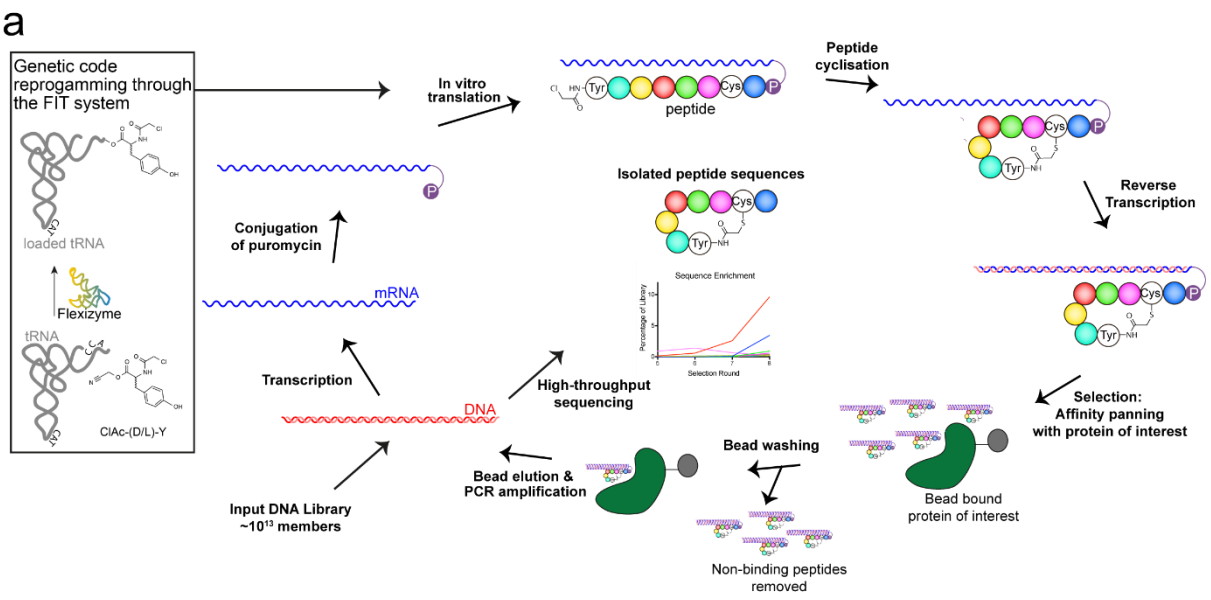


b

m = 3-7
n = 4-7

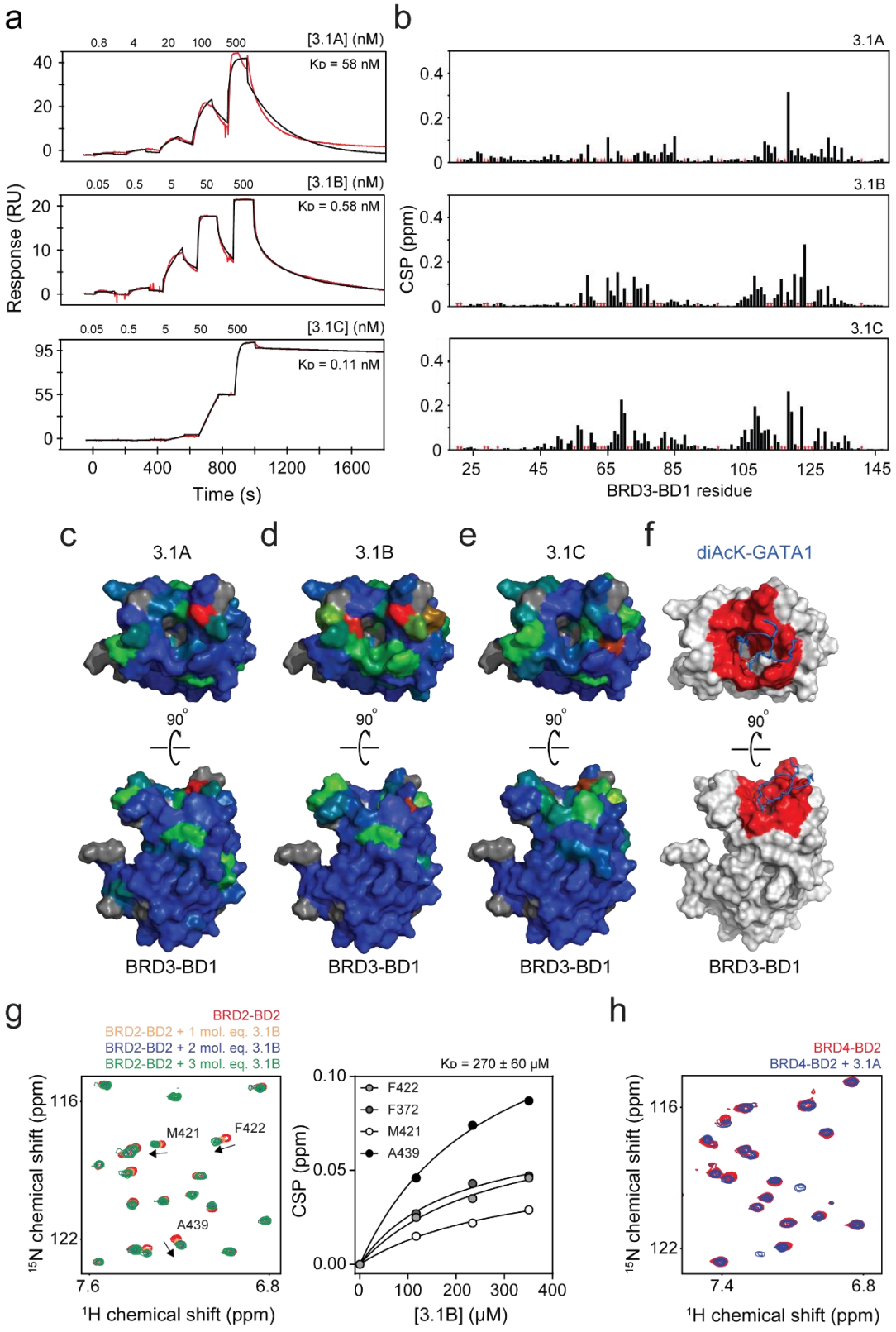
ATG(NNK)mATG(NNK)nTGTGGGTCTGGGTCTGGGTCTTAG

