Supporting Information

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SI Text

TLC Assay. Purified native SET8 and the Y334F mutant (5 μ M) were used in methyltransferase assays with the biotinylated H4-K20 peptide (500 µM). Assays contained 1% (wt/vol) BSA, 20 mM MgCl₂, 100 mM Bicine (pH 8.0), 1.0 mM [³H-methyl] AdoMet [diluted to a specific activity of 0.2 µCi/nmol with purified AdoMet (1)], 3 µM of Sulfolobus solfataricus AdoHcy hydrolase (2), and 2 units of adenosine deaminase (Sigma) in a final volume of 20 µL and incubated at 37 °C. Assays were terminated by the addition of an equal volume of 200 mM MES, pH 5.1 before the addition of a 2-fold molar excess of immobilized avidin resin (Pierce Biotechnology). The resin was then collected by centrifugation, washed three times with 300 mM NaCl, and boiled in 2% SDS for 5 min to liberate the bound histone H4 peptide. The recovered samples were then hydrolyzed under acidic conditions (6 M HCl) at 110 °C for 24 h. After treatment with KOH to remove the SDS, the samples were taken to dryness in vacuo. Subsequently, the samples were reconstituted with 50 μ L of 100 mM Bicine, pH 8.0 and standards of lysine and monomethylated, dimethylated, and trimethylated lysine (commercial sources; K, Sigma; Kme1 and Kme3, Bachem; and Kme2, Chem-Impex International) added to each for determining Rf positions on the silica gel TLC plates (Sigma; 20×20 cm, 250-µm layer of silica gel matrix on glass support, 5- to 17- μ m particle size) after chromatography by using methanol/ammonium hydroxide (75:25, vol/vol) and ninhydrin staining [0.25% (wt/vol) in acetone] (3). After applying the entire reaction 2 cm from the bottom and developing with solvent for \approx 3 h at 22 °C to within 1 cm of the top, the TLC plate was thoroughly dried before placing it in contact with the tritium Phosphor screen (GE Healthcare) for 15.5 days in the dark. Rf regions corresponding with the methylated lysine derivatives were excised from the plates, and the quantity of tritium incorporated during the assay was measured by liquid scintillation spectroscopy and corrected for recovery.

 Rebouche CJ, Broquist HP (1976) Carnitine biosynthesis in Neurospora crassa: Enzymatic conversion of lysine to epsilon-N-trimethyllysine. J Bacteriol 126:1207–1214.

Chirpich TP, Zappia V, Costilow RN, Barker HA (1970) Lysine 2,3-aminomutase. Purification and properties of a pyridoxal phosphate and S-adenosylmethionine-activated enzyme. J Biol Chem 245:1778–1789.

Collazo E, Couture JF, Bulfer S, Trievel RC (2005) A coupled fluorescent assay for histone methyltransferases. Anal Biochem 342:86–92.

