## Supporting information

Dynamics of the HAT lysine-rich loop in the catalytic core of CBP

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## Supporting Tables

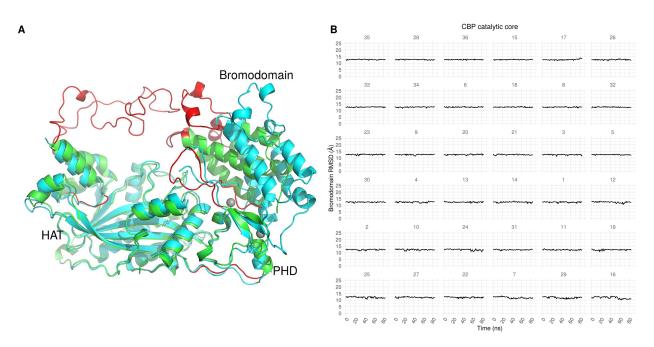
Threshold for unbinding	K1595ac in catalytic core
10 Å	2  ns
12 Å	3  ns
14 Å	4  ns
$16 \text{ \AA}$	5  ns

**Table S1:** Characteristic unbinding times of K1595ac from the bromodomain in the catalytic core, obtained from a single-exponential fit. The threshold distances used to define an unbinding event range from 10 to 16 Å. The unbinding times are overall similar, thus the analysis is robust with respect to the chosen threshold.

Threshold for unbinding	AIL endecamer	Histone peptide
10 Å	96  ns	1061  ns
12 Å	107  ns	1083  ns
14 Å	109  ns	1097  ns
16 Å	118  ns	1147  ns

Table S2: Characteristic unbinding times of the acetylated lysine in the bromodomain complexes with AIL endecamer and histone peptide, obtained from a single-exponential fit. The threshold distances used to define an unbinding event range from 10 to 16 Å. Unbinding of the AIL endecamer is always one order of magnitude faster than the histone peptide.

## **Supporting Figures**



**Figure S1:** A) Overlap of the CBP crystal structure 5U7G (cyan) and the equilibrated model of the CBP catalytic core (green). All the segments with flexible backbone in the simulations are colored in red. This includes the hinge regions, *i.e.* short loops connecting the structured domains. The bromodomain is the most mobile component as it shifts closer to the HAT domain when inserting K1595ac in the bromodomain pocket. A small shift is observed also for the HAT helix from which the AIL protrudes. The zinc ions (silver spheres) overlap between the two structures. B) Time series of the root-mean-square-deviation (RMSD) of the bromodomain  $\alpha$ -helices (C $\alpha$  atoms) after fitting to the HAT domain (C $\alpha$  atoms) of the crystal structure. The RMSD does not change between bound and unbound replicas, meaning that the bromodomain does not shift its position relative to the HAT upon AIL unbinding.

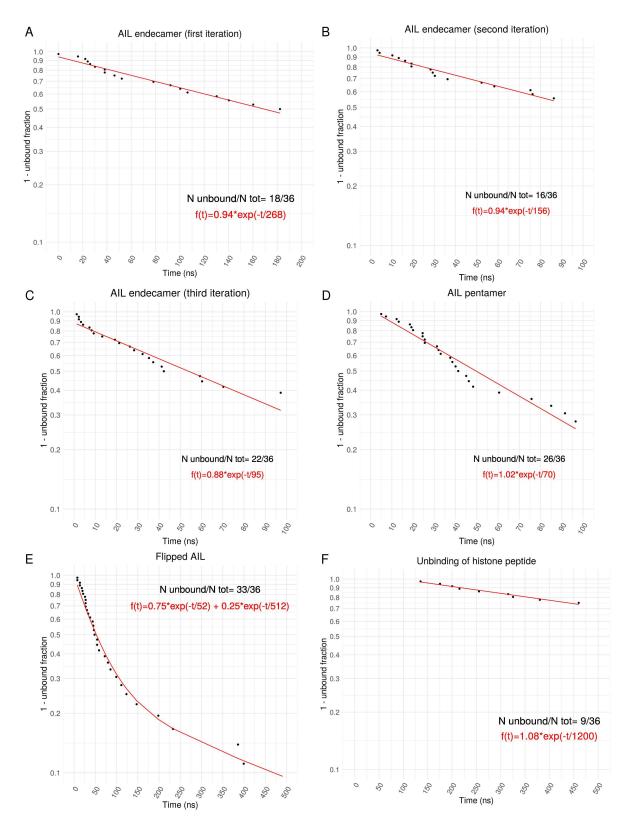


Figure S2: Kinetics of complete peptide unbinding in all the bromodomain-peptide complexes, shown in the individual timescales of sampling. Only the flipped AIL is fit with a double-exponential function, in which the first and second terms represent the fast and slow unbinding phases, respectively. All of the AIL peptides unbind faster than the histone peptide.

