Regulation of S-Adenosylhomocysteine Hydrolase by Lysine Acetylation*

Yun Wang‡, Jennifer M. Kavran‡, Zan Chen‡, Kannan R. Karukurichi‡, Daniel J. Leahy‡§, and Philip A. Cole‡§

From the Departments of ‡Pharmacology and Molecular Sciences and §Biophysics and Biophysical Chemistry, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205

Background: S-Adenosylhomocysteine hydrolase (SAHH) regulates methyltransferase reactions and is acetylated on two lysines.

Results: We prepared acetylated semisynthetic SAHH and determined the impact of acetylation on structure and activity.

Conclusion: Lys acetylation of SAHH changes hydrogen bonding patterns in its NAD\(^+\) binding regions and reduces its catalytic activity.

Significance: Acetylation of SAHH may serve to regulate global alterations in cellular methylation.

S-Adenosylhomocysteine hydrolase (SAHH) is an NAD\(^+\)-dependent tetrameric enzyme that catalyzes the breakdown of S-adenosylhomocysteine to adenosine and homocysteine and is important in cell growth and the regulation of gene expression. Loss of SAHH function can result in global inhibition of cellular methyltransferase enzymes because of high levels of S-adenosylhomocysteine. Prior proteomics studies have identified two SAHH acetylation sites at Lys\(^{401}\) and Lys\(^{408}\) but the impact of these post-translational modifications has not yet been determined. Here we use expressed protein ligation to produce semisynthetic SAHH acetylated at Lys\(^{401}\) and Lys\(^{408}\) and show that modification of either position negatively impacts the catalytic activity of SAHH. X-ray crystal structures of 408-acetylated SAHH and dually acetylated SAHH have been determined and reveal perturbations in the C-terminal hydrogen bonding patterns, a region of the protein important for NAD\(^+\) binding. These crystal structures along with mutagenesis data suggest that such hydrogen bond perturbations are responsible for SAHH catalytic inhibition by acetylation. These results suggest how increased acetylation of SAHH may globally influence cellular methylation patterns.

S-Adenosylhomocysteine hydrolase (SAHH)\(^2\) is the cellular enzyme responsible for catalyzing the hydrolytic breakdown of S-adenosylhomocysteine (AdoHcy) to adenosine and L-homocysteine (Hcy) in mammals (see Fig. 1) (1). AdoHcy is the product of S-adenosyl-L-methionine (AdoMet)-dependent biocatalytic methyltransferases including those that act on proteins and DNA. It has been established previously that genetic knockdown of SAHH or its chemical inhibition by 3-deazaneplanocin A leads to accumulation of AdoHcy and generalized product inhibition of AdoMet-dependent methyltransferases (2, 3). One of the enzymes that seems particularly sensitive to AdoHcy feedback inhibition is EZH2, the histone lysine methyltransferase component of the Polycomb repressor complex 2 responsible for Lys\(^27\) methylation of histone H3, a signature mark of heterochromatic gene silencing (2–4). Recent studies indicate that SAHH inhibition by 3-deazaneplanocin A can reactivate developmental genes in acute myeloid leukemia cells, halting cellular proliferation (3, 4).

SAHH dysfunction is linked to numerous pathologies such as neurological and vascular disorders, myopathy, fatty liver, cancer, renal insufficiency, and diabetic nephropathy (5–13). Deletion of SAHH in mice is embryonically lethal (14). Human SAHH deficiency is a rare genetic disorder and is characterized by up to 140-fold elevated plasma AdoHcy levels with a significant decrease in the SAM/AdoHcy ratio, typically leading to severe pathological consequences and death in childhood (6, 15–17).

SAHH is a highly conserved, NAD\(^+\)-dependent, homotetrameric enzyme that shows >70% amino acid sequence identity from yeast to human (18). The functional tetramer of SAHH is composed of four identical ~48-kDa subunits each of which contains an N-terminal substrate binding domain, a catalytic domain, and a C-terminal tail. Unlike typical NAD\(^+\)-dependent dehydrogenases, where each cofactor is bound by one monomer, NAD\(^+\) binding to SAHH requires contributions from neighboring subunits via their C-terminal tails (19–22). The catalytic mechanism of SAHH involves NAD\(^+\)-mediated oxidation of the 3'-hydroxy group to the ketone followed by \(\beta\)-elimination of homocysteine, generating an exo-methylene intermediate (Fig. 1). Stereospecific Michael addition of water to the exo-methylene intermediate followed by NADH-mediated reduction of the 3-ketone furnishes adenosine and regenerates NAD\(^+\) (Fig. 1), which is tightly bound and serves a catalytic role in the overall reversible reaction (1, 23, 24).

