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Binding Mode of Acetylated Histones to Bromodomains: Variations on a Common Motif

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Bromodomains, epigenetic readers that recognize acetylated lysine residues in histone tails, are potential drug targets in cancer and inflammation. Herein we review the crystal structures of human bromodomains in complex with histone tails and analyze the main interaction motifs. The histone backbone is extended and occupies, in one of the two possible orientations, the bromodomain surface groove lined by the ZA and BC loops. The acetyl-lysine side chain is buried in the cavity between the four helices of the bromodomain, and its oxygen atom accepts hydrogen bonds from a structural water molecule and a conserved asparagine residue in the BC loop. In stark contrast to this common binding motif, a large variety of ancillary interactions emerge from our analysis. In 10 of 26 structures, a basic side chain (up to five residues up- or downstream in sequence with respect to the acetyl-lysine) interacts with the carbonyl groups of the C-terminal turn of helix αB. Furthermore, the complexes reveal many heterogeneous backbone hydrogen bonds (direct or water-bridged). These interactions contribute unselectively to the binding of acetylated histone tails to bromodomains, which provides further evidence that specific recognition is modulated by combinations of multiple histone modifications and multiple modules of the proteins involved in transcription.

1. Introduction

Histone proteins arrange DNA into nucleosomes, forming the chromatin.[1] Diverse modifications of histone proteins influence chromatin packing, dynamics, and gene expression. Acetylation of lysine residues in histone tails is a frequent post-translational modification of these highly conserved proteins.[2] Bromodomains, four-helix bundles of about 110 residues, are one of the two protein families described so far to bind peptides with one or more acetyl-lysine residues, the other family being the evolutionary conserved YEATS domain.[3] There are 61 human bromodomains, and up to six of these modules are present in bromodomain-containing proteins. The binding affinity of histone tails and bromodomains is modest, and ranges in most cases between 10 and 1000 μM.[4] A relatively weak affinity is usually associated with multiple binding modes and/or a diverse set of weak noncovalent interactions. Thus, a systematic structural analysis is expected to shed light on the main interaction motifs between acetylated histone tails and bromodomains. Herein we review the crystal structures of human bromodomains in complex with acetylated histone tails. As of April 2015, there are 26 crystal structures of 11 different bromodomains, 3 histones (H4, H3, and H2A), and 16 patterns of acetylation (8 monoacetylation, 6 diacetylation, and 2 triacetylation). Five complexes determined by solution NMR spectroscopy are also available, but are not included in this study because in most of them the 20 deposited conformers show large structural disorder.

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In all crystal structures, the left-handed four-helix bundle fold[3a] of the bromodomain is conserved (helices αZ, αA, αB, and αC; Figure 1a). The acetyl-lysine binding site is a mainly hydrophobic pocket formed on one of the two ends of the bundle by the loop connecting the helices αZ with αA (called the ZA loop), and the loop between helices αB and αC (BC loop).[5] The backbone of the histone peptide is extended around the anchor acetyl-lysine, which is buried in the binding pocket (Figure 1b). The peptide backbone occupies the surface groove lined by the ZA and BC loops in either of two opposite orientations, N to C or C to N. Besides the well-described binding mode of the acetyl-lysine,[5,6] which is present in all structures, our analysis reveals additional interaction motifs between histone peptides and bromodomains. These interactions can be classified by three main types: 1) polar interactions between the carbonyl groups of the C-terminal turn of helix αB and a positively charged side chain of the histone, 2) direct and water-mediated hydrogen bonds between polar groups in the acetyl-lysine (Kac) binding site and the backbone of the histone tail, and 3) salt bridges between acidic side chains of the bromodomain and basic side chains of the histone tail.

2. The Conserved Binding Motif

The 11 bromodomains analyzed here are BRD4(1) (the first bromodomain of the BRD4 protein), CREBBP, ATAD2A, BRD2(1), BRD2(2), TIF1α, BPTF, BAZ2A, BAZ2B, BRPF1, and TRIM33 (Table 1). In all available crystal structures, the Kac side chain of the histone tail is anchored in an elongated pocket located between the ZA and BC loops of the bromodomain by a conserved network of hydrogen bonds and van der Waals interac-

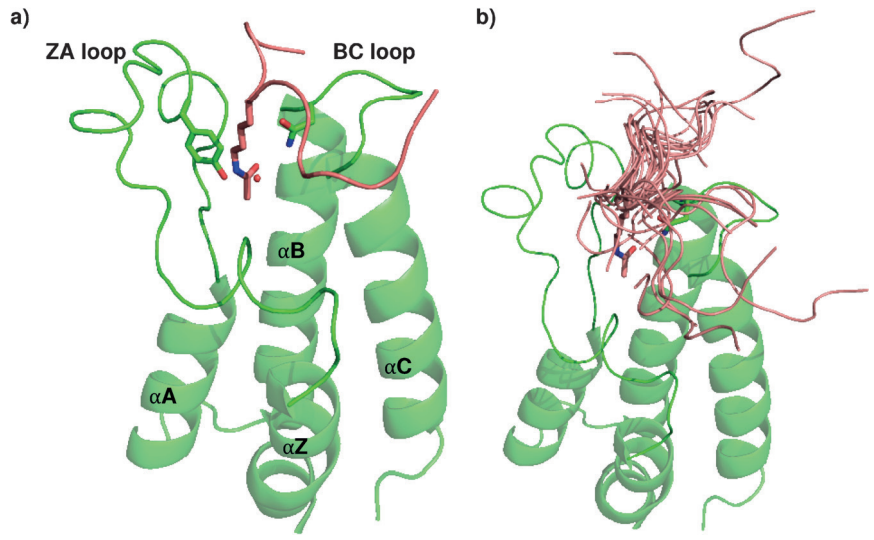

Figure 1. a) Structure of the complex of the BRD4(1) bromodomain (green) with the H4(1–12)K5acK8ac peptide (salmon). The cartoon and ribbon model of this representative complex (PDB code 3UVW)[6d] is supplemented by the side chains of the Kac, the almost strictly conserved Tyr in the ZA loop, the conserved Asn in the BC loop (sticks), as well as the structural water that bridges the Kac and Tyr side chains (red sphere). b) Binding mode of histone tails obtained by structural alignment of all complexes of human bromodomains with histone tails to the BRD4(1)–H4 complex. The bromodomain structure (green) is shown only for the BRD4(1) bromodomain. All bromodomain images were rendered using PyMOL version 1.7.0, Schrödinger LLC.

