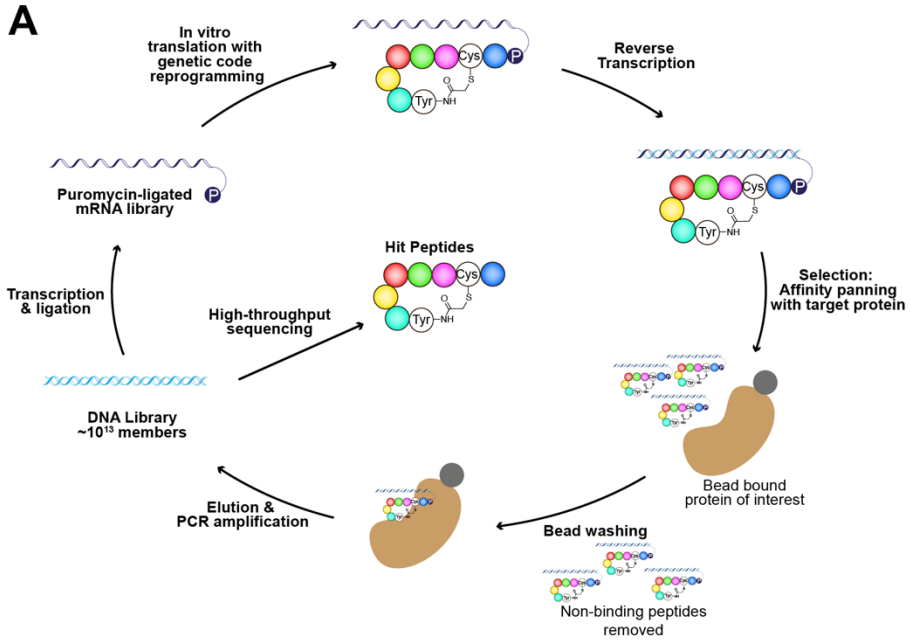
SUPPLEMENTARY FIGURES



B

Peptide Sequence (GSGSGS)- Linker Stop Codon

ATG(NNK)$_m$ATG(NNK)$_n$TGTGGGTCTGGGTCTGGGTCTTAG

Initiator = ClAc-Trp m = 3-7 n = 4-7

1st	2nd U	2nd C	2nd A	2nd G	3rd
U	Phe	Ser	Tyr	Cys	U
					C
					A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
					C
					A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
					C
					A
	KAc	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
					C
					A
	Val	Ala	Glu	Gly	G

Figure S1. Overview of the RaPID system, related to STAR methods. A. Schematic of the RaPID selection scheme. **B.** Library design and codon assignment used in the RaPID selections.