Peptide Sequence Linker Stop Codon

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

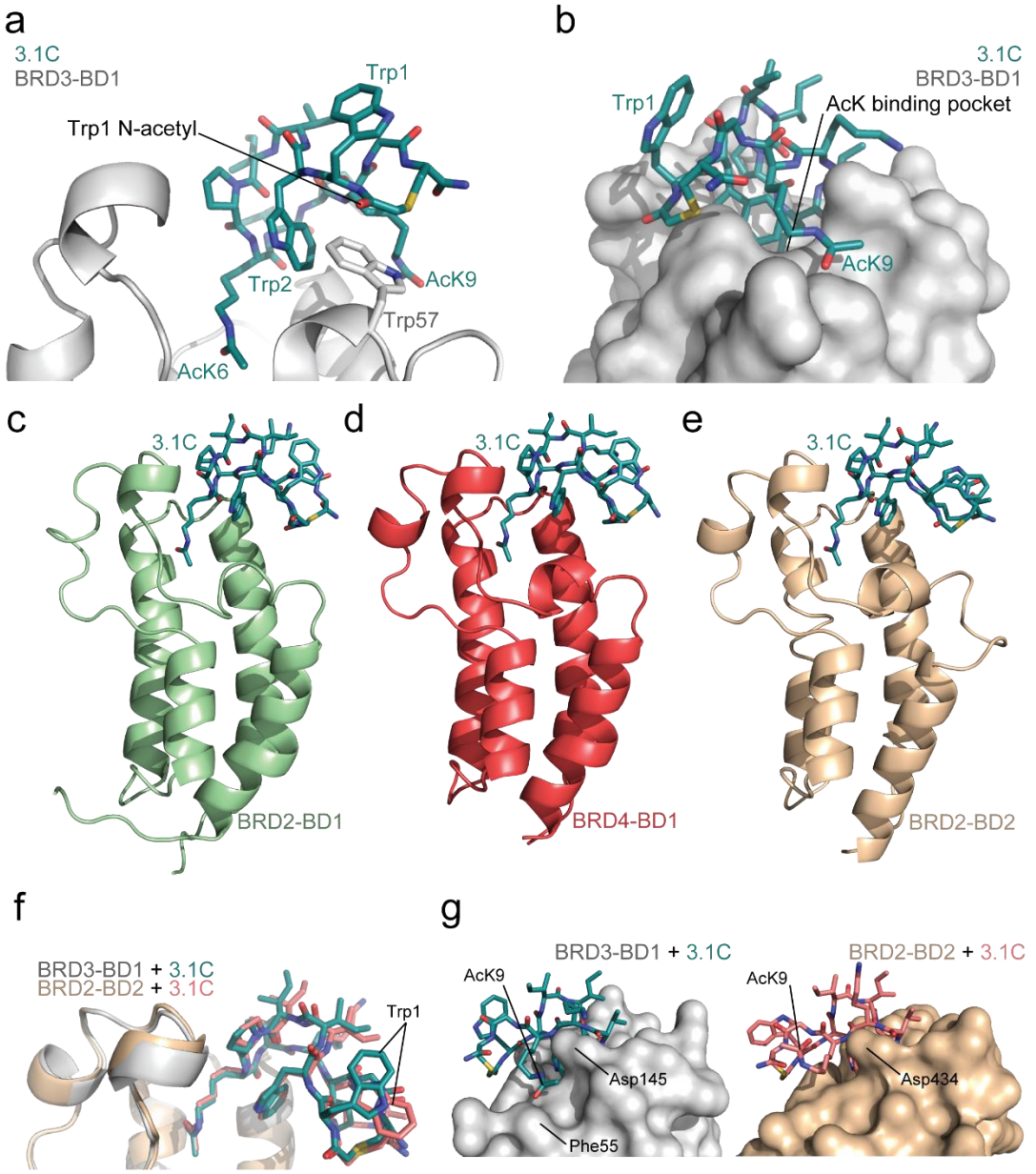
Initiator = ClAc-Trp

Supplementary Figure 1. Overview of the RaPID system. A. Schematic of the RaPID selection scheme. B. Codon assignment used in the RaPID selections.