Whereas SAHH is recognized as a key enzyme in controlling gene expression and a potential drug target for the design of...
Regulation of S-Adenosylhomocysteine Hydrolase by Acetylation

**Peptide Synthesis and Purification**—The C-terminal fragment of SAHH (amino acid 396–432 with 396 replaced by Cys, CAHLGKLNVKTLYQLTEKQQAQLGMSCDGPFPDPHY) with or without acetyl groups at Lys401 and Lys408 were synthesized using the standard Fmoc solid phase synthesis strategy on a PS3 peptide synthesizer from Protein Technologies (Tucson, AZ) on 0.1–0.2 mmol scale. Fmoc-protected amino acids were mixed with 4 eq of the coupling reagent 1-(bis(dimethylamino)methylene)-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophospho and double coupled to Wang resin (Novabiochem) containing the protected C-terminal amino acid. The Fmoc protecting group was removed by addition of 20% piperidine in dimethylformamide. A total of four peptides were produced including unacetylated, two singly acetylated, and the bi-acetylated forms. After completion, peptides were cleaved from the resin using reagent K (TFA/thioanisole/water/phenol/ethane dithiol (82.5:5:5:5:2.5, v/v)) for 3–4 h at room temperature. After precipitation with ice-cold diethyl ether, peptides were purified by reversed phase HPLC using a preparative C_{18} column and a gradient of increasing acetonitrile versus water, each containing 0.05% trifluoroacetic acid. Pure fractions were pooled, concentrated by rotor vapor, lyophilized, and stored at −20 °C.

Purified peptides were run on an analytical C_{18} reversed-phase HPLC column (Agilent Eclipse XDB-C18) to confirm purity. Peptide powder was dissolved in HPLC aqueous buffer A (water containing 0.05% trifluoroacetic acid). The elution was monitored at 214 nm and carried out at a flow rate of 1 ml/min over a multistage gradient: 5–60% buffer B (acetonitrile containing 0.05% TFA) for 0–65 min, 60–100% B for 65–70 min, 100% B for 70–80 min, 5–100% B for 80–84 min, and 5% B for 84–94 min. Synthetic peptides were shown to be >95% pure by analytical HPLC and the correct structures were confirmed by MALDI-TOF mass spectrometry (Applied Biosystems, Voyager DE-STR).

**DNA Plasmid Construction**—The full-length human SAHH open reading frame (Invitrogen, IOH14308) was subcloned into pTXB1 vector (New England Biolabs) using NdeI and SapI restriction sites. The pTXB1 vector encodes the GyrA intein domain tag for affinity chromatography. DNA sequencing was confirmed by MALDI-TOF mass spectrometry (Applied Biosystems, Voyager DE-STR).

**Preparation of Semisynthetic SAHH Proteins**—The truncated SAHH-intein-CBD fusion protein was produced in Escherichia coli BL21(DE3) liquid cultures grown at 37 °C in LB media in shaker flasks on a liter scale, followed by induction at A_{600} = 0.5 with 0.5 mM isopropyl thiogalactoside at 16 °C for 16–20 h before pelleting the cells by centrifugation (5000 × g). Cells were resuspended in lysis buffer containing 25 mM HEPES, 500 mM NaCl, 1 mM EDTA, 1 mM Tris(2-carboxyethyl)phosphine, 10% glycerol, pH 7.5) and then lysed by passage through a chilled French press cell. The cell lysate maintained at 4 °C was