tions (Figure 2a). The oxygen atom of the Kac acetyl group acts as hydrogen bond acceptor for two highly conserved residues of the bromodomain. It interacts directly with the amide group of the conserved Asn residue of the BC loop (e.g., Asn140 in BRD4(1) and Asn1064 in ATAD2A), and it forms a water-bridged polar interaction with the hydroxy group of the highly conserved Tyr residue located in the ZA loop (e.g., Tyr97 in BRD4(1) and Tyr1021 in ATAD2A; Figure 2a). Throughout the text, the three-letter code and one-letter code are used for the bromodomain and histone residues, respectively. The scatter plot of the distance of the acetyl oxygen atom from its two donors shows that the hydrogen bond with the water molecule has a shorter distance than that with the conserved Asn (Figure 2b). This observation is consistent with the optimal distances between heavy atoms in O–HN and O–HO hydrogen bonds[7] and a similar analysis of 40 complexes of BRD2(1) with different fragments.[8] Thus, the higher degree of conservation of the ZA loop Tyr (58 of 61 human bromodomains) than the BC loop Asn (48 of 61) is congruent with the greater strength of O–HO hydrogen bonds than O–HN hydrogen bonds.[7] Furthermore, mutagenesis experiments of the bromodomain of BRD2 have shown that the mutation of the conserved Tyr113 to Ala abolished the binding of H4K12ac similarly to the mutation of the conserved Asn156 to Ala.[6a] In addition, in the structures of the complexes of the TRIM33 bromodomain and H4, the hydrogen bond to the conserved Asn is not present (PDB codes 3U5O and 3U5P).[9]

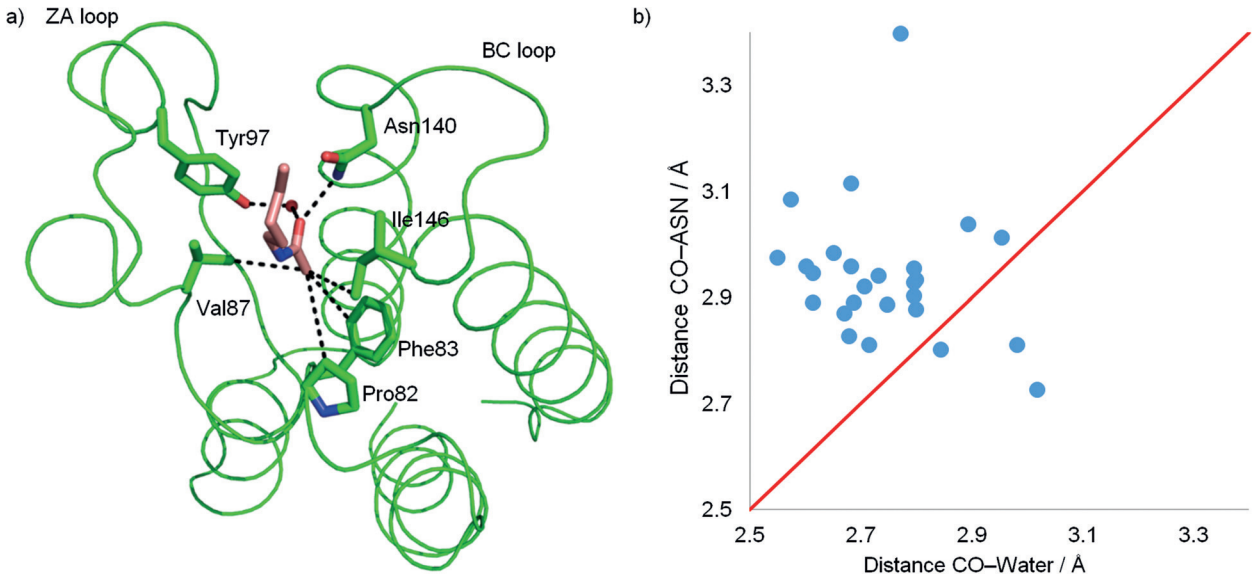

Figure 2. a) Representative example (PDB code 3UVY)[6d] of the conserved binding mode of the Kac side chain (salmon) in the Kac binding site of the BRD4(1) bromodomain (green). The conserved Asn in the BC loop (Asn140 in BRD4(1)) forms a direct hydrogen bond with the acetyl oxygen atom of Kac (black dashed lines), while the highly conserved Tyr in the ZA loop (Tyr97) is involved in a water-bridged hydrogen bond (dashed lines and red sphere); van der Waals contacts between the methyl group of Kac and hydrophobic residues are also shown (dashed lines). b) Scatter plot of the distances between the acetyl oxygen atom of the Kac side chain and the water molecule bridging to the conserved Tyr and the side chain amide nitrogen atom of the conserved Asn. The Kac O–H2O distance is almost always shorter (i.e., stronger hydrogen bond) than the Kac O–N Asn distance.

Table 1. Summary of the interactions between acetylated histone peptides and bromodomains.

