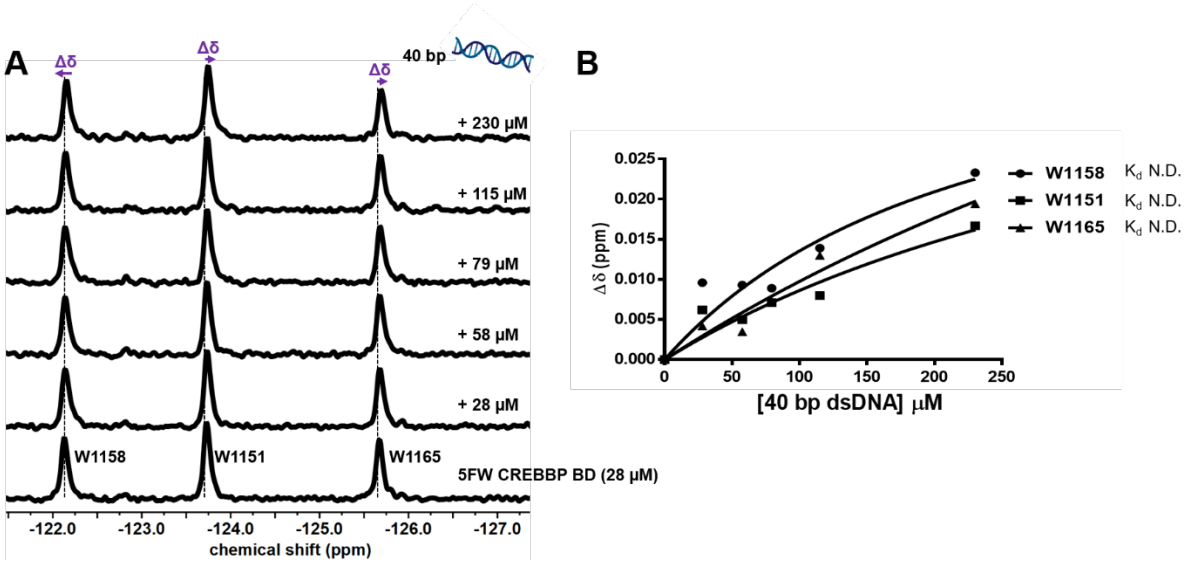


Figure S49: PrOF NMR experiments with 40 bp dsDNA binding to 5FW CREBBP. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 28 µM 5FW CREBBP. W1151, W1158, and W1165 are tryptophans situated on the αB-helix of CREBBP BD, colored red. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