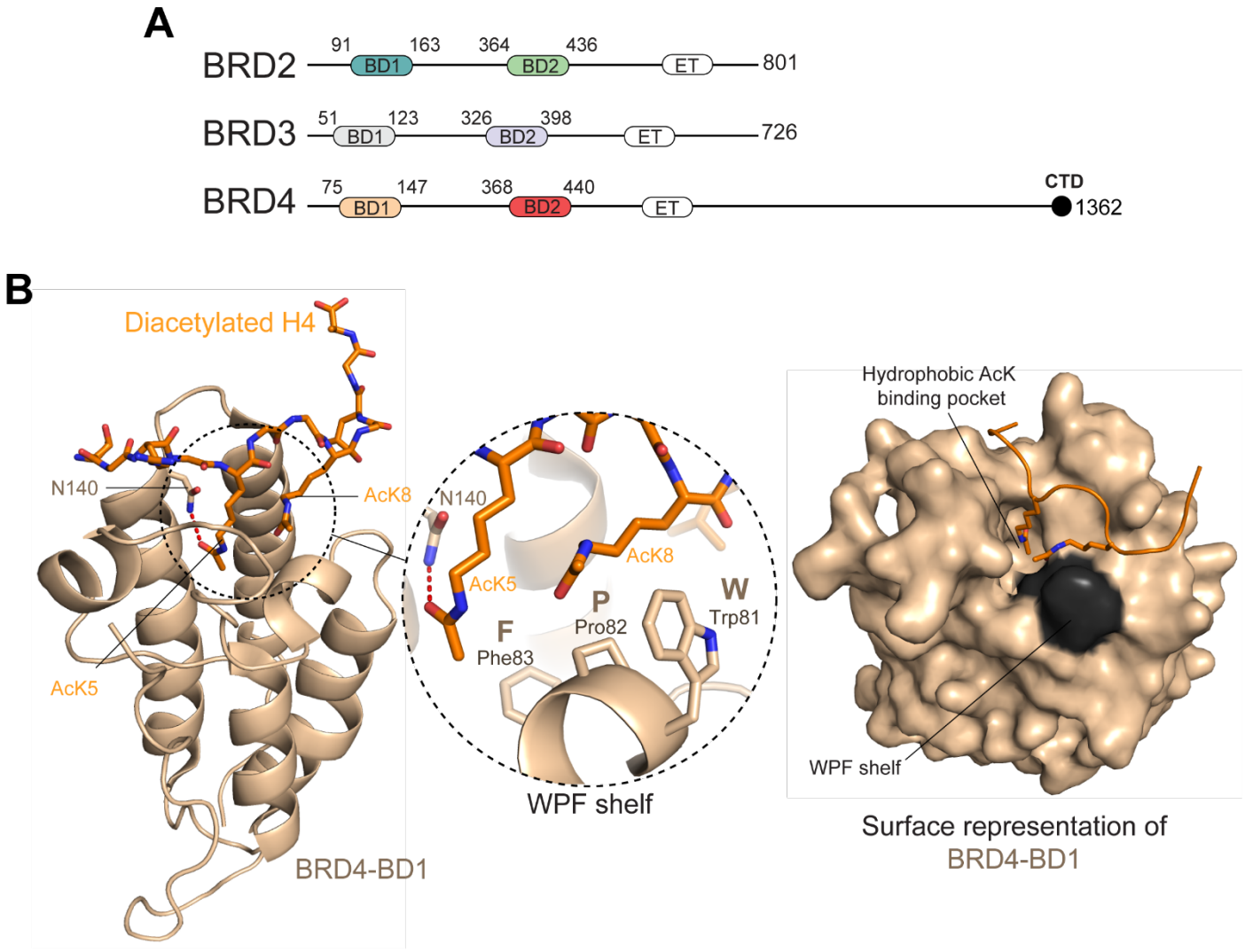


Figure S2. Domain architecture of the Bromodomain and ExtraTerminal domain (BET) family of proteins, related to STAR methods. A. Domain topology of human BRD2, BRD3, and BRD4. The first (BD1) and second (BD2) bromodomains, extraterminal domains (ET), and C-terminal domains (CTD) are indicated. Residue ranges of the BDs are indicated, and the colouring shown for each individual BD is used throughout the text. **B.** The X-ray crystal structure of BRD4-BD1 (*wheat*) in complex with a diacetylated histone H4 peptide (*orange*, PDB ID: 3UVW). *Left*: Ribbon representation of the BRD4-BD1:diacetylated histone H4 complex. The conserved asparagine (Asn140) and the *N*-terminal AcK residue from the diacetylated histone H4 motif (AcK5) with which Asn140 makes a hydrogen bond are displayed as sticks and the hydrogen bond formed between them is indicated by the orange dashed line. A close-up of the WPF shelf that forms significant van der Waals interactions with *C*-terminal AcK (AcK8) of the diacetylated motif in histone H4 is shown in the diagram enclosed in the dashed circle. *Right*: Surface representation of BRD4-BD1 showing the hydrophobic cavity of the BD that is occupied by the *N*-terminal AcKs of diacetylated motifs. The WPF shelf with which the *C*-terminal AcKs of diacetylated motifs interact is coloured in *dark grey*.