PDB code	Ref.	BrD	Histone[a]	ZA loop[b]	Macrodipole αB[c]	BC loop[d]	Direction[e]
2DVQ	[6a]	BRD2(1)	H4(1–15)**K12ac**	S1, R3	K8	L10, G11, G13, A15	N→C
2DVR	[6a]	BRD2(1)	H4(1–15)K5ac**K12ac**		K8	L10, G11, G13, A15	N→C
2DVS	[6a]	BRD2(1)	H4(10–21)**K12ac**				N→C
2E3K ch. 1[f]	[6b]	BRD2(2)	H4(1–15)**K5ac**K12ac			R3,[g] G7, K8,[g] G9	N→C
2E3K ch. 2[f]	[6b]	BRD2(2)	H4(1–15)K5ac**K12ac**			G9, G11, G13	C→N
3O34	[6c]	TIF1a	H3(13–32)**K23ac**	A25	R26	T22, A24, R26,[g,h] K27	C→N
3O35	[6c]	TIF1a	H3(23–31)**K27ac**			A25	N→C
3O36	[6c]	TIF1a	H4(14–19)**K16ac**	A15		G14	N→C
3QZS	[6f]	BPTF	H4(12–21)**K16ac**	H18	R19	R17, H18	C→N
3QZT	[6f]	BPTF	H4(12–21)**K16ac**	R17[g]		G14	N→C
3QZV	[6f]	BPTF	H4(7–17)**K12ac**	L10, G11		K8, G9	N→C
3UVW	[6d]	BRD4(1)	H4(1–12)**K5ac**K8ac	R3		S1, G2, G6, K8ac	N→C
3UVX	[6d]	BRD4(1)	H4(11–21)**K12ac**K16ac			G13, G14, K16ac, R17	N→C
3UVY	[6d]	BRD4(1)	H4(15–25)**K16ac**K20ac			R17,[g] H18, K20ac, V21	N→C
3UW9 ch. 1[f]	[6d]	BRD4(1)	H4(7–17)**K8ac**K12ac				N→C
3UW9 ch. 2[f]	[6d]	BRD4(1)	H4(7–17)K8ac**K12ac**			G11	C→N
4N3W	[6e]	CREBBP	H4(5–25)**K20ac**	H18, V21	R17	H18	N→C
4N4F	[6e]	CREBBP	H4(5–25)**K12ac**K16ac	L10, G11	K8	G9, L10	N→C
4QBM	[14]	BAZ2A	H4(14–22)**K16ac**K20ac	H18, L22	R19	R17, R19,[g] K20ac, V21	C→N
4QC1	[14]	BAZ2B	H3(9–19)**K14ac**		R17	G13, A15, P16, R17,[g] K18	C→N
4QC3 ch. 1[f]	[14]	BAZ2B	H4(7–13)**K8ac**K12ac	L10			C→N
4QC3 ch. 2[f]	[14]	BAZ2B	H4(7–13)K8ac**K12ac**			G11	N→C
4QUT	NA	ATAD2A	H4(9–16)**K12ac**			G9, G11	N→C
4QUU	NA	ATAD2A	H4(3–16)**K5ac**K8acK12ac			R3,[g] G6	N→C
4QYD	[6g]	BRPF1	H4(4–17)**K12ac**	L10, G11	K8	G9, G11	N→C
4QYL	[6g]	BRPF1	H2A(1–12)**K5ac**	R3, Q6		G2, G4, G8, A10	N→C
4TT2	[6h]	ATAD2A	H4(1–20)**K5ac**		R3	R3	N→C
3U5O	[9]	TRIM33	H3(1–22)K14ac**K18ac**	R17[g]			C→N
3U5P	[9]	TRIM33	H3(1–28)K14ac**K18ac**K23ac	R17[g]			C→N

[a] Sequence of the histone peptide in the crystal structure. The Kac in the acetyl-lysine binding site is in boldface (for details, see Section 2. *The Conserved Binding Motif*). [b] Backbone polar groups of the histone that are involved in direct or water-bridged hydrogen bonds with polar groups in the ZA loop (for details, see Section 5.1. *Backbone hydrogen bonds*). Distance threshold for hydrogen bonds of 3.8 Å. [c] Histone basic side chain that acts as a cap for the C-terminal turn of the αB helix (for details, see Section 4. *Electrostatic Interactions with the C-Terminal Turn of Helix αB*). [d] Same as [b] for the BC loop. [e] Directionality of the histone peptide in the binding groove (for details, see Section 3. *Orientation of the Histone Backbone*). [f] Two identical bromodomains bind to a single diacetylated peptide. [g] Salt bridge between the basic side chain of the histone and an acidic side chain of the bromodomain (for details, see Section 5.2. *Charged interactions*). [h] Residue at the C-terminal turn of the B helix just upstream of the BC loop. Note that for [b] and [d], the backbone polar groups of Kac are involved 21 times out of 29 in direct (6 cases) or water-bridged (15 cases) hydrogen bonds with polar groups of ZA and BC loops.

The methyl group at the tip of the Kac side chain is involved in optimal van der Waals contacts with hydrophobic residues in the ZA loop (Figure 2a). Some of these hydrophobic residues are conserved in the human bromodomain family. In particular, 55 of 61 human bromodomains have a Phe residue in the conserved Trp-Pro-Phe triad of the ZA loop (e.g., Phe83 in BRD4(1) and Phe1009 in ATAD2A, and the remaining four bromodomains have a Leu), 42 human bromodomains have a Val residue four positions downstream of the conserved Phe (e.g., Val87 in BRD4(1) and Val1013 in ATAD2A, and only one bromodomain has a hydrophilic residue at this position), and 31 human bromodomains have a Pro in the Trp-Pro-Phe triad (e.g., Pro82 in BRD4(1) and Val1008 in ATAD2A, and only seven bromodomains have a hydrophilic residue at this position). Interestingly, the crystal structures of BRD4(1) with propionyl-lysine and butyryl-lysine[10] indicate that the hydrophobic subpocket can accommodate aliphatic groups slightly larger than the single methyl group of the acetyl-lysine.