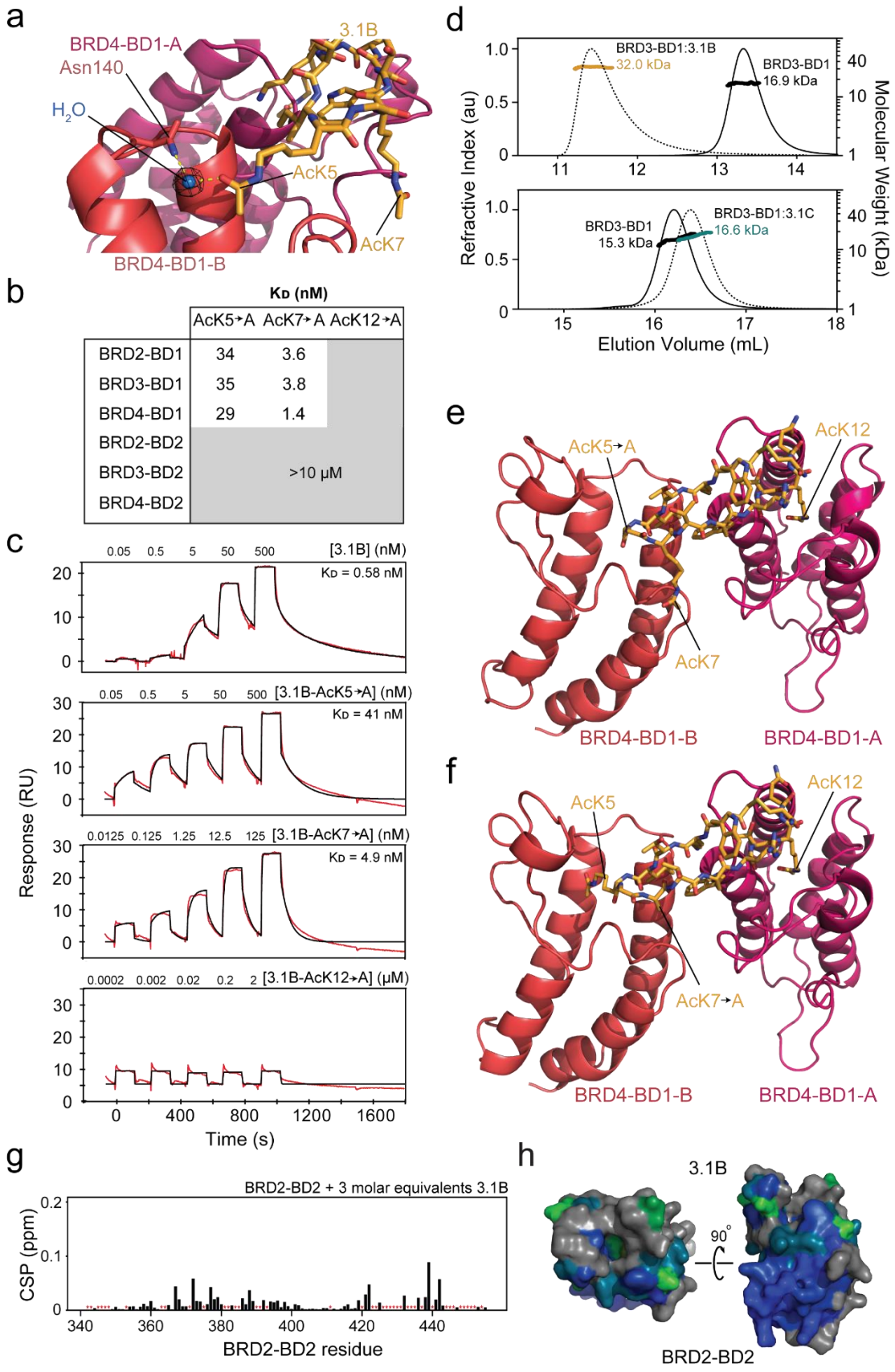
Supplementary Figure 2. A. RaPID peptides bind BRD3-BD1 in the canonical AcK-binding pocket. a. Representative SPR sensorgrams for BRD3-BD1 binding to **3.1A** (*upper panel*), **3.1B** (*middle panel*), and **3.1C** (*bottom panel*). **b.** Chemical shift perturbations in the ^{15}N-HSQC of BRD3-BD1 upon formation of complexes with either **3.1A** (*upper panel*), or **3.1B** (*middle panel*), or **3.1C** (*lower panel*). The red stars indicate unassigned residues. **c.** CSPs observed upon formation of the **3.1A** complex mapped onto the structure of BRD3-BD1 (PDB: 3S91)[1]. The degrees of CSP are displayed