**EXPERIMENTAL PROCEDURES**

**Reagents**—Mercaptoethylsulfonate was purchased from Sigma. All Fmoc-derivatives were from EMD (Billerica, MA). Other reagents were at the highest grade obtainable from commercial sources.
centrifuged for 30 min (27,000 \times g) to remove cell debris and insoluble materials and then the supernatant was loaded onto a column containing chitin beads (5 ml/liter of *E. coli* culture) and incubated at 4 °C for 30 min. After the SAHH-intein-CBD fusion protein was bound to chitin beads, the column was drained by gravity and washed with 20 column volumes of wash buffer I (50 mM HEPES, 500 mM NaCl, 0.1 mM EDTA, pH 7.5) followed by wash buffer II (50 mM HEPES, 150 mM NaCl, 0.1 mM EDTA, pH 7.5). The column was then quickly equilibrated with 200 mM 2-mercaptoethanesulfonate and protease inhibitor (Roche Complete mini) in cleavage buffer (0.2 mM potassium phosphate, 0.1 mM EDTA, pH 7.5) followed by wash buffer I (50 mM HEPES, 500 mM NaCl, 0.1 mM EDTA, pH 7.5). After the addition of 5 ml (per liter *E. coli* culture) of cleavage buffer, the column was purged with N₂ gas and sealed. Upon apparent completion of the cleavage reaction as gauged by Coomassie-stained 10% SDS-PAGE (−40 h at room temperature), the eluent was collected in 2-ml fractions and the column was further eluted with 1–2 more column volumes of cleavage buffer. Fractions that contained significant SAHH levels were concentrated 5–10-fold and subjected to buffer exchange (0.2 mM potassium phosphate, 0.1 mM EDTA, 200 mM mercaptoethylsulfonate, 1 mM NAD⁺, protease inhibitor, pH 7.0) to make a peptide solution with a final concentration ~500 μM. The peptide solution was cleared by centrifugation at 20,627 \( \times g \) in a microcentrifuge for 10 min to remove any precipitation. The concentrated, truncated SAHH C-terminal thioester protein (amino acids 1–395) prepared as described above was added to the N-Cysptide solution to achieve a final SAHH protein concentration of about 50 μM, ~1:10 protein:peptide ratio. The tube containing the ligation reaction was sealed after blanketing with N₂ gas. Ligation progress was routinely monitored every 12–16 h by Coomassie-stained 10% SDS-PAGE until ~90% completion (about 36 h at room temperature). If observed, precipitation during the ligation reaction was removed by centrifugation every 12–16 h.

Upon completion of the ligation reaction, the ligation mixture was loaded onto a Superdex 200 size exclusion chromatography column for further purification using purification buffer (20 mM K₂HPO₄, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.2). The FPLC flow rate was 0.4 ml/min and 1.5-ml fractions were collected. Gel filtration standard (Bio-Rad) was run under the same conditions. Fractions that were >95% pure by stained 10% SDS-PAGE were pooled and concentrated using an Amicon protein concentrator (10 kDa cutoff) to ~1 mg/liter of SAHH (Roche Complete mini) in cleavage buffer for 16 h at room temperature to lead to the production of purified WT and mutant r-SAHH (>90% pure by Coomassie-stained 10% SDS-PAGE), flash frozen, and stored at ~80 °C until reconstitution at >1 mg/ml.

**Reconstitution of SAHH/NAD⁺** —Reconstitution of the NAD⁺ form of SAHH was carried out as described previously (33). Briefly, SAHH was treated with 80% (v/v) ammonium sulfate in reconstitution buffer (25 mM K₂HPO₄, 5 mM DTT, 1 mM EDTA) to precipitate the purified SAHH over three successive cycles (pH 2.9, 2.9, and then pH 7.2). The precipitated protein was collected by centrifugation and dissolved in reconstitution buffer at pH 7.2. NAD⁺ (1 mM) was incubated with 0.1 mM SAHH overnight at 4 °C. The reconstituted NAD⁺ form of SAHH was further purified by a Superdex 200 column (Amer sham Biosciences Sciences, 10/300GL) as described above.

**SAHH Enzymatic Assays** —Measurement of SAHH activity in the forward (hydrolytic) direction was performed using an indirect, continuous assay by quantifying the production of homocysteine free thiol using Ellman’s reagent (5,5'-dithiobis(nitrobenzoic acid)) (33–35) in the presence of adenosine deaminase to prevent reaction reversal. To 200 μl of the enzyme solution containing 0.15–0.3 μM SAHH and 0.8 units of adenosine deaminase (Roche Applied Science) in assay buffer (50 mM potassium phosphate buffer, pH 7.2, 1 mM EDTA, 1 mM NAD⁺) was added 50 μM of varying concentrations of AdoHcy (15.6–1000 μM) with 250 μM 5,5'-dithiobis(nitrobenzoic acid) in the assay buffer. The reaction mixture was mixed rapidly and monitored at 412 nm continuously at 37 °C using a UV spectrophotometer (Beckman DU640) containing a Peltier temperature controller. The initial rate was obtained by calculating the slope after the reaction reached 37 °C and entered a linear region after a short lag phase (<2 min). An extinction coefficient of 13,600 M⁻¹ cm⁻¹ for 5-thio-2-nitrobenzoate, the product of the 5,5'-dithiobis(nitrobenzoic acid) reaction, was used to calculate the amount of homocysteine formed (33–35).