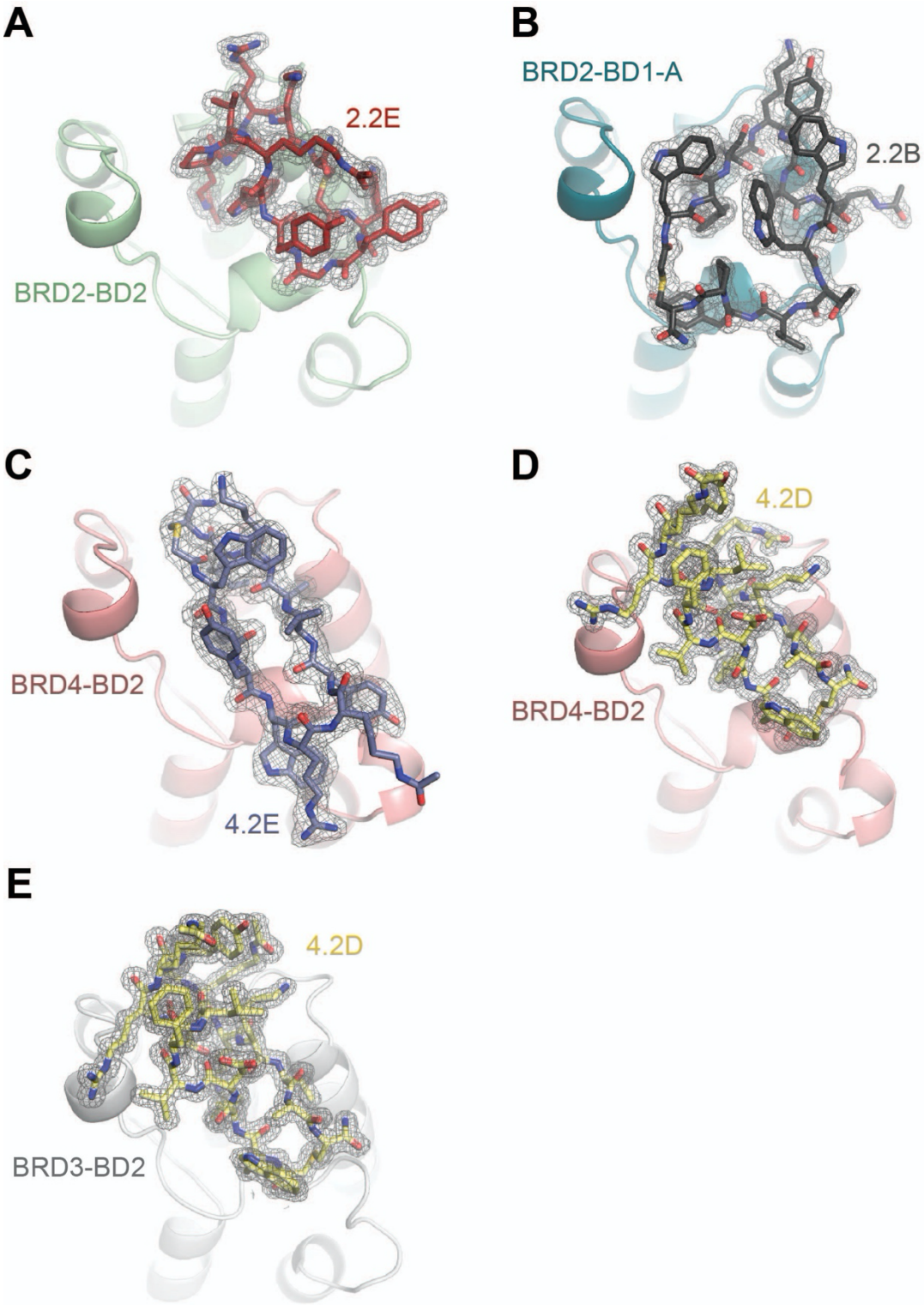


Figure S3. Electron density maps (2Fo-Fc, contour level 1.0 σ) of the final refined structures, related to Figures 2, 3, 6 and 7. A. 2Fo-Fc map of peptide **2.2E** in complex with BRD2-BD2. **B**. 2Fo-Fc map of peptide **2.2B** in complex with BRD2-BD1. **C**. 2Fo-Fc map of peptide **4.2E** in complex with BRD4-BD2. **D**. 2Fo-Fc map of peptide **4.2D** in complex with BRD4-BD2. **E**. 2Fo-Fc map of peptide **4.2D** in complex with BRD3-BD2.