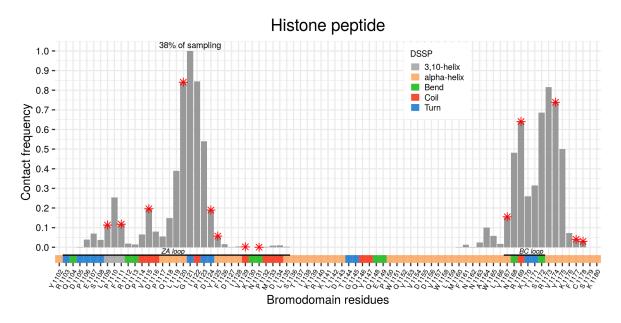
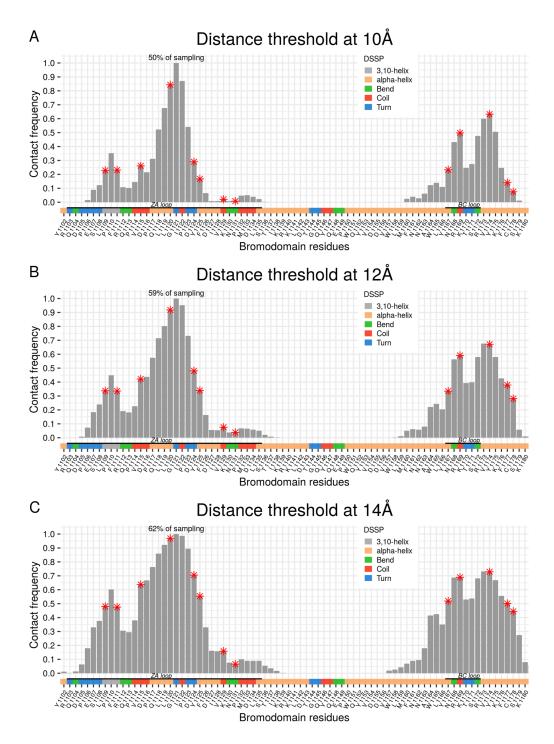


Figure S3: Contact analysis between the bromodomain backbone nitrogen atoms and all heavy atoms of the histone peptide. To define a contact, the same distance threshold of 8 Å as for the AIL endecamer in the main text is used. The percentage of sampling refers to the canonical and intermediate bound states (the K1595ac distance to the structural water is below 20 Å).



**Figure S4:** Contact analysis between the bromodomain backbone nitrogen atoms and all heavy atoms of the AIL endecamer, in a range of distance thresholds that define a contact between 10 to 14 Å. The percentage of sampling refers to the canonical and intermediate bound states.