In the three crystal structures of bromodomains with the isolated uncapped Kac amino acid (PDB codes 3P1C,[6d] 4NR9,[11] and 4QSP[12]), the binding mode of the acetyl group is identical to that in the complexes with the histone tails. The binding affinity of the uncapped Kac amino acid for five different bromodomains ranges between 1 and 7 mM in chemiluminescence experiments using AlphaScreen technology.[13] The contribution of the Kac side chain in the context of the histone tail might be significantly higher than the affinity of the uncapped Kac because the (partial) desolvation of the two formal charges of the uncapped, zwitterionic Kac penalizes the binding of the isolated Kac amino acid.

3. Orientation of the Histone Backbone

Twenty-three of the 26 crystal structures contain an acetylated histone peptide interacting with only one bromodomain. In these 23 structures, the histone peptide binds in two different orientations with respect to the Kac binding site. According to a directionality given by the binding trench of the bromodomain from the αB to the αZ helix (Figure 1), the histone peptide is directed in an N→C and C→N orientation 17 and 6

times, respectively (Table 1). The binding mode of the Kac side chain is not influenced by the orientation of the peptide backbone in the studied structures. Notably, two orientations are observed for the BPTF bromodomain and the histone peptide H4(12–21)K16ac in two crystal structures with different space groups (PDB codes 3QZS and 3QZT, respectively).[6f] The peptide is oriented in opposite directions in the two crystal structures, despite nearly identical bromodomain conformation (RMSD of the Cα atoms of only 0.5 Å).[6f] This observation provides evidence for nonspecific recognition of H4K16ac by BPTF and a possible context-dependent binding orientation that is modulated by the concomitant binding of additional histone tail marks to other domains of BPTF, e.g., its plant homeodomain (PHD) finger.[6f]

The three remaining crystal structures (BRD4(1), BRD2(2), and BAZ2B) contain diacetylated histone tails interacting with two bromodomains (i.e., two molecules of the same bromodomain), so that the peptide docks in an N→C orientation in one of the two bromodomains and C→N in the other. In BRD4(1) (PDB code 3UW9)[6d] and BAZ2B (PDB code 4QC3),[14] the H4(7–13)K8acK12ac peptide contains the three-residue segment G-L-G between the two Kacs; in BRD2(2) (PDB code 2E3K)[6b] the H4(1–15)K5acK12ac peptide includes the six-residue stretch G-G-K-G-L-G between the two Kacs. In both cases, the sequence linker is glycine rich and thus flexible. Overall, these observations argue in favor of a high adaptability of bromodomains and a context dependency for binding acetylated peptides.

4. Electrostatic Interactions with the C-Terminal Turn of Helix αB

In 10 of 26 structures, a basic side chain up to five residues upstream or downstream in sequence with respect to the Kac is involved in hydrogen bonds with the free carbonyl groups of the C-terminal turn of helix αB (Table 1 and Figure 3). This helical-capping interaction is a favorable monopole–dipole interaction in which the positive charge of the amino or guanidinium group (of histone K or R residues, respectively) acts as monopole while the backbone carbonyl groups of helix αB form the negative pole of the macrodipole. The presence of a helical macrodipole close to the conserved Asn residue is part of the global bromodomain fold and not restricted to some members of the family. It could be an important secondary interaction hotspot for the recognition of histone tails by these modules. The single point mutation R17A (where R17 acts as C-terminal cap for helix αB; PDB code 4QC1)[14] abolished the binding of the histone peptide H3(9–19)K14ac to BAZ2B in an isothermal titration calorimetry experiment.[14] Using the same experimental technique, the R19A mutation of H4(14–22)K16ac and BAZ2A (PDB code 4QBM)[14] led to the same result.[14] Similarly, the single point mutation K8A (where K8 acts as C-terminal cap for helix αB, PDB code 2DVQ)[6a] decreased the binding affinity of the histone peptide H4(1–15)K12ac for BRD2(1) by a factor of three in a surface plasmon resonance binding assay.[6a] Thus, the capping interaction with helix αB is frequent but not essential; its high frequency is likely to be a consequence of the conserved four-helix bundle fold of bromodomains. In an inverse sense, it is interesting to note that in the nucleosome core particle, the N-terminal turn of some of the histones helices (α1 of H2B, H3, and H4; α2 of H2A, H2B, H3, and H4) are capped by the phosphate groups of DNA, which is an interaction between negatively charged groups and the positive pole of the helical macrodipole of the conserved histone folds.[1] These macromolecular complexes suggest that α-helical protein domains involved in transcription share electrostatic helical-capping interactions.

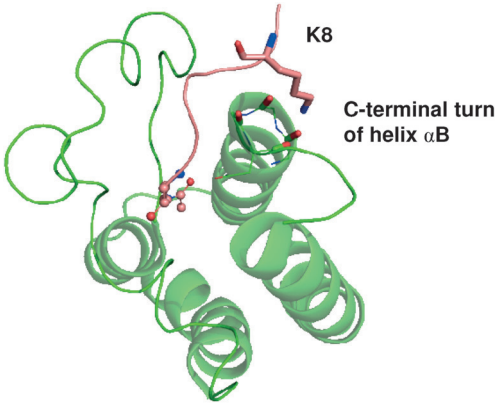

Figure 3. Representative example of a polar interaction between a histone basic side chain, K8, (salmon sticks) and the C-terminal turn of helix αB (green, carbonyl groups in sticks) as observed in the complex of CREBBP with H4(5–25)K12acK16ac (PDB code 4N4F).[6e] The charged amino group of the Lys side chain acts as a C-terminal cap which is an interaction with the helical macrodipole. The Kac residue (balls and sticks) and the conserved Asn (thin sticks) are also shown.

5. Additional Interactions

Besides the interactions involving the acetyl-lysine side chain and the capping interaction with helix αB (both mentioned above), a large variety of ancillary interactions emerge from our analysis of the 26 crystal structures. Acetylated peptide binding to bromodomain is stabilized, at least in part, by heterogeneous polar interactions that include intermolecular hydrogen bonds (mainly involving the backbone of the histone tail and polar groups of the BC loop) and salt bridges (Table 1).