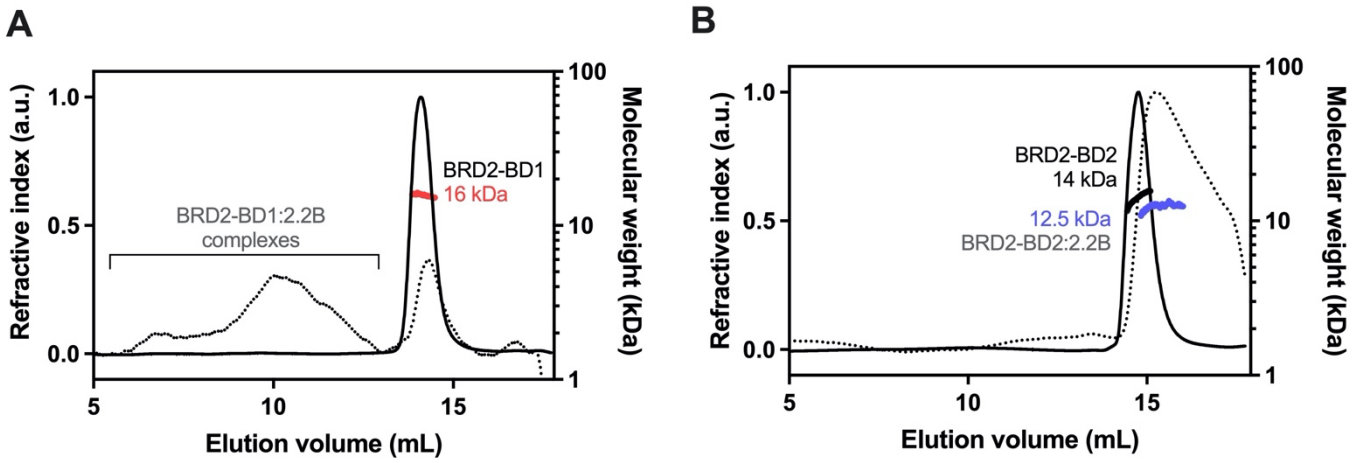


Figure S4. SEC-MALLS analysis of 2.2B in complex with BRD2-BD1 and BRD2-BD2, related to Figures 1 and 3. A. Size exclusion chromatogram for BRD2-BD1 alone (solid line) and in the presence of 1.5 molar equivalents of **2.2B** (dashed line). The calculated molecular weight from MALLS analysis is also shown for BRD2-BD1 alone (red bold line). **B**. Size exclusion chromatogram for BRD2-BD2 alone (solid line) and in the presence of 1.5 molar equivalents of **2.2B** (dashed line). The calculated molecular weight from MALLS analysis is also shown for BRD2-BD2 alone (black bold line) and in complex with **2.2B** (purple bold line)