Human Mouse	VDEKKPEVKV V <mark>E</mark> EKKPEVKV	EVKEEEESSS EAKEEEENSSS	N <mark>GTASQSTSP</mark> N <mark>DTASQSTSP</mark>	1080 SQPRKKIFKPI SQPRKKIFKPI	1090 EELRQALMPTI EELRQALMPTI	1100 LEALYRQDPE: LEALYRQDPE:	1110 SLPFRQPVDP SLPFRQPVDP
Human Mouse	1120 QLLGIPDYFD QLLGIPDYFD	1130 IVKNPMDLST IVKNPMDLST	1140 IKRKLDTGQY IKRKLDTGQY	1150 QEPWQYVDDV QEPWQYVDDV	1160 ₩LMFNNAWLYN ₩LMFNNAWLYN	1170 NRKTSRVYKF( NRKTSRVYKF(	1180 CSKLAEVFEQ CSKLAEVFEQ
Human Mouse	1190 EIDPVMQSLG EIDPVMQSLG	1200 YCCGRKYEFS YCCGRKYEFS	1210 PQTLCCYGKQ PQTLCCYGKQ	1220 LCTIPRDAAY LCTIPRDAAY	1230 Y S Y Q N R Y H F C I Y S Y Q N R Y H F C I	1240 EKCFTEIQGEI EKCFTEIQGEI	<sup>1250</sup> NVTLGDDPSQ NVTLGDDPSQ
Human Mouse	1260 PQTTISKDQF PQTTISKDQF	1270 EKKKNDTLDP EKKKNDTLDP	1280 EPFVDCKECG EPFVDCKECG	1290 RKMHQICVLH RKMHQICVLH	1300 YDIIWPSGFV( YDIIWPSGFV(	1310 CDNCLKKTGRI CDNCLKKTGRI	1320 PRKENKFSAK PRKENKFSAK
Human Mouse	1330 RLQTTRLGNH RLQTTRLGNH	1340 LEDRVNKFLR LEDRVNKFLR	1350 RQNHPEAGEV RQNHPEAGEV	1360 FVRVVASSDK FVRVVASSDK	<sup>1370</sup> IVEVKPGMKSI IVEVKPGMKSI	1380 RFVDSGEMSES RFVDSGEMSES	1390 SFPYRTKALF SFPYRTKALF
Human Mouse	1400 AFEEIDGVDV AFEEIDGVDV	<sup>1410</sup> CFFGMHVQEY CFFGMHVQNT	GSDCPPPNTR ALIA <mark>P</mark> HQIQG	1430 RVÝISYLDSII RVYISYLDSII	1440 HFFRPRCLRTA HFFRPRCLRTA	<sup>1450</sup> AVYHEILIGYI AVYHEILIGYI	1460 LEŸVKKLGYV LEYVKKLGYV
Human Mouse	1470 TGHIWACPPS TGHIWACPPS	1480 EGDDYIFHCH EGDDYIFHCH	1490 PPDQKIPKPK PPDQKIPKPK	1500 RLQEWYKKMLI RLQEWYKKMLI	<sup>1510</sup> DKÁFAERIIH DKAFAERII <mark>N</mark> I	1520 DYKDIFKQA <mark>T</mark> I DYKDIFKQA <mark>N</mark> I	1530 EDRLTSAKEL EDRLTSAKEL
Human Mouse	1540 PYFEGDFWPN PYFEGDFWPN	<sup>1550</sup> VLEESIKELE VLEESIKELE	1560 QEEEERKKEE QEEEERKKEE	1570 STAASET <mark>TEG</mark> STAASET <mark>P</mark> EG	1580 SQGDSKNAKKE SQGDSKNAKKE	1590 X N N K K T N K N K S X N N K K T N K N K S	1600 SSÍSRANKKK SSISRANKKK
Human Mouse	<sup>1610</sup> PSMPNVSNDL PSMPNVSNDL	1620 SQKLYATMEK SQKLYATMEK	1630 HKEVFFVIHL HKEVFFVIHL	1640 HAĠPVINTLPI HAGPVI <mark>STQ</mark> PI	1650 PIVDPDPLLS( PIVDPDPLLS(	1660 CDLMDGRDAFI CDLMDGRDAFI	1670 LTLARDKHWE LTLARDKHWE
Human Mouse	1680 FSSLRRSKWS FSSLRRSKWS	1690 TLCMLVELHT TLCMLVELHT	1700 QGQDRFVYTC QGQDRFVYTC	1710 NECKHHVETRV NECKHHVETRV	<sup>1720</sup> √HĊTVCEDYDI √HCTVCEDYDI	1730 LCINCYNTKSI LCINCYNTKSI	1740 H <mark>A</mark> HKMVKWGL H <mark>T</mark> HKMVKWGL

Figure S5: Alignment of the CBP catalytic core sequences from human and mouse organisms. The residue numbering on top refers to the human CBP sequence. The sequence of the catalytic core used in this study starts from residue Lys1082 until residue Asp1701. The residues that differ between the two organisms are indicated in white background. The bromodomain binding site does not have any differing residues. The AIL differs by only one residue (Thr1575 in human CBP is Pro1576 in mouse CBP).