	spec	specificity		
PKMT	Site	Methyl	Res.	
HsSET7/9	H3-K4	Mel	214	ERVYVAESLISSAGECLFSKVAVGPNTVMSFYNGVRUTHQEVDSRDWALNGNTLSLDEETVIDVPEP
HsSMYD3		Me 3	4	LKVEKFATAN RENGLRAVTPLRPGELLFRSD PLAYTVCKGSRGVVCDRCLLGKEKLMRCSQCR (123)
HsMLL1		Me3	3829	EAVGVYRSPIHCRCLFCKRNIDAGEMVIEYAGNVIRSIQTDKREKYYDSKGIG-CYMFRIDDSE
ScSET1		Me3	938	KPVMFARSAIHNWGLYALDSIAAKEMIIEYVGERIRQPVAEMREKRYLKNGIGSSYLFRVDENT
HsSUV39H1	H3-K9	Me3	243	YDLCIFRTDDG-RCWGVRTLEKIRKNSFVMEYVGEIITSEEAERRGQIYDRQGATYLFDLD(5)-Y
HsSUV39H2		Me3	250	YSLCIFRTSNG-RCWCVKTLVKIKRMSFVMEYVGEVITSEEAERRGQFYDNKGITYLFDLD(5)-F
NcDIM-5		Me3	162	VPLQIFRTKDRGWGVKCPVNIKRGQFVDRYLGEIITSEEADRRRAESTIARRKDVYLFALDKFS-(12)PL
SpCLR4		Me3	328	LPLEIFKTKEKGWGVRSLRFAPAGTFITCYLGEVITSAEAAKRDKNYDDDGITYLFDLDMFD(3)YE
HsSETDB1		Me2	803	VRLOLFKTONKCWGIRCLDDIAKGSFVCIYAGKILTDDFADKEGLEMGDEYFANLDHIE (345) CY
HsGLP		Me2/3	1095	ARLQLYRTRDMCWCVRSLQDIPPGTFVCEYVGELISDSEADVREEDSYLFDLD(5)VY
HsG9a		Me2/3	1038	VRLQLYRTAKMCWCVRALQTIPQGTFICEYVCELISDAEADVREDDSYLFDLD(5)VY
AtKYP		Mel	446	FNLEVFRSAKKGWAVRSWEYIPAGSPVCEYIGVVRRTADVDTISDNEYIFEID(36)EF
AtSUVH6		Mel	616	LPLEIFKTKSRCWCVRCLKSIPIGSFICEYVGELLEDSEAERRIGNDEYLFDIG(31)GF
HsEZH2	H3-K27	Me3	612	KHLLLAPSDVAGWGIFIKDPVQKNEFISEYCGEIISQDEADRRGKVYDKYMCSFLFNLNNDF
SCSET2	H3-K36	Me 3	120	APIAIFKTKHKCYCVRAEQDIEANOFIYEYKCEVIEEMEFRDRLIDYDQRHFKHFYFMMLQNGE
HsSMYD2		Me2	7	GGLERFCSPGKCRGLRALOPFOVGDLLFSCPA(10)GNHCEYCFTRKEGLSKCGRCKQAFYCNVECQK(110)LS
HSSET8	H4-K20	Mel	216	EGMKIDLIDGKCRCVIATKOFSRGDFVVEYHGDLIEITDAKKREALYAQDPSTGCYMYYFQYLSKTY
HsSUV4H201		Me2/3	198	LPCNRYSSEQNCAKIVATKEWKRNDKIELLVCCHAELSEIEENMLLRHGENDFSVMYSTRK
Hacer7/0	H2 F4	No1	202	
HECHVD3	H3-K4	Mel	100	
HeMII 1		Mes	2002	MOLYGYGYGIPSISLENHSCHWCSI VFNGPR
ASMELI CoCETI		Mes	1003	VDAIMIGN - AARFINISCHPICISKVINIDG - VRI IVIAURNIIRGESIIDIRFIEDA
Necini2011	W2	Meg	210	IDAIRTOS - TARFINISCOPICIARIIRAGO - RRR - IVIIALROPASSOTI DIR BRED
HSSUV39HI Necini20H2	H3-K9	Me3	217	
NeDIM E		Meg	241	VDARYIGA VSHFVNHCDHALQVFNVFIDA - LDIRIPRIANS IRINAGISJI DIQUKUSGD
RCDIM-5		Meg	206	
NeCETDR1		Meg	1210	VDQNIED-VSRTATESTITISSVIUMGRAIIDDETAIRDEVELDITETARAADS TAXVEDAVSRTATESTITISSVIUMUNIDEDWARDAGATSITISVUVPUGSV
HeGLD		Me2/3	1156	TRADEVAL - USEFINIUSEDNU VOVEVAL - ODU BEDIAFESTNI TRADICI SU UNIVERSIV
HeCos		Me2/3	1000	
AFEAD		Me1	539	
ALKIP		Mel	704	TDAGS 104 - FART INTEGENLE VQCVLSSR QDIALARVVLI FARDIN SPINOSTI DI GIALDSV
HoF7U2	W2 - W27	Me1	675	IDARSKON - VSKI INDESTRUIADAV HIDD EDSTITAVNI PADINIFICEN CONTAINADOV
CeCET2	H3 K26	Mes	105	VDAIRGN
BCBE12	H3-K30	Meg	104	TORTINGS - MARCHING CONTRICTION WAT - ALK AGTAQUAN ACCOUNT OF INVORTIGA
Hacking	H4 800	mez Mol	294	
HESEIG	H4-K20	Mel /2	264	VDATRETNE-IGRATINEBRCGCCTRLHDTDGVPHLILIASKUTAGGSTLLDTGDRSKAS
HSSUV4H201		Me2/3	260	CAQLWLGE AAFINHDCRIMCKFVSTGRDT ACWKALRDEPGEDISC XXGDGFFGE