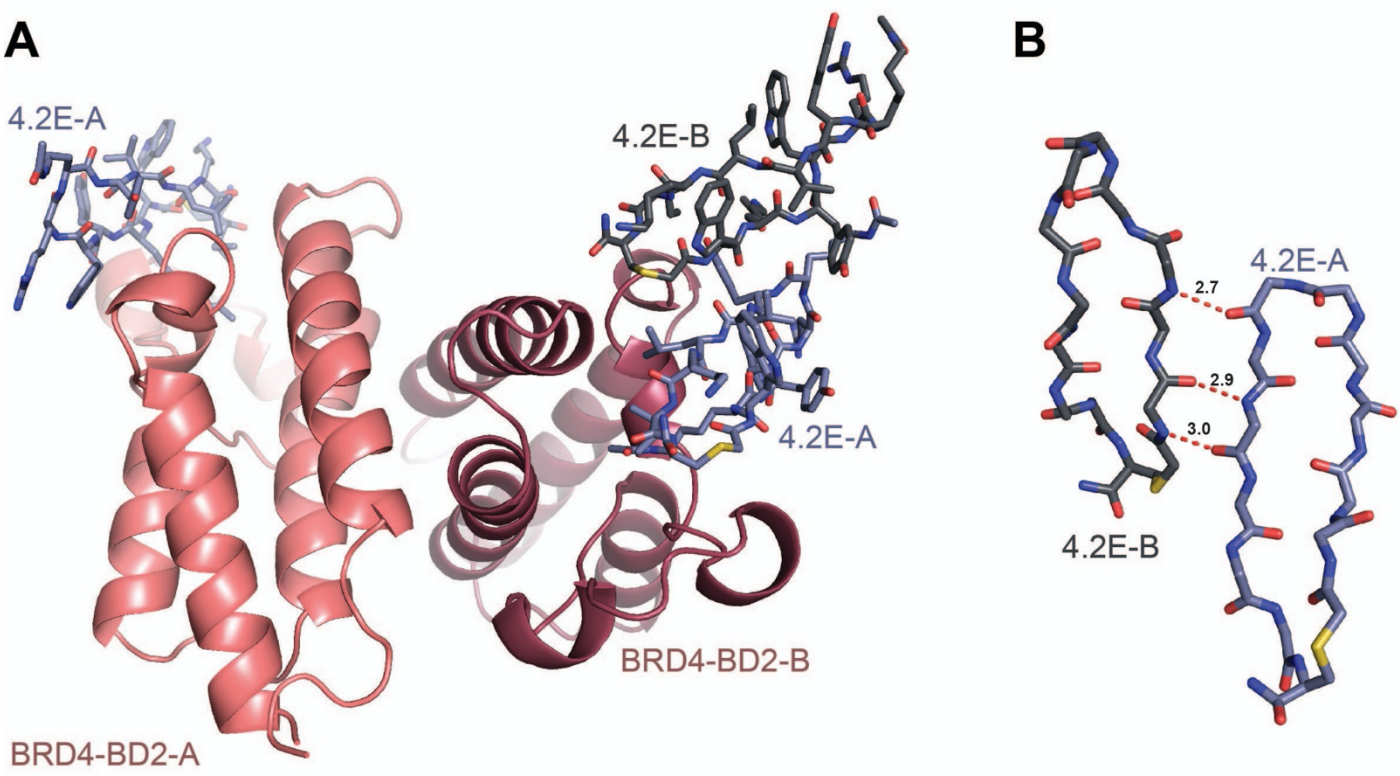


Figure S5. Asymmetric unit of the BRD4-BD2:4.2E complex, related to Figure 6. A. The asymmetric unit shows two copies of BRD4-BD2, each bound in an identical fashion to a molecule of **4.2E** (**4.2-A**). An additional peptide, **4.2E-B**, is observed interacting with the **4.2-A** molecule that is bound to BRD4-BD2-B in the asymmetric unit. **B**. Inter-mainchain hydrogen bonding between **4.2E-A** and **4.2E-B** (red dashed lines).