5.1. Backbone hydrogen bonds

In all crystal structures there are multiple hydrogen bonds between the backbone of residues in the vicinity of the Kac (±5 residues) and backbone or side chain atoms of residues of the BC and ZA loops. They form a complex network of polar interactions and are of two types: direct or water-mediated hydrogen bonds (Figure 4). These networks of hydrogen bonds exhibit variability in number and positions along the histone peptide sequence. Many of them involve the polar groups of glycine residues in the histones, which are particularly abundant in H2A and H4 histone tails. In only two cases (PDB codes 2DVQ[6a] and 4QYD[6g]), residues located further than five positions away from the Kac are involved in hydrogen bonds with the bromodomain. In contrast to the abundance of peptide/

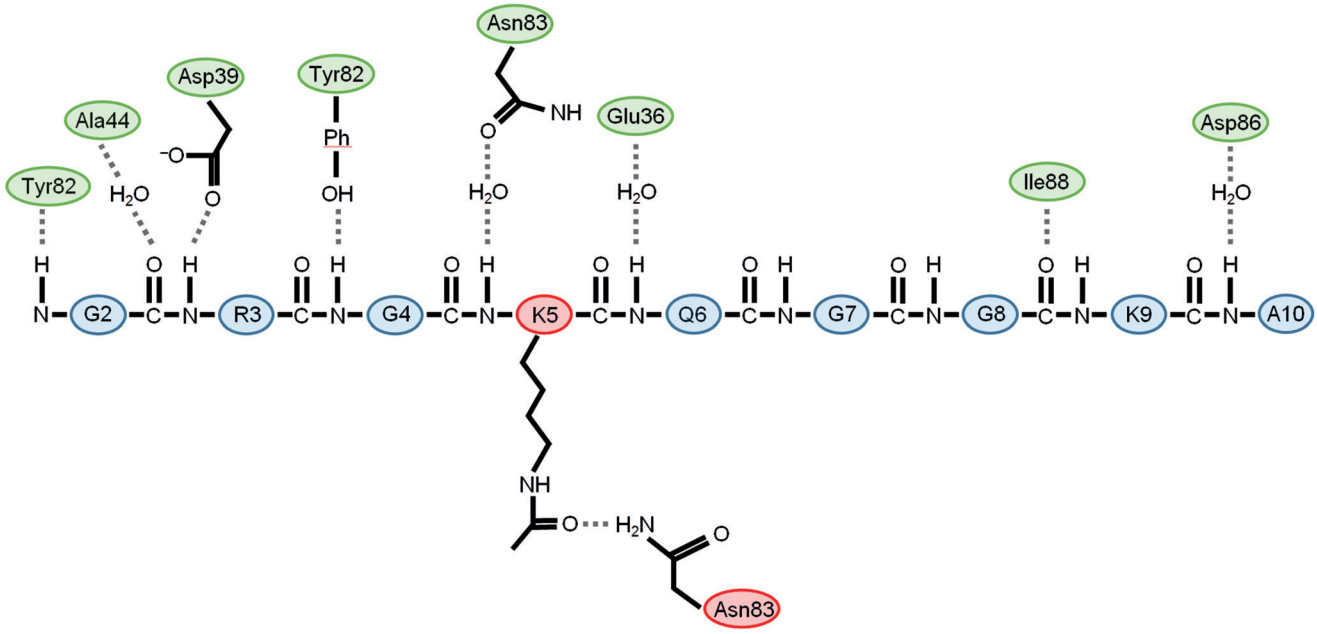

Figure 4. Representative example of multiple polar interactions between histone peptide and bromodomain polar groups in the binding groove. The plot shows a two-dimensional representation of the hydrogen bonds (dotted lines) between the backbone of H2A(1–12)Kac5 (residues with blue labels) and polar groups in the Kac binding site of the BRPF1 bromodomain (green labels) (PDB code 4QYL).[6g] Water-bridged polar interactions are labelled (H_2O). The side chain of Asn83 acts as donor for a hydrogen bond with the acetyl group of Kac5 (red labels).

bromodomain hydrogen bonds, there are very few intrapeptide backbone hydrogen bonds.

We previously investigated the binding of the tetrapeptide Kac-G-G-Kac (from the diacetylated histone tail H4(5–8)K5acK8ac) into the BRD4(1) and CREBBP bromodomains by using molecular dynamics (MD) simulations.[15] Each system was simulated in parallel with two different force fields to assess potential systematic errors due to the choice of force field. In all simulations, i.e., irrespective of the force field and bromodomain, we observed direct and water-bridged hydrogen bonds between the backbone polar groups of the histone tetrapeptide and residues in the ZA and BC loops. Moreover, the simulations unmasked an alternative binding mode in which the Kac NH group acts as donor for a hydrogen bond with the backbone carbonyl group of the Pro residue of the conserved Trp-Pro-Phe triad (i.e., Pro82 in BRD4(1) and Pro1110 in CREBBP) which is located at the N-terminal part of the ZA loop.[15] This simulation result suggests that the polar groups on the periphery of the Kac binding site not only provide favorable hydrogen bond donors and acceptors for the main binding mode, but might also facilitate the process of binding of the histone tail. Notably, the backbone carbonyl group of the Pro residue of the conserved Trp-Pro-Phe triad can also interact with hydrogen bond donors of small-molecule ligands as observed for BAZ2B[11] and BRD4(1).[16]

5.2. Charged interactions

There are ten salt bridges between basic side chains of the peptide and acidic side chains of the bromodomain in the 26 structures (Figure 5). Seven of these salt bridges involve residues of the BC or ZA loops and are exposed to the solvent so that their contribution to the free energy of binding is likely to be small because of the desolvation penalty and freezing of degrees of freedom (i.e., rotameric states) of the side chains. In the remaining three cases, the salt bridge competes with the interaction between the positive moiety of the histone tail and the helix macrodipole. Interestingly, in 20 of the 26 structures, positively charged side chains are present in the histone sequence up to five residues away from Kac but are not involved in salt bridges with the bromodomain. Many of them are not resolved in the crystal structure which indicates that they are very flexible and point mainly toward the solvent.