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Fig. S1. Sequence alignment of the SET domains of representative histone lysine methyltransferases. The enzymes are categorized according to their site specificity, and their reported product specificities are also delineated. Sequence identity and conservation are delineated by black and gray text backgrounds, respectively. The conserved active-site tyrosine that hydrogen-bonds to the lysine or methyllysine *e*-amine group is denoted in green, and the invariant tyrosine residue within the lysine-binding channel is indicated in cyan. Phe/Tyr switch residues are highlighted in red. In SET7/9, Tyr-305 has been shown to function as the Phe/Tyr switch residue. Although not conserved at the primary sequence level, the position of the phenol side chain of Tyr-305 is structurally conserved with the Phe/Tyr switch residues in other histone methyltransferases (Fig. S2 *D* and *E*). The product specificity "ME2/3" signifies PKMTs that exhibit weak trimethyltransferase activity. Species abbreviations: *At, Arabidopsis thaliana; Hs, Homo sapiens; Nc, Neurospora crassa; Sc, Saccharomyces cerevisiae;* and *Sp, Schizosaccharomyces pombe*.



Fig. S2. Binary and ternary complexes of SET domain PKMTs highlighting the structural conservation of the active-site water molecule. The residues comprising the active site of each enzyme (gray carbons atoms), cofactor (green carbons), water (red), and peptidyl lysine or monomethyllysine (yellow carbons) are illustrated. Conventional hydrogen bonds are depicted as dashed orange lines, and CH^{...}O hydrogen bonds are rendered as cyan dashes. Cut-off distances for conventional and CH^{...}O hydrogen bonds are 3.3 and 3.7 Å, respectively. (A) GLP–AdoHcy complex (Protein Data Bank ID code 2IGQ). (B) G9A–AdoHcy (Protein Data Bank ID code 208J). (C) SUV39H2–AdoMet (Protein Data Bank ID code 2R3A). (D) SET7/9–AdoMet (Protein Data Bank ID code 1N6A). (E) SET7/9–TAF10K189me1–AdoHcy (Protein Data Bank ID code 2F69). (F) SET8–H4K20me1–AdoHcy (Protein Data Bank ID code 2BQZ).



SET8 Y334F-H4K20me2-AdoHcy (kinked K20me2 side chain)

Fig. S3. The SET8 Y334F mutant bound to AdoHcy and the H4K20me2 peptide with the dimethyllysine side chain in a kinked conformation. Residues and hydrogen bonds are illustrated as in Fig. S2. Inset for the Y334F mutant represent the $F_O - F_C$ omit map electron density for the H4K20me2 side chain contoured at 2.0 σ .



SET8 Y334F-H4K20me2-AdoHcy GLP-H3K9me2-AdoHcy

Fig. S4. Superimposition of the active sites of SET8 Y334F–H4K20me2–AdoHcy and GLP–H3K9me2–AdoHcy (Protein Data Bank ID code 2RFI) product complexes. The SET8 Y334F and GLP complexes are distinguished by green and violet carbon atoms, respectively. Hydrogen bonds are depicted as in Fig. S2.