Measurement of SAHH activity in the reverse (AdoHcy synthesis) direction was performed using a direct, discontinuous assay by quantifying AdoHcy formation from adenosine and homocysteine by reversed phase HPLC (36). SAHH (~0.1 μM) was incubated with 1 mM adenosine and 5 mM homocysteine in 200 μl of assay buffer at 37 °C for 0, 10, 20, 40, and 60 min and the reaction was quenched by addition of 10 μl of 6 N trifluoroacetic acid. Quenched reaction mixtures were filtered through
Regulation of S-Adenosylhomocysteine Hydrolase by Acetylation

an Amicon microconcentrator (3 kDa cutoff) to remove the protein precipitate. The resulting mixtures were diluted 1:1 in HPLC aqueous buffer A (water containing 0.05% trifluoroacetic acid) and analyzed by HPLC using a C18 reversed-phase column (Agilent Eclipse XDB-C18). The elution was carried out at a flow rate of 1 ml/min over a multistage gradient: 0.5–7% buffer B (acetonitrile containing 0.05% trifluoroacetic acid) for 0–45 min, 7–100% B for 45–50 min, and 100% B for 50–60 min. The peaks corresponding to AdoHcy and adenosine were monitored at 254 nm. The concentration of AdoHcy was determined by calculating the percentage of AdoHcy in the reaction mixture at specific time points with less than 20% substrate conversion. The kcat values were obtained from the slopes from plots of [AdoHcy]/[E] versus time using linear regression curves. All reaction rates were measured in duplicate on two separate occasions and replicate measurements typically agreed within 20%.

Western Blot—Western blots were performed using dry transfers to PVDF via an Iblot dry blotting system (Invitrogen) and blocked with 5% bovine serum albumin in Tris-buffered saline in TWEEN 20 for 1 h at room temperature. Primary anti-acetyl-lysine antibody (ab21623, Abcam) was applied using 1:2000 dilution and incubated overnight at 4 °C. Secondary anti-rabbit IgG horseradish peroxidase-linked antibody (GE Healthcare, NA934V) was used at 1:10,000 dilution. Enhanced chemiluminescence Western blotting detection reagents (Amersham Biosciences) were employed in combination with a Western blot imager (Syngene PXI 6).

Protein Crystallization—Reconstituted acetylated semisynthetic SAHH was concentrated to 10–15 mg/ml and dialyzed against crystallization buffer (10 mM Tris, 1 mM EDTA, pH 7.2). Crystals of acetylated semisynthetic SAHH were grown by the hanging-drop method by mixing 2 μl of SAHH with 1 μl of a well solution containing 200 mM sodium formate, 5–20% PEG 3350, and 100 mM BisTris, pH 6.0–6.5. SAHH AcK408 crystals were grown without seeding. Bi-acetylated SAHH was crystallized by dropwise addition of 20% PEG 3350, 200 mM sodium formate, 100 mM BisTris, pH 6, and 1 mM NAD+ protected in a stepwise fashion. The crystals were first stabilized with 12% glycerol. Stabilized crystals were harvested by dropwise addition of the same buffer supplemented with 12% glycerol. Stabilized crystals were harvested and flash frozen in liquid nitrogen. Data were collected at beamline 7.2 at the Stanford Synchrotron Radiation Light Source and processed with HKL2000 (37). Molecular replacement was performed with PHASER (38) using the structure of human SAHH as a search model (39) and diffraction data extending to 2.8 Å. Two independent subunits of SAHH were found in the crystallographic asymmetric unit with a final translational z-score of 66.4. Model building and structure refinement were performed iteratively using COOT (40) and PHENIX (41), respectively.

X-ray Crystallographic Data Collection and Structure Determination—Both bi-acetylated and AcK408 crystals were cryo-protected in a stepwise fashion. The crystals were first stabilized by dropwise addition of 20% PEG 3350, 200 mM sodium formate, 100 mM BisTris, pH 6, and 1 mM NAD+ and then cryoprotected by dropwise addition of the same buffer supplemented with 12% glycerol. Stabilized crystals were harvested and flash frozen in liquid nitrogen. Data were collected at beamline 7.2 at the Stanford Synchrotron Radiation Light Source and processed with HKL2000 (37). Molecular replacement was performed with PHASER (38) using the structure of human SAHH as a search model (39) and diffraction data extending to 2.8 Å. Two independent subunits of SAHH were found in the crystallographic asymmetric unit with a final translational z-score of 66.4. Model building and structure refinement were performed iteratively using COOT (40) and PHENIX (41), respectively.