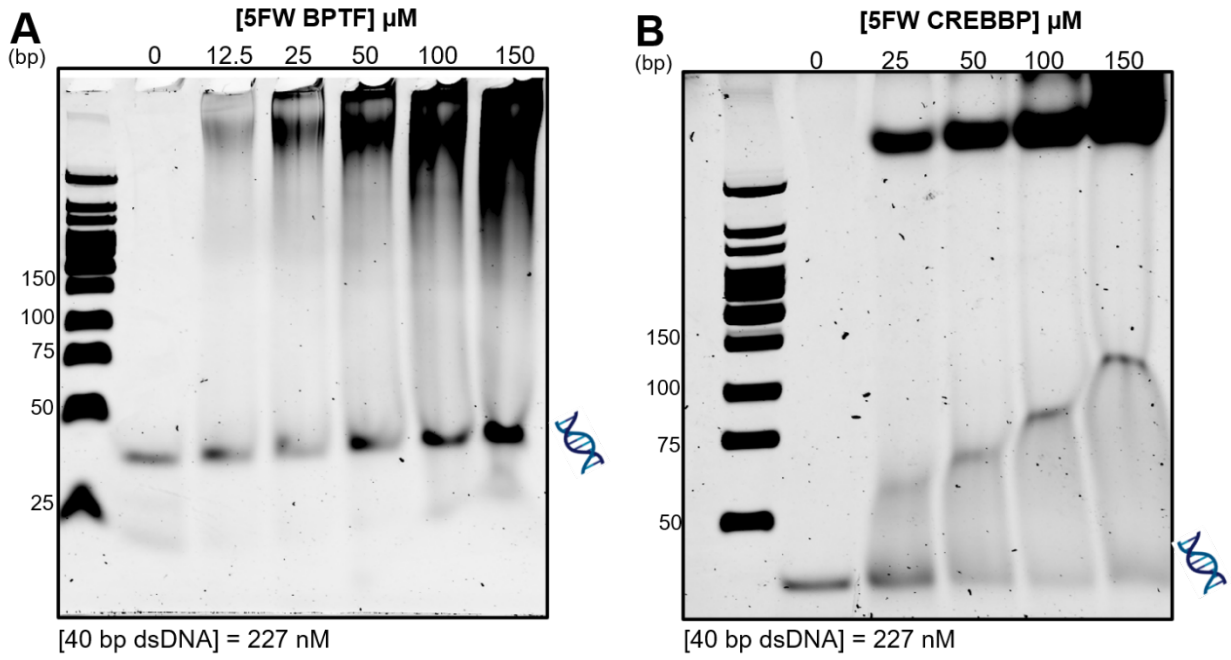


Figure S50: EMSA titrations of (A) 5FW BPTF and (B) 5FW CREBBP with 40 bp dsDNA.

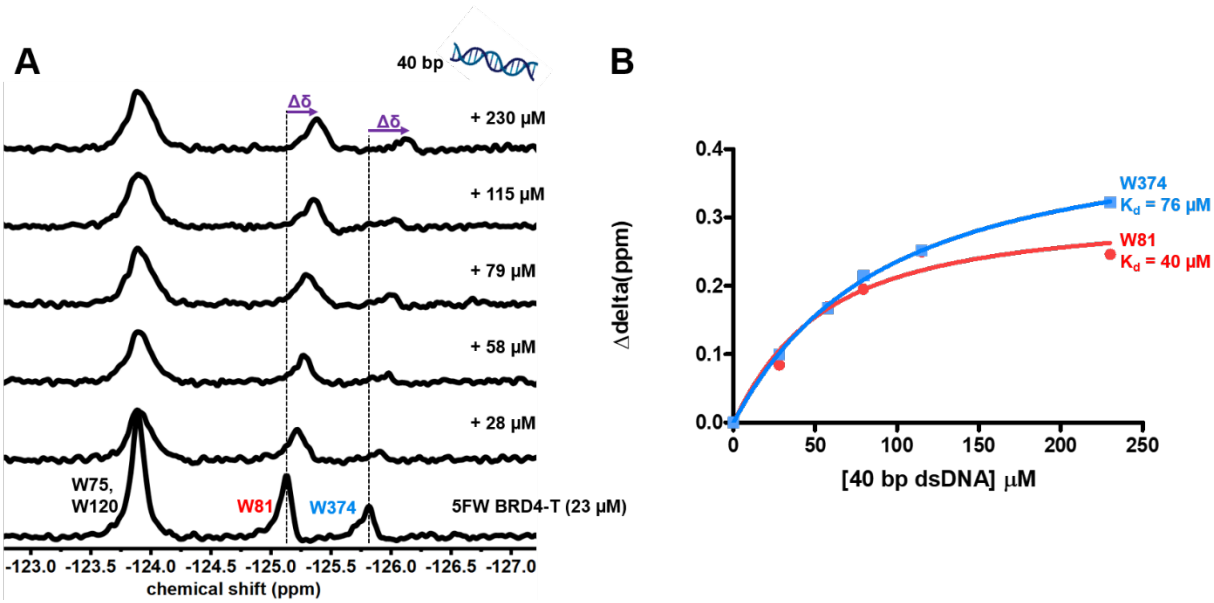


Figure S51: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRD4-T. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 23 µM 5FW BRD4-T. W81 and W374 are the WPF shelf tryptophans in BD1 and BD2, colored red and blue respectively. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