SET8-H4K20-AdoHcy SET8 Y245F-H4K20-AdoHcy

Fig. S5. Superimposition of the active sites of native SET8 and the SET8 Y245F mutant bound to the H4K20 peptide and AdoHcy. The lysine-binding channel of the enzyme is depicted as in Fig. S2. The Y245F mutation, H4K20, and the water molecules corresponding to this complex are depicted in magenta. Orange and magenta dashed lines illustrate hydrogen bonds that are observed in native SET8 and the Y245F mutant, respectively, and hydrogen bonds common to both complexes are shown as red dashes. (*Inset*) The $F_O - F_C$ omit map density (contoured at 2.0 σ) of the H4K20 side chain in the SET8 Y245F ternary complex.



Fig. S6. Schematic representation of hydrogen bonding in the active site of SET8 Y334F during monomethyltransfer and dimethyltransfer. (*A*) In monomethylation, the Tyr-245 hydroxyl group and the active-site water molecule in the enzyme function as hydrogen-bond acceptors to align the deprotonated K20 ε -amine for an S_N2 methyltransfer reaction with the methyl group of AdoMet (same color scheme as in Fig. S2). After methyltransfer, the K20me1 side chain is bound in a protonated state through these two hydrogen bonds. (*B*) During dimethylation, the K20me1 side chain is bound in an alternative conformation with its methyl group positioned in the solvent binding pocket where it forms a CH^{...}O hydrogen bond with Ile-297 in the enzyme. The deprotonated state owing to the hydrogen bond with the Tyr-245 hydroxyl group. The dimethyl product is bound in a protonated state owing to the hydrogen bond to this tyrosine.



SET8-H4K20me2-AdoHcy Model

Fig. 57. Model of native SET8 bound to the H4K20me2 peptide. The coordinates of the SET8–H4K20–AdoHcy (Protein Data Bank ID code 1ZKK) and SET Y334F–H4K20me2–AdoHcy complexes were superimposed to generate the docking of the K20me2 in the active site of the native enzyme. Residues and hydrogen bonds are illustrated as in Fig. S2. The interaction distances between the K20me2 methyl group bound in the solvent pocket and the oxygen atoms of the active-site water molecule and Tyr-334 OH group in SET8 are denoted by dashed blue lines with distances shown.





WT SET8-H4K20-AdoHcy





SET8 Y334F-H4K20-AdoHcy





SET8 Y334F-H4K20me2-AdoHcy

Fig. S8. Dimensions of the lysine-binding channels of native SET8 and the Y334F mutant. (*A*) Two views of the molecular surface of the lysine-binding channel of wild type (WT) SET8 bound to AdoHcy (green carbon atoms) and the H4K20 peptide (yellow carbons). For clarity, only the K20 side chain of the substrate is shown. The Phe/Tyr switch residue Tyr-334 and its contributions to the channel are illustrated in magenta. The active-site water molecule bound in the solvent pocket is rendered as a dotted van der Waals sphere in red. The right and left panels are separated by an approximate 45° rotation along the vertical axis. In the right panel, the cofactor is omitted, and the midpoint of the lysine binding channel and the solvent pocket are labeled for clarity. (*B*) Active site of SET8 Y334F–H4K20–AdoHcy complex illustrated as in *A*.

Table S1. Crystallographic data and refinement statistics for SET8 mutant complexes