TABLE 1

| Enzyme | Forward reaction | Forward reaction, kcat/Km | Reverse reaction, kcat/Km |
|--------|------------------|--------------------------|--------------------------|
| r-SAHH | 0.79 ± 0.04 | 13.9 ± 3.2 | 0.057 | 2.0 ± 0.10 |
| N27K r-SAHH | 0.40 ± 0.01 | 23.1 ± 2.6 | 0.017 | NP* |
| D293A r-SAHH | 0.56 ± 0.03 | 33.4 ± 5.3 | 0.017 | NP |
| s-SAHH | 1.04 ± 0.04 | 12.9 ± 1.7 | 0.081 | 3.1 ± 0.09 |
| AcK396-s-SAHH | 0.34 ± 0.01 | 12.3 ± 1.6 | 0.028 | 1.2 ± 0.02 |
| AcK401/408-s-SAHH | 0.30 ± 0.02 | 8.8 ± 2.2 | 0.034 | 1.0 ± 0.02 |
| biAcK401/408-s-SAHH | 0.36 ± 0.01 | 23.6 ± 1.8 | 0.015 | 1.2 ± 0.01 |

*NP, not performed.

RESULTS

SAHH Semisynthesis—To generate unacylated and 401- and 408-acetylated forms of SAHH, we elected to use expressed protein ligation, a protein semisynthesis method that involves replacing the natural C terminus of a protein of interest with a synthetic peptide (Fig. 2) (42). One requirement of expressed protein ligation is the need for a cysteine at the ligation junction (42). As there was not a natural Cys conveniently located, we examined several sites for Cys replacement. We found that replacement of Glu396 with Cys was relatively well tolerated for expression and catalytic properties as reflected in the parameters of semisynthetic SAHH compared with WT recombinant SAHH (see below, Table 1).
To perform expressed protein ligation, cDNA for the C terminally deleted form of SAHH (amino acids 1–395, t-SAHH) was subcloned in-frame to a gyrase intein-chitin binding domain containing vector, allowing for thioester generation at the C terminus of the recombinant protein fragment of SAHH. Separately, solid phase synthesis was performed to prepare highly purified N-Cys containing 37-mer synthetic peptides corresponding to the SAHH C-terminal residues amino acids 396–432 with or without side chain acetyl groups on Lys401 or Lys408 (see Fig. 3). Ligation of the recombinant SAHH thioester fragment of amino acids 1–395 (t-SAHH) to the N-Cys peptides proceeded smoothly in the presence of 1 mM NAD⁺/H⁺ cofactor, which enhanced the yield of the desired semisynthetic proteins, presumably by stabilizing the SAHH-folded protein structure. The four semisynthetic SAHH proteins, s-SAHH (unacetylated), AcK⁴⁰¹-s-SAHH (acetylated at Lys⁴⁰¹), AcK⁴⁰⁸-s-SAHH (acetylated at 408), and bi-AcK⁴⁰¹/⁴⁰⁸-s-SAHH (acetylated at both Lys⁴⁰¹ and Lys⁴⁰⁸) were further purified by size exclusion chromatography (Fig. 4), demonstrating that each was tetrameric. In contrast, truncated SAHH (t-SAHH) was monomeric. Coomassie-stained SDS-PAGE and mass spectrometry showed that each of the semisynthetic SAHH proteins was >90% pure (Fig. 5). In addition, the presence of the acetyl-Lys modifications in AcK⁴⁰¹-s-SAHH, AcK⁴⁰⁸-s-SAHH, and bi-AcK⁴⁰¹/⁴⁰⁸-s-SAHH but not s-SAHH was confirmed by Western blot with anti-acetyl-Lys antibody (see Fig. 5B).