through a blue-green-red gradient: the blue portions of the structure do not undergo any CSPs and the red sections represent the residues that display the greatest CSPs. Grey regions indicate unassigned residues. **d.** CSPs observed upon the **3.1B** complex mapped onto the structure of BRD3-BD1 (PDB: 3S91)[1]. The degrees of CSP are displayed through a blue-green-red gradient: the blue portions of the structure do not undergo any CSPs and the red sections represent the residues that display the greatest CSPs. Grey regions indicate unassigned residues. **e.** CSPs from the **3.1C** complex are mapped onto the structure of BRD3-BD1 (PDB: 3S91)[1]. The degrees of CSP are displayed through a blue-green-red gradient: the blue portions of the structure do not undergo any CSPs and the red sections represent the residues that display the greatest CSPs. Grey regions indicate unassigned residues. **f.** Structure of the BRD3-BD1 complex with a diacetylated-GATA1 peptide (2L5E, the peptide is shown in *blue* and the BD is shown in *grey*)[2], highlighting BD residues (*red*) that directly contact the peptide. **g.** Overlaid section of ^{15}N-HSQC spectra of BRD2-BD2 alone (*red*) or in the presence of one (*yellow*), two (*blue*), or three (*green*) molar equivalents of **3.1B**. Signals that undergo the greatest CSPs in the are labelled in the protein alone spectrum. Binding curves were derived by tracking the combined ^{15}N/^{1}H chemical shift perturbations of the signals undergoing the greatest CSPs. The data were fitted to a simple 1:1 Langmuir binding isotherm using Graphpad. **h.** Overlaid ^{15}N-HSQC spectra of BRD4-BD2 alone (*red*) or in the presence of one (*blue*) molar equivalent of **3.1A**.



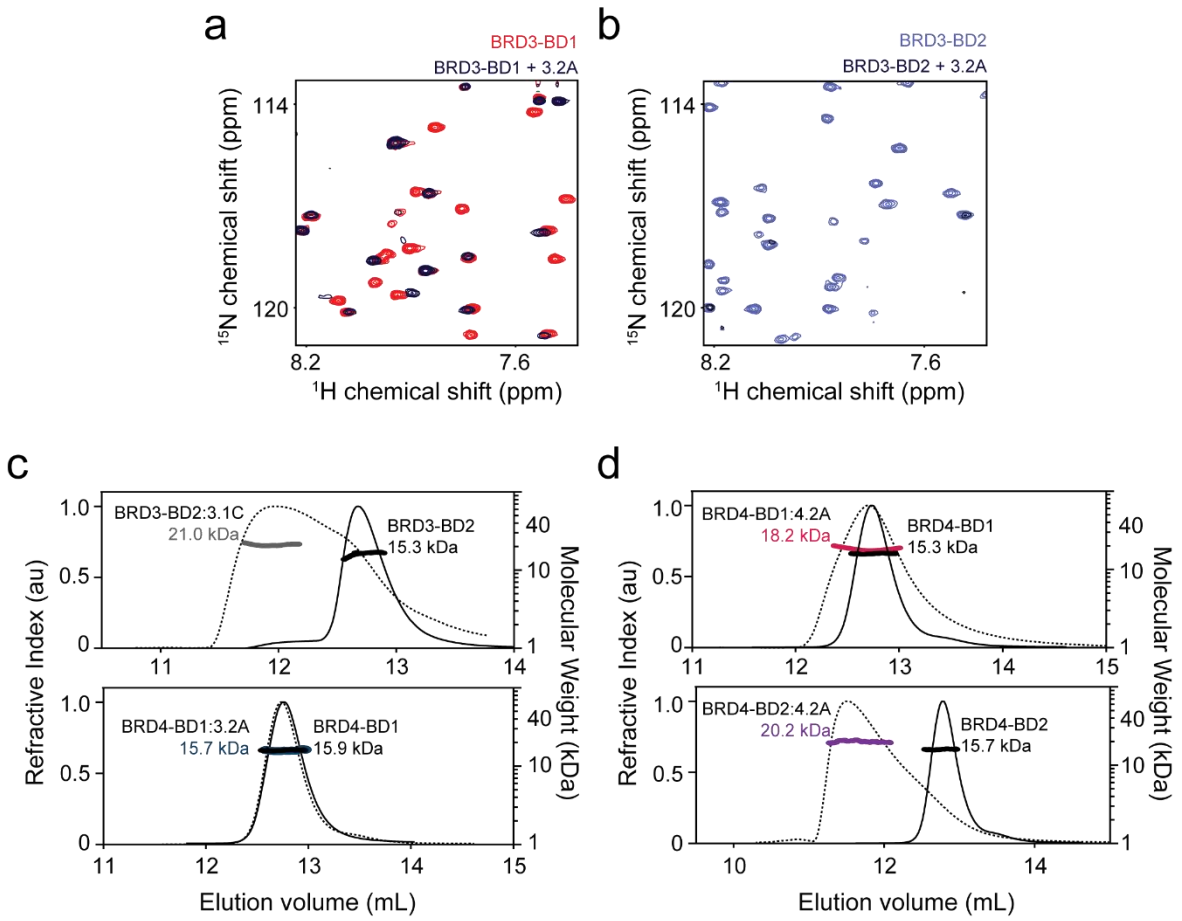
Supplementary Figure 3. X-ray crystal structures of BRD2-BD1, BRD4-BD1 and BRD2-BD2 bound to 3.1C. a.