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$\begin{tabular}{ c c c c c } \hline H4K20/AdOHcy & AdOHcy & AdOHcy & AdOHcy & AdOHcy & H4K20/AdOH \\ \hline Synchrotron & APS & APS & APS & ESRF \\ Beamline & 17.1D & 22.1D & 17.1D & BM30A \\ \hline Synchrotron & APS & APS & APS & ESRF \\ \hline Space group & P1 & & & & & & & & & & & & & & & & & $					
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Statistic	H4K20/AdoHcy	H4K20me1/ AdoHcy	H4K20me2/ AdoHcy	SET8-Y245F, H4K20/AdoHcy
Beamine 17-ID 22-ID 17-ID BM30A Crystal parameters Space group P1 Space group P1 Unit cell ************************************	Synchrotron	APS	APS	APS	ESRF
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	Total reflections	363,128	439,614	604,630	334,404
R_{sym} (%)2.7 (14.6)6.7 (52.8)2.8 (13.1)4.1 (17.3) $l/sigma$ (l)38.2 (10.6)22.5 (8.4)30.4 (9.9)31.1 (8.4)Completeness (%)95.2 (92.2)95.0 (91.8)94.3 (86.6)94.1 (90.6)Refinement statistics </td <td>Unique reflections</td> <td>91,908</td> <td>55,907</td> <td>204,489</td> <td>90,904</td>	Unique reflections	91,908	55,907	204,489	90,904
I /sigma (I) $38.2 (10.6)$ $22.5 (8.4)$ $30.4 (9.9)$ $31.1 (8.4)$ Completeness (%) $95.2 (92.2)$ $95.0 (91.8)$ $94.3 (86.6)$ $94.1 (90.6)$ Refinement statisticsResolution range (Å) $12.9 - 1.6$ $23.7 - 1.5$ $47.4 - 1.25$ $38.9 - 1.6$ Reflections ($F_0 > 2\sigma$) $87,774$ $53,407$ $183,160$ $87,679$ Final modelrmsd with native SET8 0.18 1.13^* 0.55 0.30 Protein/peptide atoms $5,122/308$ $2,566/135$ $5,117/324$ $5,138/286$ AdoHcy104 52 104104Water $1,022$ 227 864 589 R factors tRworking 16.2 19.3 18.1 21.5 Rrive 20.6 22.0 20.7 25.6 rmsBond length (Å) 0.010 0.019 0.013 0.016 Bond angles (\circ) 1.254 1.895 1.573 1.600 Average B factors (Å^2)Protein/peptide atoms $16.2/17.3$ $32.5/34.2$ $34.5/35.6$ $6.7/8.4^{*}$ AdoHcy 15.1 23.3 38.8 6.6^{*} Water 30.7 33.6 40.1 17.4^{*}	R _{sym} (%)	2.7 (14.6)	6.7 (52.8)	2.8 (13.1)	4.1 (17.3)
Completeness (%)95.2 (92.2)95.0 (91.8)94.3 (86.6)94.1 (90.6)Refinement statistics $38.9 - 1.6$ $37.7 - 1.5$ $47.4 - 1.25$ $38.9 - 1.6$ Resolution range (Å) $12.9 - 1.6$ $23.7 - 1.5$ $47.4 - 1.25$ $38.9 - 1.6$ Reflections ($F_0 > 2\sigma$) $87,774$ $53,407$ $183,160$ $87,679$ Final model $rmsd with native SET8$ 0.18 1.13^* 0.55 0.30 Protein/peptide atoms $5,122/308$ $2,566/135$ $5,117/324$ $5,138/286$ AdoHcy 104 52 104 104 Water $1,022$ 227 864 589 R factors [†] R 22.0 20.7 25.6 R working 16.2 19.3 18.1 21.5 Bond length (Å) 0.010 0.019 0.013 0.016 Bond angles (\circ) 1.254 1.895 1.573 1.600 Average B factors (Å^2) 15.1 23.3 38.8 6.6^* Water 30.7 33.6 40.1 17.4^*	l /sigma (l)	38.2 (10.6)	22.5 (8.4)	30.4 (9.9)	31.1 (8.4)