FIGURE 3. SAHH C-terminal 37-mer peptides with Lys or acetyl-Lys were synthesized using the Fmoc solid phase peptide strategy and purified by HPLC. Their masses were confirmed by MALDI-TOF. A, MALDI-TOF spectra of synthesized C-terminal 37-mer peptides. Unacetylated C-terminal synthetic peptide, \( m/z = 4254 \) (calculated \( m/z \): 4255.2); AcK⁴⁰¹ C-terminal synthetic peptide, \( m/z = 4297 \) (calculated \( m/z \): 4297.2); AcK⁴⁰⁸ C-terminal synthetic peptide, \( m/z = 4295 \) (calculated \( m/z \): 4297.2); and bi-AcK⁴⁰¹/⁴⁰⁸ C-terminal synthetic peptide, \( m/z = 4337 \) (calculated \( m/z \): 4339.2). B, analytical reversed phase HPLC chromatograms of C-terminal synthetic peptides confirm >95% purity.
Enzymatic Analysis of Semisynthetic SAHH Proteins—The catalytic activity of SAHH was measured in both the forward (hydrolase) and reverse (AdoHcy synthesis) directions. To determine SAHH activity in the thermodynamically disfavored forward direction, it is necessary to add adenosine deaminase to breakdown adenosine as it is formed to prevent the back reaction (18, 33–36). Measurement of SAHH activity in the forward direction was performed by monitoring homocysteine production quantified via its free thiol by reaction with Ellman’s reagent in a continuous assay. Measurement of the reverse reaction was performed using a direct, discontinuous assay that permits simultaneous quantification of AdoHcy and adenosine. Although more robust than the measurement of free thiol that can air-oxidize, this HPLC assay is less sensitive and in our hands was only amenable to $k_{cat}$ measurement with saturating substrate concentration because measurements at low substrate concentration show poor signal-to-noise. These experiments showed that the kinetic parameters of unacetylated semi-
Regulation of S-Adenosylhomocysteine Hydrolase by Acetylation

A. representative steady-state kinetic plots for semi-synthetic SAHH forward reactions: (blue s-SAHH ●), AcK\textsuperscript{401}-s-SAHH (green ▼), AcK\textsuperscript{408}-s-SAHH (red ▲), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); B, representative time course plots for semisynthetic SAHH reverse reactions at saturating substrate concentrations: s-SAHH (blue ●), AcK\textsuperscript{401}-s-SAHH (red ▲), AcK\textsuperscript{408}-s-SAHH (green ▼), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); C, representative HPLC traces for semisynthetic SAHHS reverse reactions at saturating substrate concentrations. From top to bottom: enzyme reaction mixture at t = 0 min, s-SAHH reaction mixture at t = 20 min, and AcK\textsuperscript{401}-s-SAHH reaction mixture at t = 20 min. X, Y, and Z represent peaks for NAD\textsuperscript{+}, adenosine, and AdoHcy from left to right, respectively.

FIGURE 6. Measurements of catalytic activity of semisynthetic SAHHS. A, representative steady-state kinetic plots for semi-synthetic SAHH forward reactions: (blue s-SAHH ●), AcK\textsuperscript{401}-s-SAHH (green ▼), AcK\textsuperscript{408}-s-SAHH (red ▲), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); B, representative time course plots for semisynthetic SAHH reverse reactions at saturating substrate concentrations: s-SAHH (blue ●), AcK\textsuperscript{401}-s-SAHH (red ▲), AcK\textsuperscript{408}-s-SAHH (green ▼), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); C, representative HPLC traces for semisynthetic SAHHS reverse reactions at saturating substrate concentrations. From top to bottom: enzyme reaction mixture at t = 0 min, s-SAHH reaction mixture at t = 20 min, and AcK\textsuperscript{401}-s-SAHH reaction mixture at t = 20 min. X, Y, and Z represent peaks for NAD\textsuperscript{+}, adenosine, and AdoHcy from left to right, respectively.

Regulation of S-Adenosylhomocysteine Hydrolase by Acetylation

A semisynthetic SAHH (s-SAHH), which contains a E396C replacement, were very similar to fully recombinant WT SAHH (r-SAHH), with k\textsubscript{cat} (0.79 s\textsuperscript{-1} versus 1.04 s\textsuperscript{-1}, r-SAHH versus s-SAHH) and AdoHcy K\textsubscript{m} (13.9 versus 12.9 \textmu M, r-SAHH versus SAHH) values that are within 30% (see Table 1).