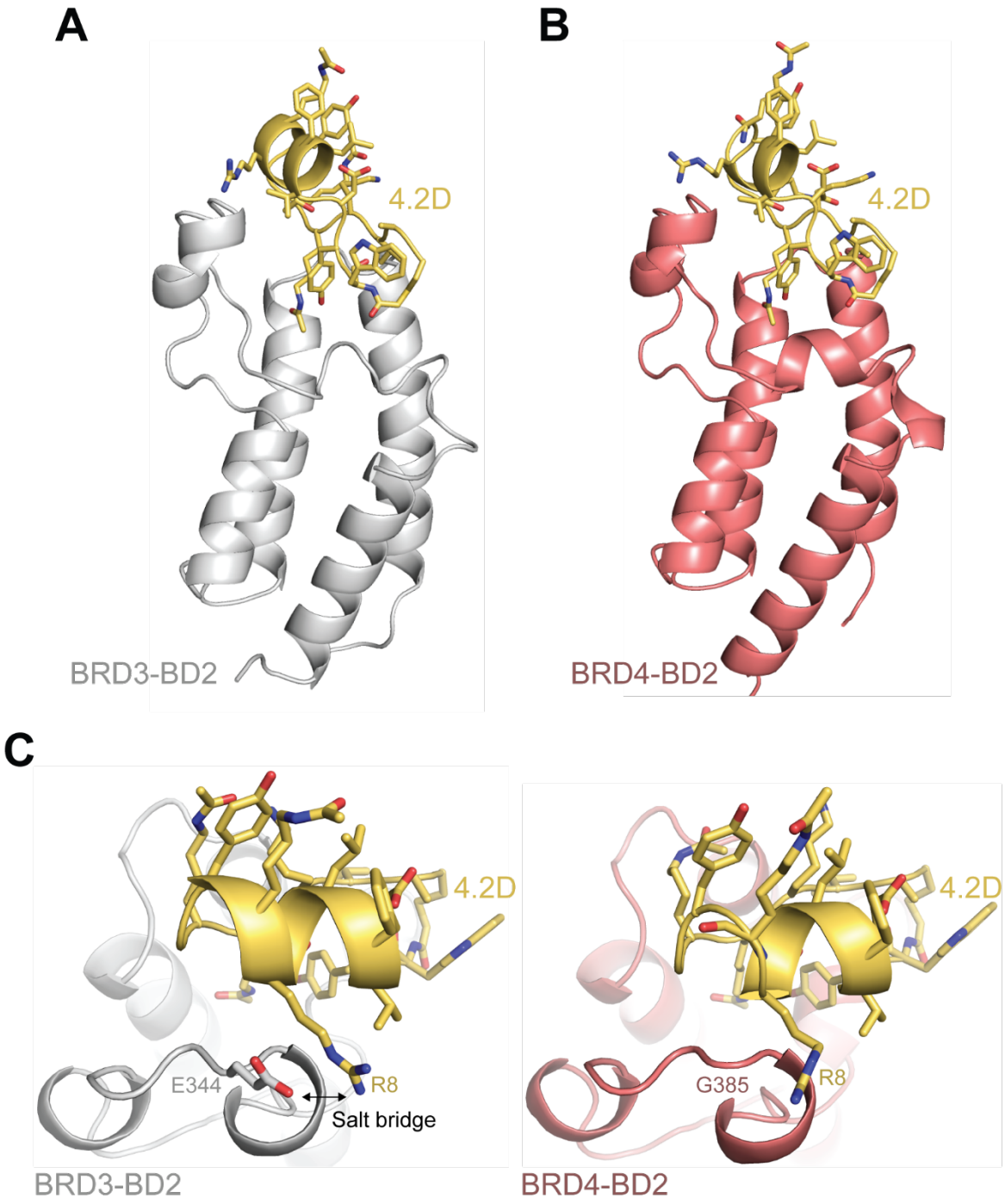


Figure S6. Comparison of the BRD3-BD2:4.2D and BRD4-BD2:4.2D complexes, related to Figure 7. The peptide is shown in yellow, BRD3-BD2 is shown in grey, and BRD4-BD2 is shown in salmon. **A**. Ribbon representation of the structure of BRD3-BD2 bound of **4.2D** (1.5 Å resolution; PDB ID: 8CV5). **B**. Ribbon representation of the structure of BRD4-BD2 in complex with BRD4-BD2 shown in the same orientation as the structure in **A**. An overlay of α-carbons of both structures shown in **A** and **B** has an RMSD of 0.32 Å). **C**. *Left panel:* Close-up view of the BRD3-BD2:**4.2D** interaction. The sole residue, E344, in BRD3-BD2 that differs from BRD4-BD2 within the binding sphere of the interaction is displayed. This residue forms a salt bridge with R8 of **4.2D**. *Right panel*: Close-up view of the BRD4-BD2:**4.2D** interaction. The position of the sole residue, G385, in BRD4-BD2 that differs from BRD3-BD2 within the binding sphere of the interaction is shown. This residue does not form any contacts with **4.2D**.

SUPPLEMENTARY TABLES

Table S1. Peptide details and dissociation constants for the cyclic peptides selected for study from RaPID selections against BRD2-BD2, BRD3-BD2, and BRD4-BD2, related to Figures 1, 4 and 5.

				K_D (nM)[a]					
		Peptide	Sequence	Enrichment	2.1	3.1	4.1	2.2	3.2	4.2
RaPID Selection	BRD2-BD2	2.2A	WNGWW**K**IP**K**Q**K**C	2.3%	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.3	0.2 ± 0.08	2 ± 3	2 ±0.6
		2.2B	WYS**K**KYA**K**WWTVYPC	1.4%	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.5	20 ± 5	50 ± 40	200 ± 180
		2.2C	WHYWLLR**K**GKIHKLRC	1.3%	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
		2.2D	WSGYW**K**IP**K**QLC	1.2%	1 ± 0.4	0.9 ± 0.4	2 ± 0.3	0.4 ± 0.3	8 ± 0.1	6 ± 1
		2.2E	WYGYW**K**VP**K**RKC	0.6%	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	2 ± 1	60 ± 2	34 ± 6
	BRD3-BD2	3.2A	W**K**KAILPG**K**ILKTLHIC	3.1%	[b]	[b]	9 ± 20	[b]	[b]	[b]
		3.2B	WSWLCK**K**YNLIHC	2.6%	20 ± 20	30 ± 30	20 ± 8	40 ± 50	10 ± 8	30 ± 20
		3.2C	W**K**QWK**K**YGLKIC	2.0%	[b]	[b]	[b]	90 ± 50	30 ± 10	10 ± 10
		3.2D	WISYQ**K**YRLW**K**C	0.2%	[b]	[b]	[b]	300 ± 8	5 ± 0.5	100 ± 20
		3.2E	WNV**K**YW**K**KYNIKC	0.1%	[b]	[b]	[b]	20 ± 2	6 ± 3	3 ± 2
		3.2F	WLYYQ**K**W**K**LF**K**C	0.1%	[b]	1000 ± 1000	[b]	7 ± 2	2 ± 3	4 ± 2
		3.2G	WLFY**KK**WRLY**K**C	0.2%	[b]	[b]	[b]	9 ± 7	2 ± 0.1	7 ± 0.8
	BRD4-BD2	4.2A	W**K**NWCWL**K**R**K**LLLRC	20.0%	50 ± 30	3 ± 1	30 ± 30	100 ± 100	20 ± 10	30 ± 10
		4.2B	WAYHTIRL**K**WR**W**PSISC	9.5%	20 ± 0.03	[b]	[b]	3 ± 6	100 ± 10	1 ± 0.04
		4.2C	W**K**GYLCLR**K**RIQRTYNC	4.6%	[b]	[b]	[b]	20 ± 30	20 ± 100	40 ± 40
		4.2D	WYDVFLTR**K**YG**KK**KVAC	3.8%	90 ± 20	10 ± 7	40 ± 50	20 ± 7	20 ± 3	0.3 ± 0.05
		4.2E	W**K**YWR**K**YVLKIC	2.5%	[b]	30 ± 10	[b]	2 ± 0.4	20 ± 0.7	0.9 ± 0.2
		4.2F	WLLFYNH**K**LIRRLL**K**C	1.5%	[b]	1000 ± 1000	1000 ± 1000	200 ± 80	20 ± 3	40 ± 20
		4.2G	W**K**SWG**K**HFLLVC	1.0%	400 ± 400	400 ± 200	[b]	200 ± 30	20 ± 0.8	20 ± 0.1