a. The structure of BRD3-BD1 (*grey*) in complex with **3.1C** (*teal*) showing the sandwich of Trp57 from the BD between the Trp2 sidechain and the N-acetyl moiety of Trp1 from **3.1C**. **b.** Surface representation of BRD3-BD1 (*grey*) when in complex with **3.1C** (*teal*). AcK9 of **3.1C** interacts with a groove formed on the surface of the BD between the αZ and αD helices at a location distal to the AcK binding pocket. **c.** Ribbon representation of Ribbon representation of the X-ray crystal structure of BRD2-BD1 (*pale green*) bound to **3.1C** (*teal*, 2.3 Å resolution, PDB ID 6U61). **d.** Ribbon representation of the X-ray crystal structure of BRD4-BD1 (*coral red*) bound to **3.1C** (*teal*, 1.7 Å resolution, PDB IDs 6U6K). **e.** Ribbon representation of the X-ray crystal structure of BRD2-BD2 (*wheat*) bound to **3.1C** (*teal*, 1.5 Å resolution, PDB ID 6U71). **f.** Overlay of the BRD3-BD1:**3.1C** complex with the BRD2-BD2:**3.1C** complex. One of the major differences between the two peptide structures, the 180° flipping of the Trp1 sidechain, is indicated. **g.** Comparison of the orientation of AcK9 of **3.1C** and the surface representation the two BDs from the BRD3-BD1:**3.1C** and BRD2-BD2:**3.1C** complexes. BRD2-BD2 lacks the groove that AcK9 rests upon in the BRD3-BD1:**3.1C** structure leading to the residue being oriented upwards into the solvent in the BRD2-BD2:**3.1C** structure.