Interestingly, the presence of acetylation at either Lys\textsuperscript{401} (AcK\textsuperscript{401}-s-SAHH, k\textsubscript{cat} of 0.34 s\textsuperscript{-1}) or Lys\textsuperscript{408} (AcK\textsuperscript{408}-s-SAHH, k\textsubscript{cat} of 0.30 s\textsuperscript{-1}) in SAHH conferred 3-fold reduction in k\textsubscript{cat} relative to unacetylated s-SAHH, with less than a 30% change in AdoHcy K\textsubscript{m} among these enzyme forms (Table 1, Fig. 6). Doubly acetylated SAHH (bi-AcK\textsuperscript{401/408}-s-SAHH) showed a similar 3-fold k\textsubscript{cat} reduction and a 2-fold increase in SAH K\textsubscript{m}. The k\textsubscript{cat} effects were corroborated by analyzing the reverse reactions (Table 1, Fig. 6). Thus, the catalytic efficiency (k\textsubscript{cat}/K\textsubscript{m}) of SAHH was reduced by acetylation at either lysine position and the largest effect, 5-fold reduction, was observed in the dually acetylated enzyme.

Structure of Acetylated SAHH—We determined the crystal structures of semisynthetic SAHH bound to NAD\textsuperscript{+} with either one acetylation (AcK\textsuperscript{401}) or two acetylations (AcK\textsuperscript{401/408}) by molecular replacement and refine these structures using diffraction data extending to 2.6 and 2.3 Å, respectively (Table 2, Fig. 7). Additional electron density was observed in the adenosine binding pocket and was assigned as adenosine, which likely copurified with SAHH. The electron density for the backbone atoms of the modified residues was clear, but the electron density for the side chains was weak (Fig. 8, A and B) indicating that the side chains are poorly ordered. As the structure of the SAHH AcK\textsuperscript{408} is nearly identical to

FIGURE 6. Measurements of catalytic activity of semisynthetic SAHHS. A, representative steady-state kinetic plots for semi-synthetic SAHH forward reactions: (blue s-SAHH ●), AcK\textsuperscript{401}-s-SAHH (green ▼), AcK\textsuperscript{408}-s-SAHH (red ▲), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); B, representative time course plots for semisynthetic SAHH reverse reactions at saturating substrate concentrations: s-SAHH (blue ●), AcK\textsuperscript{401}-s-SAHH (red ▲), AcK\textsuperscript{408}-s-SAHH (green ▼), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); C, representative HPLC traces for semisynthetic SAHHS reverse reactions at saturating substrate concentrations. From top to bottom: enzyme reaction mixture at t = 0 min, s-SAHH reaction mixture at t = 20 min, and AcK\textsuperscript{401}-s-SAHH reaction mixture at t = 20 min. X, Y, and Z represent peaks for NAD\textsuperscript{+}, adenosine, and AdoHcy from left to right, respectively.

FIGURE 6. Measurements of catalytic activity of semisynthetic SAHHS. A, representative steady-state kinetic plots for semi-synthetic SAHH forward reactions: (blue s-SAHH ●), AcK\textsuperscript{401}-s-SAHH (green ▼), AcK\textsuperscript{408}-s-SAHH (red ▲), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); B, representative time course plots for semisynthetic SAHH reverse reactions at saturating substrate concentrations: s-SAHH (blue ●), AcK\textsuperscript{401}-s-SAHH (red ▲), AcK\textsuperscript{408}-s-SAHH (green ▼), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); C, representative HPLC traces for semisynthetic SAHHS reverse reactions at saturating substrate concentrations. From top to bottom: enzyme reaction mixture at t = 0 min, s-SAHH reaction mixture at t = 20 min, and AcK\textsuperscript{401}-s-SAHH reaction mixture at t = 20 min. X, Y, and Z represent peaks for NAD\textsuperscript{+}, adenosine, and AdoHcy from left to right, respectively.

FIGURE 6. Measurements of catalytic activity of semisynthetic SAHHS. A, representative steady-state kinetic plots for semi-synthetic SAHH forward reactions: (blue s-SAHH ●), AcK\textsuperscript{401}-s-SAHH (green ▼), AcK\textsuperscript{408}-s-SAHH (red ▲), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); B, representative time course plots for semisynthetic SAHH reverse reactions at saturating substrate concentrations: s-SAHH (blue ●), AcK\textsuperscript{401}-s-SAHH (red ▲), AcK\textsuperscript{408}-s-SAHH (green ▼), bi-AcK\textsuperscript{401/408}-s-SAHH (black ◦); C, representative HPLC traces for semisynthetic SAHHS reverse reactions at saturating substrate concentrations. From top to bottom: enzyme reaction mixture at t = 0 min, s-SAHH reaction mixture at t = 20 min, and AcK\textsuperscript{401}-s-SAHH reaction mixture at t = 20 min. X, Y, and Z represent peaks for NAD\textsuperscript{+}, adenosine, and AdoHcy from left to right, respectively.
SAHH AcK401/408, we will focus our discussion on the bi-acetylated structure.