6. Summary and Outlook

The analysis of 26 crystal structures of human bromodomains in complex with acetylated histone peptides reveals a common binding motif and variable ancillary interactions. The binding mode of the Kac side chain in the conserved cavity of the bromodomain four-helical fold is common to all structures. This finding is consistent with the nearly 100 crystal structures of complexes of bromodomains and small fragments that have a hydrogen bond acceptor that emulates the oxygen atom of the acetyl group.[6h,8,11,17] In addition to this anchor point, a wide variety of polar interactions are present: 1) a favorable electrostatic interaction between a basic side chain of the histone tail and the C-terminal turn of helix αB (Figure 3), 2) direct and water-mediated hydrogen bonds between the backbone of the histone peptide and polar groups in the ZA and BC loops (Figure 4 and Table 1), and 3) various salt bridges at diverse positions along the histone sequence (Figure 5). While heterogeneous hydrogen bonds and electrostatic interactions contribute to the binding affinity, the Kac side chain is

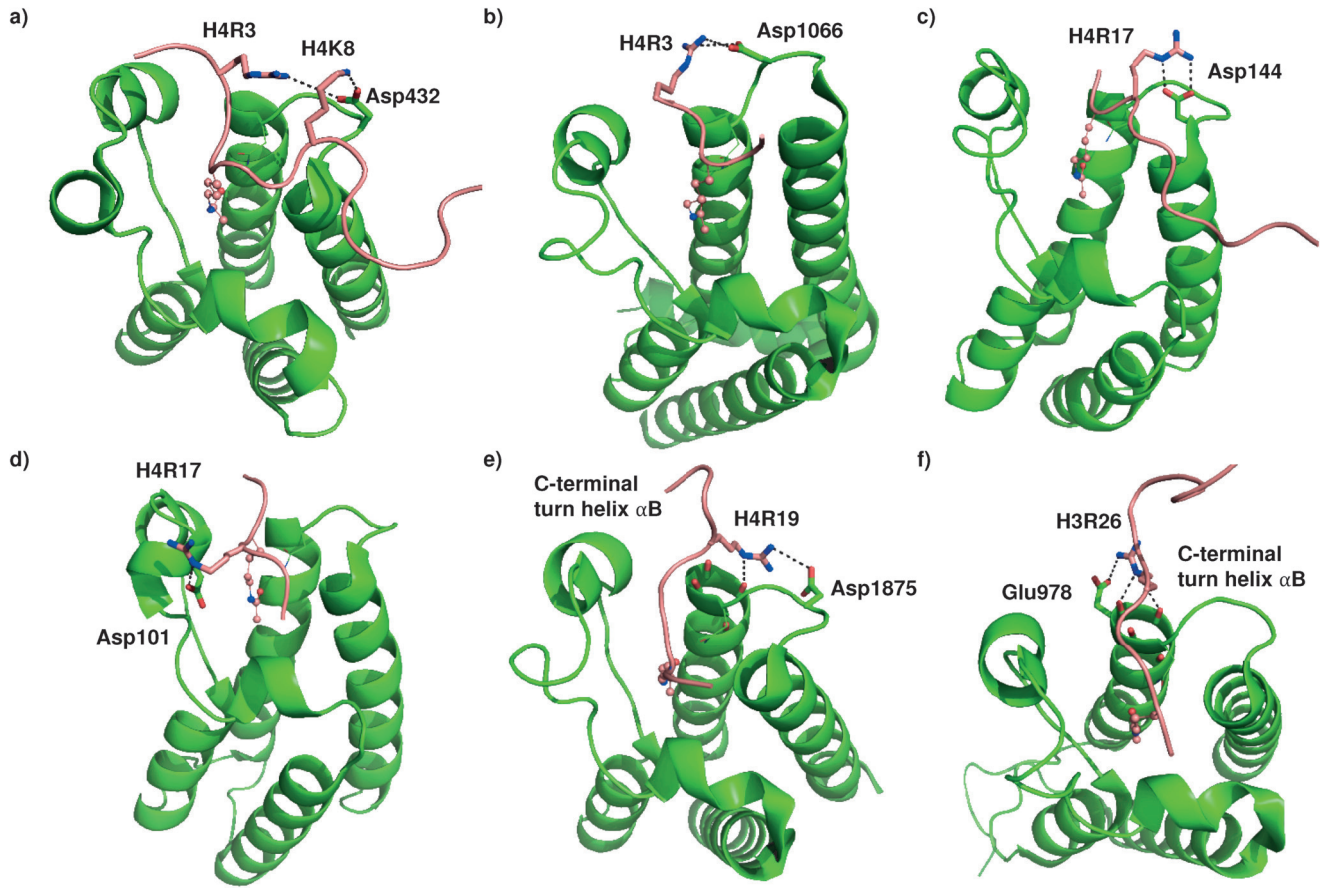

Figure 5. Representative examples of the variety of salt bridges observed in the crystal structures of complexes of acetylated histone peptides (salmon with charged side chains in sticks) and bromodomains (green, charged side chains in sticks). The interactions are shown by dashed lines. a) K8 and R3 of H4K5acK12ac and Asp432 of BRD2(2) (PDB code 2E3K);[6b] b) R3 of H4K5acK8acK12ac and Asp1066 of ATAD2A (PDB code 4QUU); c) R17 of H4K16acK20ac and Asp144 of BRD4(1) (PDB code 3UVY);[6d] d) R17 of H4K16ac and Asp101 of BPTF (PDB code 3QZT);[6f] e) R19 of H4K16acK20ac, C-terminal turn of helix αB and Asp1875 of BAZ2A (PDB code 4QBM);[14] f) R26 of H3K23ac, C-terminal turn of helix αB and Glu978 of TIF1a (PDB code 3O34).[6c]

the only group involved in a conserved binding motif. In this context, it is important to note that MD simulations have provided evidence that Kac can interact with the bromodomain in a binding mode slightly more buried than that observed in the crystal structures.[15]