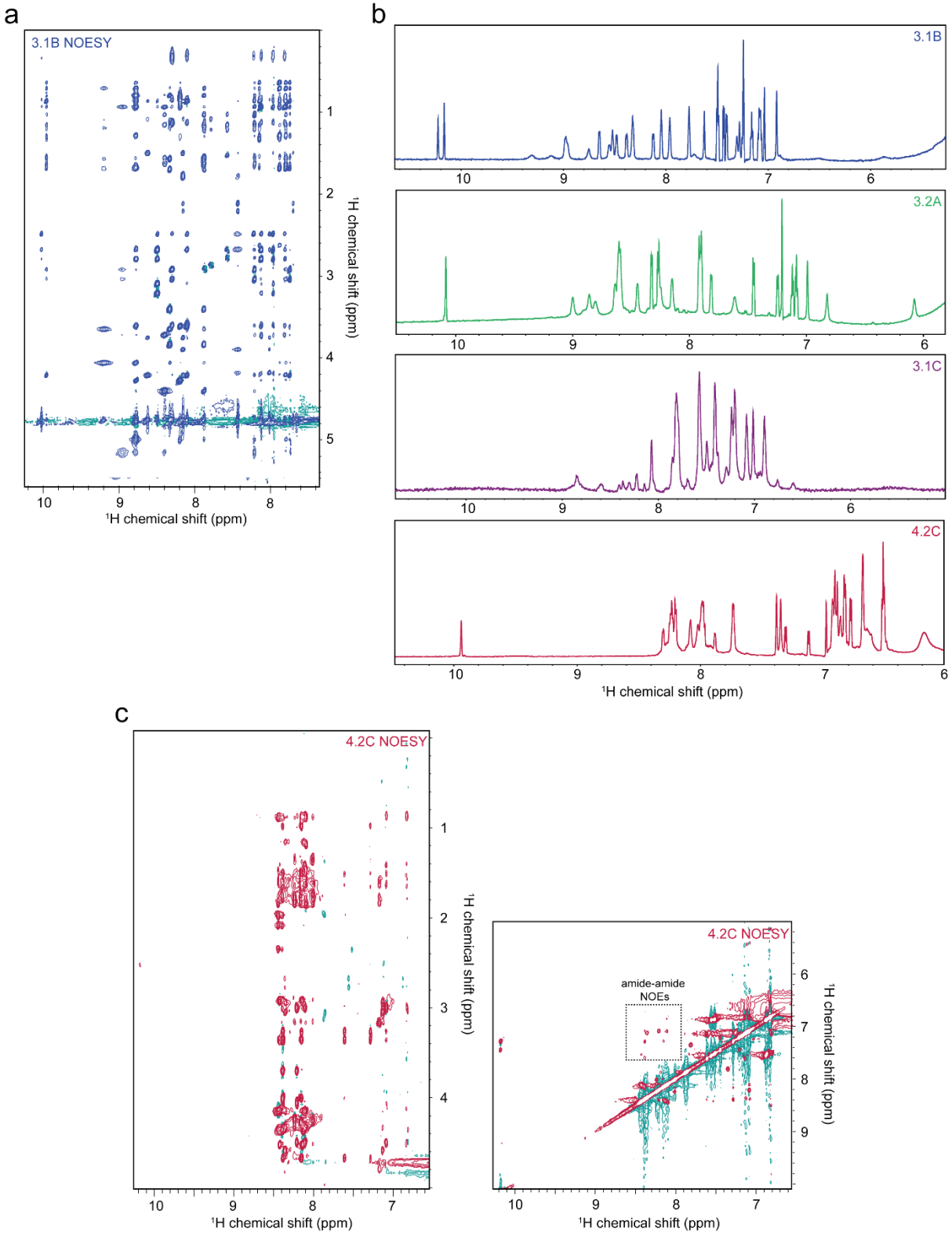


Supplementary Figure 4. Analysis of BRD3/4-BD1:3.1B complexes. a. A close up view of the water-mediated interaction between Asn140 of BRD4-BD1-B (*coral red*) and AcK5 of **3.1B** (*orange*). AcK5

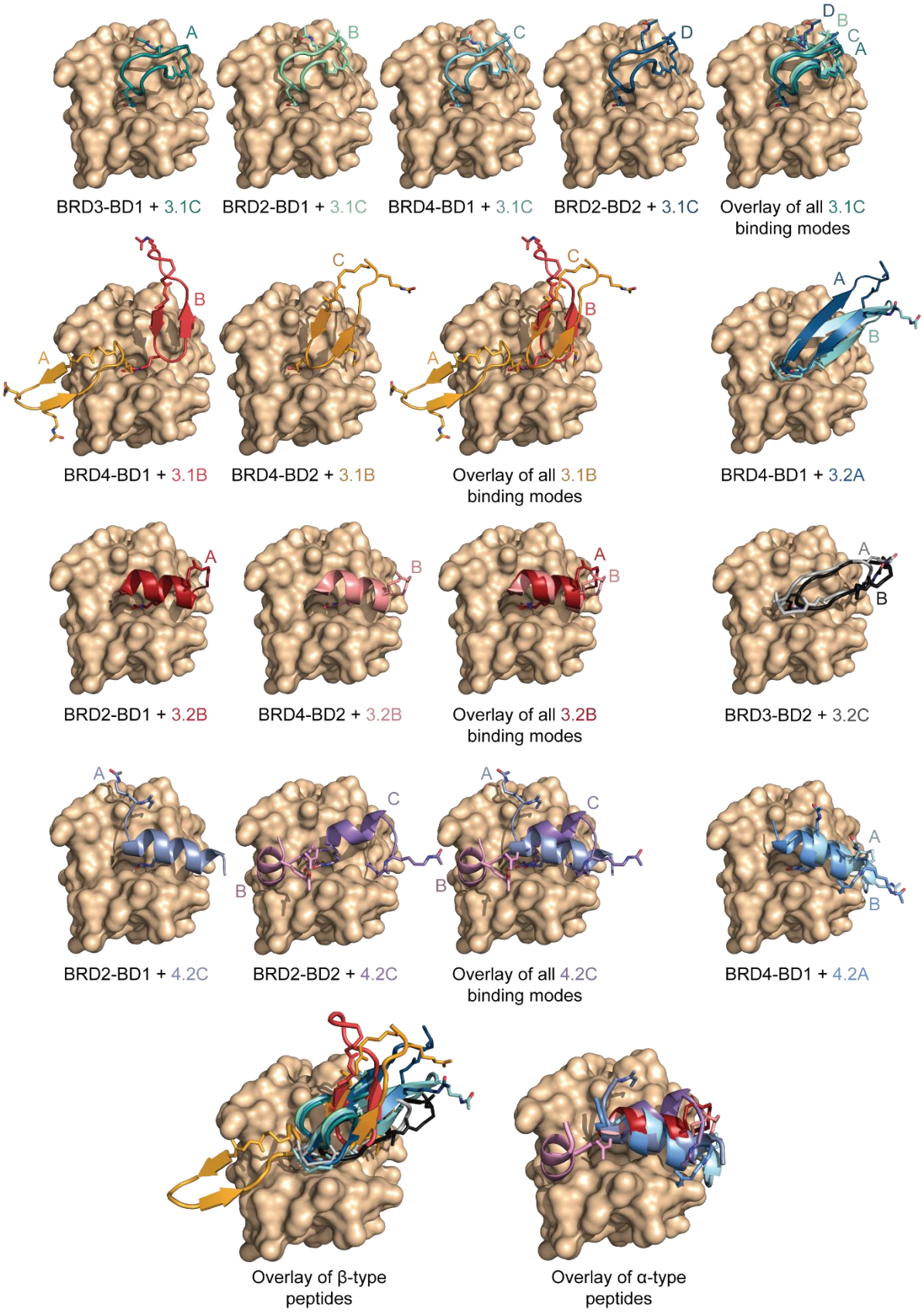
enters the binding pocket at a distinct angle to the canonical AcK binding mode and forms a hydrogen bond with a water molecule (shown as a *blue sphere*) which in turn forms a hydrogen bond with Asn140. The electron density for the water is shown and hydrogen bonding is indicated by yellow dashed lines. **b.** Dissociation constants for the binding of each of the six BDs to **3.1B** AcK mutants. **c.** SPR data (*red*) for the binding of BRD3-BD1 to **3.1B** (*top*) and to AcK5→Ala, AcK7→Ala and AcK12→Ala mutants. Fits to a simple 1:1 binding model (*black*) are shown and derived K_Ds are indicated on each plot. **d.** *Top:* Size-exclusion chromatogram for BRD3-BD1 alone (solid line) and in the presence of one molar equivalent of **3.1B** (dashed line). The calculated molecular weight trace from MALLS analysis is also shown. *Bottom:* Size-exclusion chromatogram for BRD3-BD1 alone (solid line) and in the presence of one molar equivalent of **3.1C** (dashed line). The calculated molecular weight trace from MALLS analysis is also shown. BRD3-BD1 alone elutes as a single peak with the expected mass of 17 kDa. Addition of one molar equivalent of **3.1B** (Mw =1.9 kDa) shifts the peak to an earlier elution time and yields a mass of 32 kDa, in close agreement with the expected mass of a 2:1 BD-**3.1B** complex. **e.** Ribbon diagram of the BRD4-BD1:**3.1B**_AcK5→Ala X-ray crystal structure (2.3 Å resolution, PDB ID 6U72). The peptide is shown in *orange* and the two BDs bound to the peptide are shown in pink (BRD4-BD1-A) and *coral red* (BRD4-BD1-B). The AcK5→Ala replacement and remaining AcKs are indicated. **f.** Ribbon diagram of the BRD4-BD1:**3.1B**_AcK7→Ala X-ray crystal structure (2.6 Å resolution, PDB ID 6U8G). The peptide is shown in *orange* and the two BDs bound to the peptide are shown in pink (BRD4-BD1-A) and *coral red* (BRD4-BD1-B). The AcK7→Ala replacement and remaining AcKs are indicated. **g.** Chemical shift perturbations in the ^{15}N-HSQC of BRD2-BD2 upon formation of complexes following addition of three molar equivalents of **3.1B**. The red stars indicate unassigned residues. **h.** CSPs observed upon formation of the **3.1B** complex mapped onto the structure of BRD2-BD2 (PDB: 3ONI)[1]. The degrees of CSP are displayed through a blue-green-red gradient: the blue portions of the structure do not undergo any CSPs and the red sections represent the residues that display the greatest CSPs. Grey regions indicate unassigned residues.