Refinement statisticsResolution range (Å)12.9 – 1.623.7 – 1.547.4 – 1.2538.9 – 1.6Reflections ($F_0 > 2\sigma$)87,77453,407183,16087,679Final model	Completeness (%)	95.2 (92.2)	95.0 (91.8)	94.3 (86.6)	94.1 (90.6)
Resolution range (Å) $12.9 - 1.6$ $23.7 - 1.5$ $47.4 - 1.25$ $38.9 - 1.6$ Reflections ($F_{o} > 2\sigma$) $87,774$ $53,407$ $183,160$ $87,679$ Final model </td <td>Refinement statistics</td> <td></td> <td></td> <td></td> <td></td>	Refinement statistics				
Reflections ($F_{o} > 2\sigma$)87,77453,407183,16087,679Final modelrmsd with native SET80.181.13*0.550.30Protein/peptide atoms5,122/3082,566/1355,117/3245,138/286AdoHcy10452104104Water1,022227864589R factors† R 16.219.318.121.5 R_{rree} 20.622.020.725.6rms R 0.0100.0190.0130.016Bond length (Å)0.0100.0191.5731.600Average B factors (Å ²) P 15.1 $32.5/34.2$ $34.5/35.6$ $6.7/8.4^{*}$ AdoHcy15.123.338.8 6.6^{*} Water30.733.640.117.4^{*}	Resolution range (Å)	12.9 – 1.6	23.7 – 1.5	47.4 – 1.25	38.9 – 1.6
Final model rmsd with native SET8 0.18 1.13* 0.55 0.30 Protein/peptide atoms 5,122/308 2,566/135 5,117/324 5,138/286 AdoHcy 104 52 104 104 Water 1,022 227 864 589 <i>R</i> factors [†] 7 864 589 <i>R</i> factors [†] 16.2 19.3 18.1 21.5 <i>R</i> free 20.6 22.0 20.7 25.6 25.6 rms 16.2 19.3 18.1 21.5 <i>R</i> free 20.6 22.0 20.7 25.6 25.6 rms 16.2 19.3 18.1 21.5 <i>R</i> free 20.6 22.0 20.7 25.6 rms 15.1 21.5 16.2 Bond length (Å) 0.010 0.019 0.013 0.016 Bond angles (°) 1.254 1.895 1.573 1.600 Average <i>B</i> factors (Å ²) 7 7 38.8	Reflections ($F_{o} > 2\sigma$)	87,774	53,407	183,160	87,679
rmsd with native SET8 0.18 1.13^* 0.55 0.30 Protein/peptide atoms $5,122/308$ $2,566/135$ $5,117/324$ $5,138/286$ AdoHcy104 52 104104Water $1,022$ 227 864 589 R factors† $$	Final model				
Protein/peptide atoms $5,122/308$ $2,566/135$ $5,117/324$ $5,138/286$ AdoHcy10452104104Water1,022227864589R factors† $$	rmsd with native SET8	0.18	1.13*	0.55	0.30
AdoHcy10452104104Water1,022227864589R factors* 227 864589R working16.219.318.121.5R free20.622.020.725.6rms 20.6 22.020.725.6Bond length (Å)0.0100.0190.0130.016Bond angles (\circ)1.2541.8951.5731.600Average B factors (Å ²) $25.734.2$ 34.5/35.6 $6.7/8.4^{\ddagger}$ AdoHcy15.123.338.8 6.6^{\ddagger} Water30.733.640.117.4^{\ddagger}	Protein/peptide atoms	5,122/308	2,566/135	5,117/324	5,138/286
Water1,022227864589R factorst R working16.219.318.121.5 R_{rree} 20.622.020.725.6rmsBond length (Å)0.0100.0190.0130.016Bond angles (\circ)1.2541.8951.5731.600Average B factors (Å ²)Protein/peptide atoms16.2/17.332.5/34.234.5/35.66.7/8.4 [‡] AdoHcy15.123.338.86.6 [‡] Water30.733.640.117.4 [‡]	AdoHcy	104	52	104	104