[a]The K_D values are given as the geometric mean of a minimum of three independent measurements. We estimate the uncertainty in each K_D value to be ~25%.

[b]No binding observed (meaning any interaction must have a K_D >1 μM).

[c]No data (nd) due to non-specific binding of the peptide to the SPR sensor chip.

[d]Coloured boxes in the peptide detail section indicate the peptides from each selection that are unique to this study. Details of previously studied peptides (unhighlighted) are also given. Affinities of the peptides for their cognate BDs are highlighted by coloured boxes in the K_D values section.

Table S2. Data collection and refinement statistics for the crystal structure of BRD2-BD2 in complex with 2.2E (PDB ID: 8CV7) solved using molecular replacement, related to Figure 2 and STAR methods.

	BRD2-BD2:2.2E
Data collection	
Space group	P 1 21 1
Cell dimentions	
a, *b*, *c* (Å)	52.95, 52.42, 55.99
α, *β*, *γ* (°)	90.00, 96.67, 90.00
Resolution (Å)	1.6 (1.6-1.63)
R_{merge}	0.054 (0.427)
$I/\sigma I$	18.3 (4.2)
CC(1/2)	0.999 (0.933)
Completeness (%)	98.3 (97.0)
Redundancy	7.0 (7.0)
Refinement	
Resolution (Å)	1.6
No. reflections	39545
R_{work}/R_{free}	0.1756/0.1983
No. atoms	2330
Protein	1812
Ligand/ion	248
Water	270
B-factors	
Overall	23.94
Protein	22.66
Ligand	25.12
Solvent	31.40
R.m.s. deviations	
Bond lengths (Å)	0.0069
Bond angles (°)	1.0250

*Values in the parantheses are for the highest resolution shell.
All data were collected on a single crystal.

Table S3. Data collection and refinement statistics for the crystal structure of BRD2-BD1 in complex with 2.2B (PDB ID: 8DNQ) solved using molecular replacement, related to Figure 3 and STAR methods.

	BRD2-BD1:2.2B
Data collection	
Space group	P 1 21 1
Cell dimentions	
a, *b*, *c* (Å)	41.68. 54.37, 76.93
α, *β*, *γ* (°)	90.00, 93.85, 90.00
Resolution (Å)	1.84 (1.84-1.88)
R_{merge}	0.095 (0.701)
$I/\sigma I$	6.9 (1.3)
CC(1/2)	0.996 (0.733)
Completeness (%)	98.2 (97.4)
Redundancy	3.7 (3.6)
Refinement	
Resolution (Å)	1.84
No. reflections	29500
R_{work}/R_{free}	0.2115/0.2614
No. atoms	2482
Protein	1950
Ligand/ion	330
Water	196
B-factors	
Overall	22.41
Protein	20.93
Ligand	24.89
Solvent	31.93
R.m.s. deviations	
Bond lengths (Å)	0.0070
Bond angles (°)	0.8270

*Values in the parantheses are for the highest resolution shell.
All data were collected on a single crystal.

Table S5. Data collection and refinement statistics for the crystal structure of BRD4-BD2 in complex with 4.2E (PDB ID: 8CV4) solved using molecular replacement, related to Figure 6 and STAR methods.