Supplementary Figure 5. ^{15}N-HSQC analysis of interactions with 3.2A. a. Overlaid ^{15}N-HSQC spectra of BRD3-BD1 alone (*red*) or in the presence of 1.5 molar equivalents of **3.2A** (*dark purple*). **b.** Overlaid ^{15}N-HSQC spectra of BRD3-BD2 alone (*light purple*) or in the presence of 0.5 molar equivalents of **3.2A**. There is a mass disappearance of signals upon addition of **3.2A**. **c.** *Top:* Size-exclusion chromatogram for BRD3-BD2 alone (solid line) and in the presence of 0.5 molar equivalents of **3.2C** (dashed line). The calculated molecular weight from MALLS analysis is also shown. Addition of 0.5 molar equivalents of **3.2C** leads to a shift from a peak that yields a mass of 15.3 kDa (BRD3-BD2 alone) to species with an earlier elution time that yields a molecular weight of 21.0 kDa, suggesting the formation of a weak 2:1 BD:**3.2C** complex. *Bottom:* Size-exclusion chromatogram for BRD4-BD1 alone (solid line) and in the presence of 0.5 molar equivalents of **3.2A** (dashed line). The calculated molecular weight from MALLS analysis is also shown. **d.** *Top:* Size-exclusion chromatogram for BRD4-BD1 alone (solid line) and in the presence of 0.5 molar equivalent of **4.2A** (dashed line). The calculated molecular weight trace from MALLS analysis is also shown. *Bottom:* Size-exclusion chromatogram for BRD4-BD2 alone (solid line) and in the presence of one molar equivalent of **3.1C** (dashed line). The calculated molecular weight trace from MALLS analysis is also shown. BRD4-BD1 alone elutes as a single peak with the expected mass of 15.3 kDa. Addition of 0.5 molar equivalent of **4.2A** causes peak broadening and yields a mass of 18.3 kDa, consistent with a 1:1 BD:**4.2A** complex. Addition of 0.5 molar equivalents of **4.2A** to BRD4-BD2 leads to a shift from a peak that yields a mass of 15.7 kDa (BRD4-BD2 alone) to a species with an earlier elution time that yields a molecular weight of 20.2, indicating the formation of a weak 2:1 BD:**4.2A** complex.