R factors [†] Rworking 16.2 19.3 18.1 21.5 R _{free} 20.6 22.0 20.7 25.6 rms Units Units <thunits< th=""> Units <thunits< <="" td=""><td>Water</td><td>1,022</td><td>227</td><td>864</td><td>589</td></thunits<></thunits<>	Water	1,022	227	864	589
$R_{working}$ 16.219.318.121.5 R_{free} 20.622.020.725.6rmsBond length (Å)0.0100.0190.0130.016Bond angles (°)1.2541.8951.5731.600Average B factors (Å ²)VProtein/peptide atoms16.2/17.332.5/34.234.5/35.66.7/8.4 [‡] AdoHcy15.123.338.86.6 [‡] Water30.733.640.117.4 [‡]	R factors [†]				
R _{free} 20.6 22.0 20.7 25.6 rms Bond length (Å) 0.010 0.019 0.013 0.016 Bond angles (°) 1.254 1.895 1.573 1.600 Average B factors (Å ²) 7 7 7 7 Protein/peptide atoms 16.2/17.3 32.5/34.2 34.5/35.6 6.7/8.4 [‡] AdoHcy 15.1 23.3 38.8 6.6 [‡] Water 30.7 33.6 40.1 17.4 [‡]	Rworking	16.2	19.3	18.1	21.5
rms Bond length (Å) 0.010 0.019 0.013 0.016 Bond angles (°) 1.254 1.895 1.573 1.600 Average <i>B</i> factors (Å ²) Protein/peptide atoms 16.2/17.3 32.5/34.2 34.5/35.6 6.7/8.4 [‡] AdoHcy 15.1 23.3 38.8 6.6 [‡] Water 30.7 33.6 40.1 17.4 [‡]	R _{free}	20.6	22.0	20.7	25.6
Bond length (Å) 0.010 0.019 0.013 0.016 Bond angles (°) 1.254 1.895 1.573 1.600 Average B factors (Å ²) 7 7 7 7 7 Protein/peptide atoms 16.2/17.3 32.5/34.2 34.5/35.6 6.7/8.4 [‡] AdoHcy 15.1 23.3 38.8 6.6 [‡] Water 30.7 33.6 40.1 17.4 [‡]	rms				
Bond angles (°) 1.254 1.895 1.573 1.600 Average B factors (Å ²) 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Bond length (Å)	0.010	0.019	0.013	0.016
Average B factors (Ų) 32.5/34.2 34.5/35.6 6.7/8.4* Protein/peptide atoms 16.2/17.3 32.5/34.2 34.5/35.6 6.7/8.4* AdoHcy 15.1 23.3 38.8 6.6* Water 30.7 33.6 40.1 17.4*	Bond angles (॰)	1.254	1.895	1.573	1.600
Protein/peptide atoms 16.2/17.3 32.5/34.2 34.5/35.6 6.7/8.4 [±] AdoHcy 15.1 23.3 38.8 6.6 [±] Water 30.7 33.6 40.1 17.4 [±]	Average <i>B</i> factors (Å ²)				
AdoHcy 15.1 23.3 38.8 6.6 [±] Water 30.7 33.6 40.1 17.4 [±]	Protein/peptide atoms	16.2/17.3	32.5/34.2	34.5/35.6	6.7/8.4 [‡]
Water 30.7 33.6 40.1 17.4 [±]	AdoHcy	15.1	23.3	38.8	6.6 [±]
	Water	30.7	33.6	40.1	17.4 [‡]

APS, Advanced Photon Source; ESRF, European Synchrotron Radiation Facility. Values in parentheses refer to data in the highest-resolution shell. *The higher rms value for the H4K20me1 complex is caused in part by differences in the unit cell parameters compared with the other complexes.

[†]*R* factor: $R_{\text{working}} = \Sigma ||F_0| - |F_c||/\Sigma |F_0|$; $R_{\text{free}} = \Sigma_T ||F_0| - |F_c||/\Sigma_T |F_0|$, where T is a test data set of 5% of the total reflections randomly chosen and set aside before refinement.

⁺The *B* factors of the SET8 Y245F complex differ from the other structures due to TLS refinement using Refmac. TLS groups and tensors are listed in the coordinate file.