No specific interaction motif emerges from our analysis which is consistent with the modest specificity of bromodomains for any particular pattern of acetylation. Moreover, the structural analysis reveals that the histone peptide can bind in opposite directions, N to C or C to N, in the binding groove. For instance, the same acetylated histone peptide can bind in both orientations in BPTF. Allis and co-workers postulated that the bromodomain of BPTF could recognize H4K16ac in a monovalent binding (N→C or C→N orientation) or in a bivalent binding concomitant to the binding of another epigenetic mark, H4K4me3, by another domain of BPTF, a PHD finger (compatible only with the N→C orientation).[6f] Thus, the crystal structures of bromodomain/histone tail complexes provide further evidence of a combinatorial readout of multiple histone marks in the field of epigenetics, as already postulated in 2000[18] and reaffirmed recently.[6f]

Most histone sequences studied in this review are flexible, with the presence of numerous glycine residues at positions adjacent to the Kac, as pointed out by Zhou and co-workers in a study of yeast bromodomains.[19] No histone in the studied subset has a proline residue at position Kac ±1, as observed in yeast histone binder sequences.[19] The occurrence of positively charged residues around the Kac in the binder sequence is high in the 26 studied structures, but they do not necessarily interact directly with the bromodomain. This agrees with the suggestion of electrostatic interactions primarily needed for steering the peptide to the binding site, but not necessarily present in the bound state.[19,20]

The specificity of bromodomains to acetylated histone tails is still not completely clear. Bromodomains have low affinities for acetylated histone peptides (~10–1000 μM), and the difference between specific and nonspecific binding may be modest.[21] Vollmuth and Geyer showed that BRD4 bromodomains could bind histones with propionyl-lysine with affinity and binding mode similar to that of Kac.[10] Bromodomains are also known to bind acetylated peptides in non-histone proteins, for example BRD3 to GATA1,[22] PCAF to HIV-1 Tat,[23] CREBBP to p53,[24] or NF-κB to BRD4(2).[25] Other types of interactions between bromodomains and acetylated peptides may occur. BRD4(1), the most promiscuous bromodomain for small-molecule binding,[26] can accommodate two Kac residues in

the binding pocket. The binding affinity is increased relative to the monoacetylated peptide. Moreover, another family of protein modules has been described recently to bind Kac residues of histone tails in humans, the YEATS domains.[3b] The immunoglobulin-like fold of the YEATS module is completely different from the four-helix bundle topology of bromodomains. Moreover, the Kac binding pocket in YEATS domains does not resemble the one in bromodomains.[3b]

It is likely that new crystal structures of bromodomains in complex with (histone) peptides with multiple marks will shed more light on the complexity of recognition by these reader modules. In particular, structures with more than one protein domain will play a key role in deciphering the histone code at atomic-level detail. Such in-depth knowledge will be crucial to identify small molecules that bind potently and selectively to bromodomains, which will be useful for understanding the biological role of individual members of the family of (human) bromodomains.

Keywords: bromodomains · crystal structures · epigenetics · histone binding · histones · noncovalent interactions