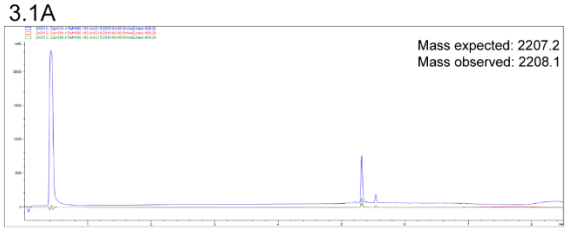


Supplementary Figure 6. Two-dimensional NOESY and one-dimensional ^{1}H-NMR spectra of selected peptides. **a.** Section of a two-dimensional NOESY spectra of **3.1B**. **b.** One-dimensional ^{1}H-NMR spectra of **3.1B**, **3.2A**, **3.1C**, and **4.2C**. **c.** Sections of two-dimensional NOESY spectra of **4.2C**. All spectra were collected at 25 ºC on an 800-MHz spectrometer.

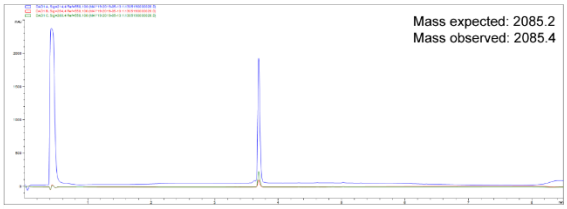


Supplementary Figure 7. RaPID cyclic peptide structure summary

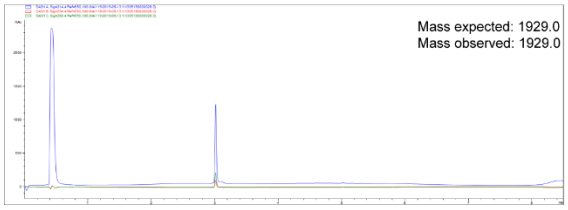
3.1A



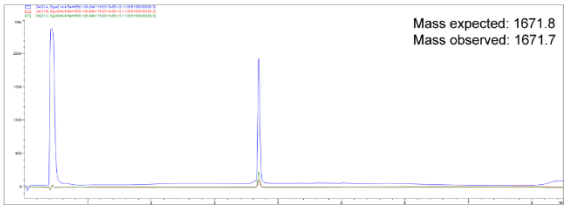
3.2A



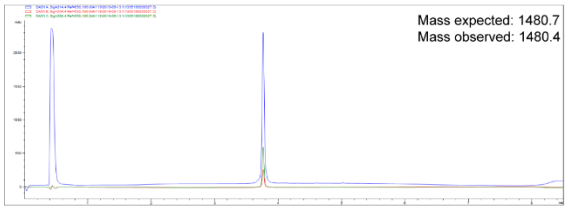
3.1B



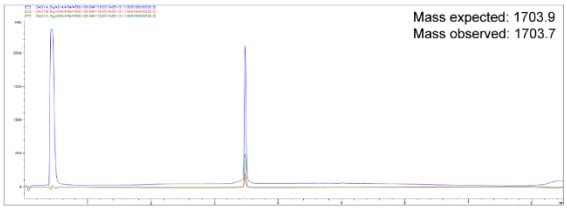
3.2B



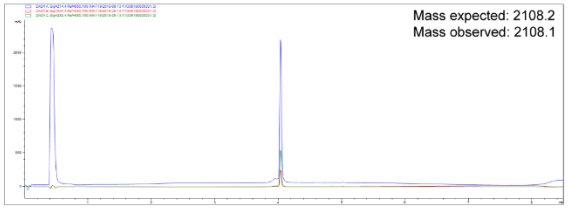
3.2C



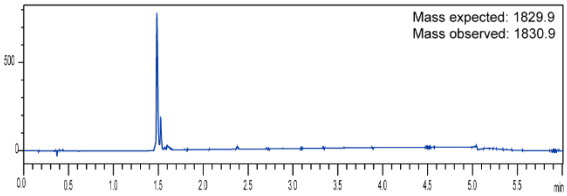
3.2C



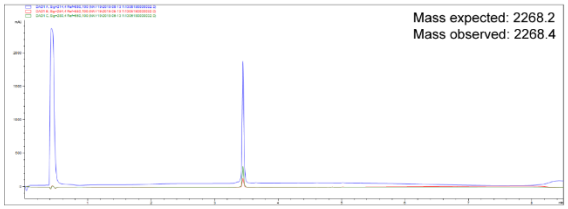
4.2A



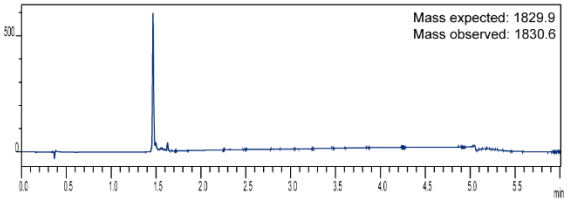
3.1B AcK5Ala



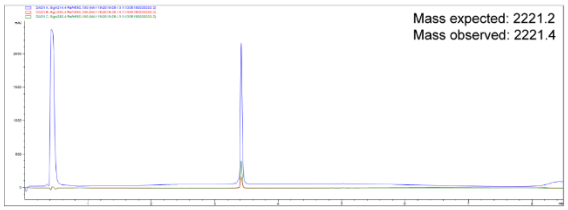
4.2B



3.1B AcK7Ala



4.2C



Supplementary Figure 8. HPLC traces of all RaPID peptides