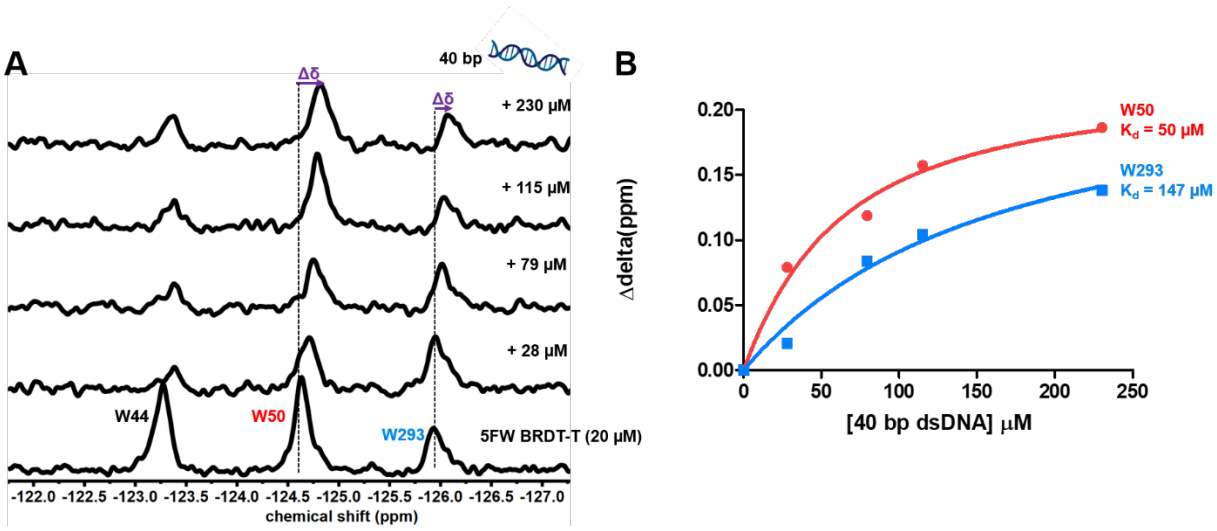


Figure S52: PrOF NMR experiments with 40 bp dsDNA binding to 5FW BRDT-T. (A) Stacked ^{19}F NMR spectra and (B) Binding isotherm of 40 bp dsDNA (28-230 µM) with 20 µM 5FW BRDT-T. W50 and W293 are the WPF shelf tryptophans in BD1 and BD2, colored red and blue respectively. The change in the chemical shift (Δδ) is shown in purple and is indicated by the dashed line.