[1] K. Luger, A. W. Mäder, R.K. Richmond, D. F. Sargent, T. J. Richmond, *Nature* **1997**, *389*, 251 – 260.
[2] K. Marushige, *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 3937 – 3941.
[3] a) C. Dhalluin, J. E. Carlson, L. Zeng, C. He, A.K. Aggarwal, M. M. Zhou, *Nature* **1999**, *399*, 491 – 496; b) Y. Li, H. Wen, Y. Xi, K. Tanaka, H. Wang, D. Peng, Y. Ren, Q. Jin, S. Y. Dent, W. Li, H. Li, X. Shi, *Cell* **2014**, *159*, 558 – 571.
[4] P. Filippakopoulos, S. Knapp, *FEBS Lett.* **2012**, *586*, 2692 – 2704.
[5] R. Sanchez, M.-M. Zhou, *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 659 – 665.
[6] a) T. Umehara, Y. Nakamura, M.K. Jang, K. Nakano, A. Tanaka, K. Ozato, B. Padmanabhan, S. Yokoyama, *J. Biol. Chem.* **2010**, *285*, 7610 – 7618; b) T. Umehara, Y. Nakamura, M. Wakamori, K. Ozato, S. Yokoyama, B. Padmanabhan, *FEBS Lett.* **2010**, *584*, 3901 – 3908; c) W. W. Tsai, Z. Wang, T. T. Yiu, K. C. Akdemir, W. Xia, S. Winter, C. Y. Tsai, X. Shi, D. Schwarzer, W. Plunkett, B. Aronow, O. Gozani, W. Fischle, M. C. Hung, D. J. Patel, M. C. Barton, *Nature* **2010**, *468*, 927 – 932; d) P. Filippakopoulos, S. Picaud, M. Mangos, T. Keates, J. P. Lambert, D. Barsyte-Lovejoy, I. Felletar, R. Volkmer, S. Muller, T. Pawson, A. C. Gingras, C. H. Arrowsmith, S. Knapp, *Cell* **2012**, *149*, 214 – 231; e) A. N. Plotnikov, S. Yang, T. J. Zhou, E. Rusinova, A. Frasca, M. M. Zhou, *Structure* **2014**, *22*, 353 – 360; f) A. J. Ruthenburg, H. Li, T. A. Milne, S. Dewell, R.K. McGinty, M. Yuen, B. Ueberheide, Y. Dou, T. W. Muir, D. J. Patel, C. D. Allis, *Cell* **2011**, *145*, 692 – 706; g) M. Y. Lubula, B. E. Eckenroth, S. Carlson, A. Poplawski, M. Chruszcz, K. C. Glass, *FEBS Lett.* **2014**, *588*, 3844 – 3854; h) G. Poncet – Montange, Y. Zhan, J. P. Bardenhagen, A. Petrocchi, E. Leo, X. Shi, G. R. Lee IV, P. G. Leonard, M. K. Geck Do, M. G. Cardozo, J. N. Andersen, W. S. Palmer, P. Jones, J. E. Ladbury, *Biochem. J.* **2015**, *466*, 337 – 346; i) P. Filippakopoulos, S. Knapp, *Nat. Rev. Drug Discovery* **2014**, *13*, 337 – 356.
[7] P. Gilli, L. Pretto, V. Bertolasi, G. Gilli, *Acc. Chem. Res.* **2009**, *42*, 33 – 44.
[8] C. W. Chung, A. W. Dean, J. M. Woolven, P. Bamborough, *J. Med. Chem.* **2012**, *55*, 576 – 586.
[9] Q. Xi, Z. Wang, A. I. Zaromytidou, X. H. Zhang, L. F. Chow-Tsang, J. X. Liu, H. Kim, A. Barlas, K. Manova-Todorova, V. Kaartinen, L. Studer, W. Mark, D. J. Patel, J. Massague, *Cell* **2011**, *147*, 1511 – 1524.
[10] F. Vollmuth, M. Geyer, *Angew. Chem. Int. Ed.* **2010**, *49*, 6768 – 6772; *Angew. Chem.* **2010**, *122*, 6920 – 6924.
[11] F. M. Ferguson, O. Fedorov, A. Chaikuad, M. Philpott, J. R. Muniz, I. Felletar, F. von Delft, T. Heightman, S. Knapp, C. Abell, A. Ciulli, *J. Med. Chem.* **2013**, *56*, 10183 – 10187.
[12] A. Chaikuad, A. M. Petros, O. Fedorov, J. Xu, S. Knapp, *MedChemComm* **2014**, *5*, 1843 – 1848.
[13] M. Philpott, J. Yang, T. Tumber, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, T. Keates, I. Felletar, A. Ciulli, S. Knapp, T. D. Heightman, *Mol. BioSyst.* **2011**, *7*, 2899 – 2908.
[14] C. Tallant, E. Valentini, O. Fedorov, L. Overvoorde, F. M. Ferguson, P. Filippakopoulos, D. I. Svergun, S. Knapp, A. Ciulli, *Structure* **2015**, *23*, 80 – 92.
[15] A. Magno, S. Steiner, A. Caflisch, *J. Chem. Theory Comput.* **2013**, *9*, 4225 – 4232.
[16] X. Lucas, D. Wohlwend, M. Hugle, K. Schmidtkunz, S. Gerhardt, R. Schule, M. Jung, O. Einsle, S. Gunther, *Angew. Chem. Int. Ed.* **2013**, *52*, 14055 – 14059; *Angew. Chem.* **2013**, *125*, 14305 – 14309.
[17] a) G. Lolli, R. Battistutta, *Acta Crystallogr. Sect. D* **2013**, *69*, 2161 – 2164; b) M. J. Harner, B. A. Chauder, J. Phan, S. W. Fesik, *J. Med. Chem.* **2014**, *57*, 9687 – 9692; c) L. Zhao, Y. Wang, D. Cao, T. Chen, Q. Wang, Y. Li, Y. Xu, N. Zhang, X. Wang, D. Chen, L. Chen, Y. L. Chen, G. Xia, Z. Shi, Y. C. Liu, Y. Lin, Z. Miao, J. Shen, B. Xiong, *J. Med. Chem.* **2015**, *58*, 1281 – 1297; d) P. V. Fish, P. Filippakopoulos, G. Bish, P. E. Brennan, M. E. Bunnage, A. S. Cook, O. Federov, B. S. Gerstenberger, H. Jones, S. Knapp, B. Marsden, K. Nocka, D. R. Owen, M. Philpott, S. Picaud, M. J. Primiano, M. J. Ralph, N. Sciammetta, J. D. Trzupek, *J. Med. Chem.* **2012**, *55*, 9831 – 9837.
[18] B. D. Strahl, C. D. Allis, *Nature* **2000**, *403*, 41 – 45.
[19] Q. Zhang, S. Chakravarty, D. Ghersi, L. Zeng, A. N. Plotnikov, R. Sanchez, M. M. Zhou, *PloS one* **2010**, *5*, e8903.
[20] N. Blöchliger, M. Xu, A. Caflisch, *Biophys. J.* **2015**, *108*, 2362 – 2370.
[21] C. W. Chung, J. Witherington, *J. Biomol. Screen.* **2011**, *16*, 1170 – 1185.
[22] R. Gamsjaeger, S. R. Webb, J. M. Lamonica, A. Billin, G. A. Blobel, J. P. Mackay, *Mol. Cell. Biol.* **2011**, *31*, 2632 – 2640.
[23] S. Mujtaba, Y. He, L. Zeng, A. Farooq, J. E. Carlson, M. Ott, E. Verdin, M. M. Zhou, *Mol. cell* **2002**, *9*, 575 – 586.
[24] S. Mujtaba, Y. He, L. Zeng, S. Yan, O. Plotnikova, Sachchidanand, R. Sanchez, N. J. Zeleznik-Le, Z. Ronai, M. M. Zhou, *Mol. cell* **2004**, *13*, 251 – 263.
[25] G. Zhang, R. Liu, Y. Zhong, A. N. Plotnikov, W. Zhang, L. Zeng, E. Rusinova, G. Gerona-Nevarro, N. Moshkina, J. Joshua, P. Y. Chuang, M. Ohlmeyer, J. C. He, M. M. Zhou, *J. Biol. Chem.* **2012**, *287*, 28840 – 28851.
[26] D. Gallenkamp, K. A. Gelato, B. Haendler, H. Weinmann, *ChemMedChem* **2014**, *9*, 438 – 464.

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