	BRD4-BD2:4.2E
Data collection	
Space group	P 21 21 21
Cell dimentions	
a, *b*, *c* (Å)	53.58, 65.96, 76.39
α, *β*, *γ* (°)	90.00, 90.00, 90.00
Resolution (Å)	1.93 (1.93-1.97)
R_{merge}	0.079 (0.749)
$I/\sigma I$	19.6 (3.4)
CC(1/2)	1.000 (0.907)
Completeness (%)	99.6 (94.4)
Redundancy	13.1 (6.6)
Refinement	
Resolution (Å)	1.93
No. reflections	20881
R_{work}/R_{free}	0.2090/0.2545
No. atoms	2389
Protein	1884
Ligand/ion	387
Water	118
B-factors	
Overall	29.38
Protein	28.31
Ligand	35.15
Solvent	27.67
R.m.s. deviations	
Bond lengths (Å)	0.0071
Bond angles (°)	1.0026

*Values in the parantheses are for the highest resolution shell.
All data were collected on a single crystal.

Table S6. Data collection and refinement statistics for the crystal structure of BRD4-BD2 in complex with 4.2D (PDB ID: 8CV6) solved using molecular replacement, related to Figure 7 and STAR methods.

	BRD4-BD2:4.2D
Data collection	
Space group	P 1 21 1
Cell dimentions	
a, *b*, *c* (Å)	29.44, 59.64, 36.27
α, *β*, *γ* (°)	90.00, 100.01, 90.00
Resolution (Å)	1.7 (1.7-1.73)
R_{merge}	0.073 (0.566)
$I/\sigma I$	15.9 (3.0)
CC(1/2)	0.999 (0.851)
Completeness (%)	97.8 (93.0)
Redundancy	7.1 (6.6)
Refinement	
Resolution (Å)	1.84
No. reflections	29500
R_{work}/R_{free}	0.2115/0.2614
No. atoms	1195
Protein	932
Ligand/ion	161
Water	102
B-factors	
Overall	19.23
Protein	19.15
Ligand	16.68
Solvent	23.95
R.m.s. deviations	
Bond lengths (Å)	0.0070
Bond angles (°)	0.8270

*Values in the parantheses are for the highest resolution shell.
All data were collected on a single crystal.

Table S7. Data collection and refinement statistics for the crystal structure of BRD3-BD2 in complex with 4.2D (PDB ID: 8CV5) solved using molecular replacement, related to Figure 7 and STAR methods.

	BRD3-BD2:4.2D
Data collection	
Space group	P 32 2 1
Cell dimentions	
a, *b*, *c* (Å)	76.07, 76.07, 57.91
α, *β*, *γ* (°)	90.00, 90.00, 120.00
Resolution (Å)	1.47 (1.47-1.49)
R_{merge}	0.083 (0.906)
$I/\sigma I$	22.8 (3.5)
CC(1/2)	1.000 (0.882)
Completeness (%)	99.9 (99.0)
Redundancy	20.3 (19.7)
Refinement	
Resolution (Å)	1.47
No. reflections	33292
R_{work}/R_{free}	0.1800/0.1967
No. atoms	1248
Protein	929
Ligand/ion	161
Water	158
B-factors	
Overall	18.72
Protein	18.18
Ligand	16.25
Solvent	24.44
R.m.s. deviations	
Bond lengths (Å)	0.0059
Bond angles (°)	1.0453

*Values in the parantheses are for the highest resolution shell.
All data were collected on a single crystal.

Table S8. Details of the precipitant conditions in which cocrystals were obtained, related to STAR methods. Protein:peptide complex with PDB ID and corresponding conditions are given.

Protein:Peptide Complex	Precipitant Conditions
BRD2-BD2:2.2E (PDB ID: 8CV7)	0.2 M sodium fluoride, 0.1 M Bis-Tris propane pH 8.5, 20% (w/v) PEG3350
BRD2-BD1:2.2B (PDB ID: 8DNQ)	0.2 M trimethylamine N-oxide dihydrate, 0.1 M tris pH 8.5, 20% (w/v) PEG2000 MME
BRD4-BD2:4.2E (PDB ID: 8CV4)	0.1 M PCTP[a] pH 4.0, 25% (w/v) PEG1500
BRD4-BD2:4.2D (PDB ID: 8CV6)	0.1 M HEPES pH 7.5, 30% (w/v) PEG1000
BRD3-BD2:4.2D (PDB ID: 8CV5)	0.2 M sodium sulfate, 20% (w/v) PEG3350

[a]PCTP: Sodium propionate, Sodium cacodylate trihydrate, Bis-Tris propane.