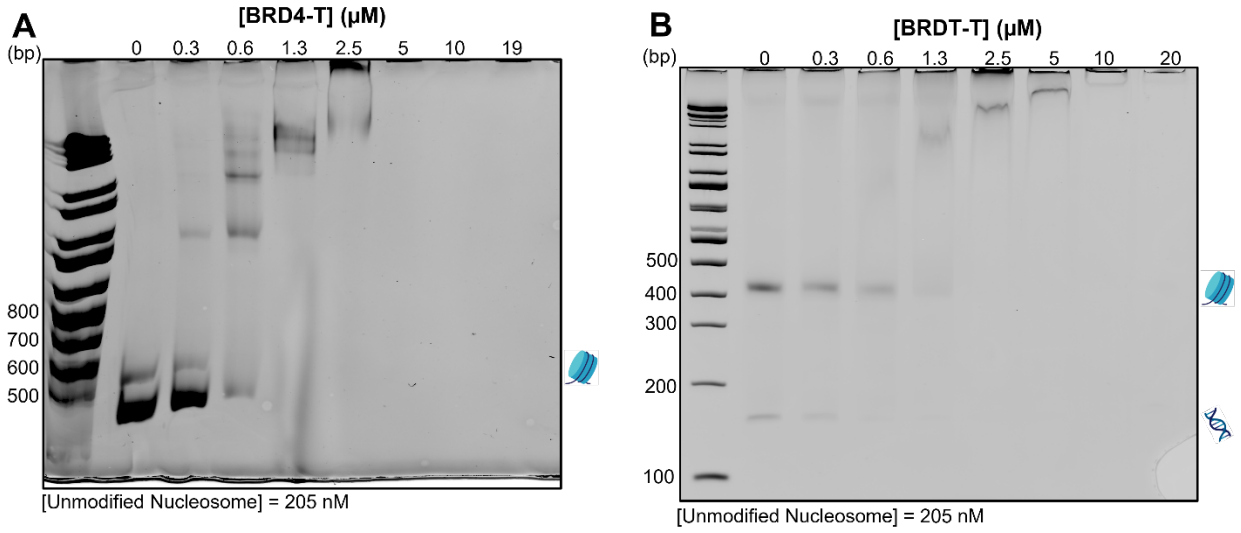


Figure S53: EMSA titrations of BRD4-T with (A) unmodified nucleosomes and (B) 40 bp dsDNA.

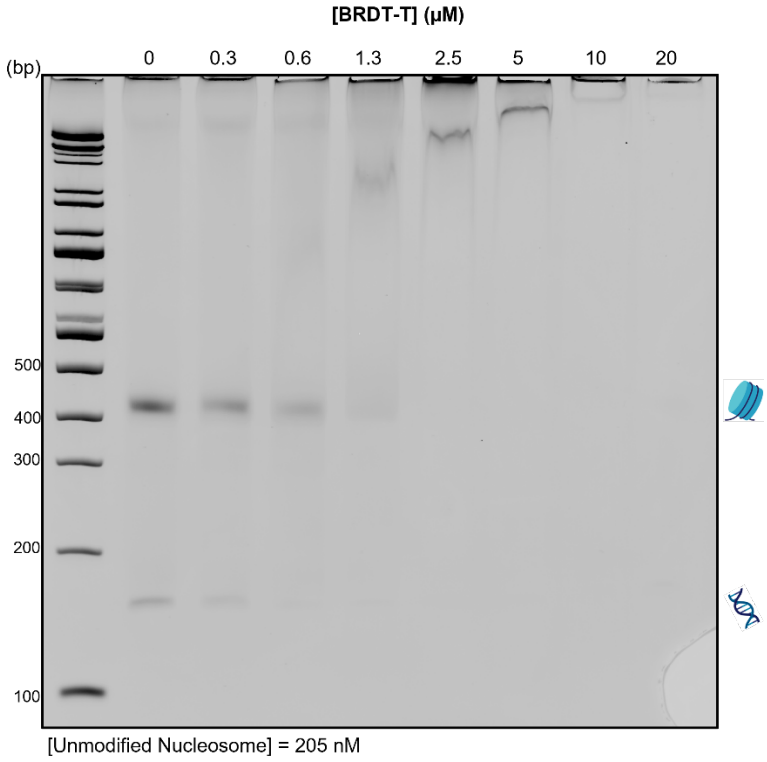


Figure S54: EMSA titration of BRDT-T with the unmodified nucleosome.

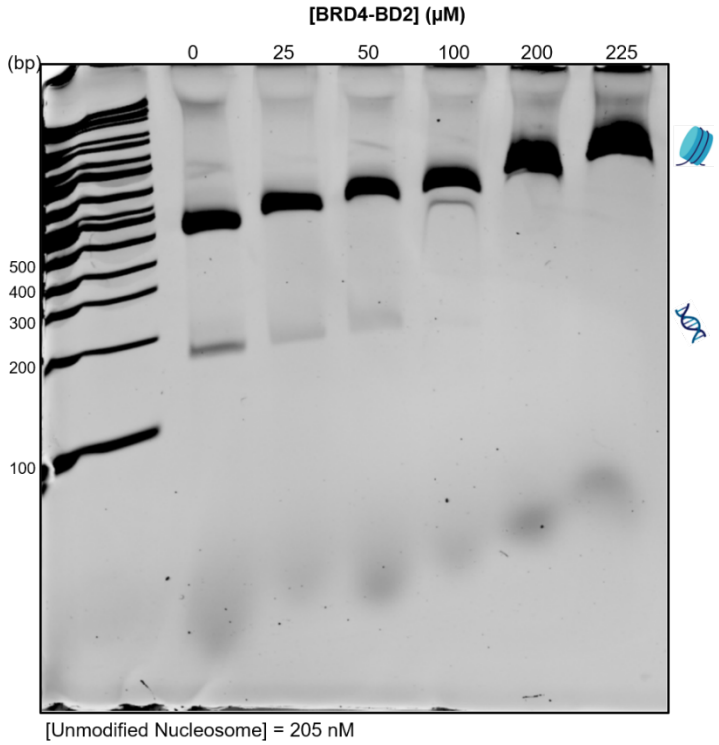
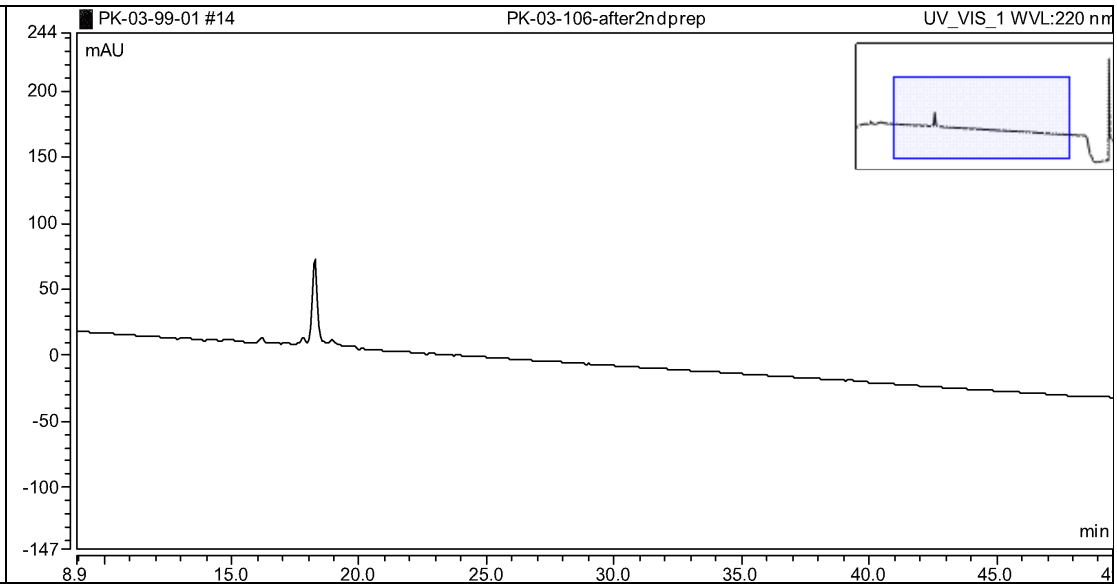


Figure S55: EMSA titration of BRD4-BD2 with the unmodified nucleosome.

HPLC analytical purity traces for H4 K5Ac,K8Ac,K12Ac,K16Ac. Peptide purity was assessed with a Dionex Ultimate RP-HPLC system using a Vydac C-18 column and a 0-50% CH_3CN gradient over 50 min. Traces are shown below.

Gradient	**Analytical trace**

0-50% CH_3CN over 60 min	

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https://doi.org/10.1021/acs.biochem.0c00159.