Acetylated SAHH crystallizes with two monomers in the asymmetric unit and the tetramer is generated by crystallographic symmetry. This tetramer superimposes with the wild-type, unmodified tetramer with a root mean square deviation of 0.45 Å over 1712 C/Hatoms (Fig. 7A). No local perturbations in the backbone positions were observed at the sites either of semisynthetic ligation or acetylations (Figs. 7 and 8), but small structural changes were clearly observed for side chains near the sites of modifications. Acetylation of Lys408 disrupts a hydrogen bond with Asp422, and both side chains adopt different conformations than when non-acetylated (Fig. 8C). Acetylation of Lys401 results in a rearrangement of a hydrogen bonding network between Lys401, Asn27, and Asp293 (Fig. 8D). In SAHH AcK401/408, the ε-amino group of AcK408 makes hydrogen bonds to the backbone carbonyl of Asn27. In the non-acetylated structure, a water mediates this interaction. Additionally, Arg34 adopts a different rotamer when Lys401 is modified and, in one chain of the asymmetric unit, the guanidine group of Arg34 is within hydrogen bonding distance of the carbonyl.

To explore the possibility that SAHH hydrogen bonding networks involving Asn27, Asp293, and Asp422 contribute to the catalytic defects conferred by 401 and 408 Lys acetylation, we attempted to generate three SAHH recombinant mutant proteins: N27K, D293A, and D422K. Although D422K r-SAHH did not express sufficient soluble protein for reconstitution and purification, N27K and D293A r-SAHH were obtained. Both N27K and D293A r-SAHH showed 3-fold reductions in catalytic efficiency ($k_{cat}/K_m$) compared with WT r-SAHH (see TABLE 2).

### TABLE 2
Data collection and refinement statistics

| Data collection | Protein  | 4PFJ | 4PGF |
|----------------|---------|------|------|
| Space group | P 2 2 2 | P 2 2 2 |
| a, b, c (Å) | 97.597, 102.740, 175.018 | 98.366, 102.727, 176.160 |
| Resolution (Å) | 50-2.6 (2.69-2.60) | 50-2.3 (2.38-2.3) |
| Rmerge (%) | 12.8 (100.0) | 16.9 (100.0) |
| Completeness (%) | 100.0 (100.0) | 99.9 (99.7) |
| Redundancy | 7.1 (7.0) | 6.5 (5.9) |
| Unique reflections | 27,640 (2717) | 39,810 (3912) |

| Refinement | Protein | Water | Other |
|-----------|---------|-------|-------|
| No. of atoms | 13,290 | 25 | 204 |
| B-factors (Å²) | 46.4 | 74.6 | 52.3 |
| Root mean square deviations | 0.005 | 0.855 | 66.7 |

$R_{merge} = \sum_j |I_j - \langle I_j \rangle| / \sum_j I_j$, where $I_j$ is the intensity of an individual reflection, and $\langle I_j \rangle$ is the mean intensity for multiply recorded reflections.

$R_{merge}$ values in parentheses are for the highest resolution shell.

$R_{merge} = \sum_j |F_o - F_c| / \sum_j F_o$, where $F_o$ is an observed amplitude and $F_c$ a calculated amplitude; $R_{merge}$ is the same statistic calculated over a subset of the data that has not been used for refinement.

SAHH AcK401/408, the ε-amino group of AcK408 makes hydrogen bonds to the backbone carbonyl of Asn27. In the non-acetylated structure, a water mediates this interaction. Additionally, Arg34 adopts a different rotamer when Lys401 is modified and, in one chain of the asymmetric unit, the guanidine group of Arg34 is within hydrogen bonding distance of the carbonyl.

To explore the possibility that SAHH hydrogen bonding networks involving Asn27, Asp293, and Asp422 contribute to the catalytic defects conferred by 401 and 408 Lys acetylation, we attempted to generate three SAHH recombinant mutant proteins: N27K, D293A, and D422K. Although D422K r-SAHH did not express sufficient soluble protein for reconstitution and purification, N27K and D293A r-SAHH were obtained. Both N27K and D293A r-SAHH showed 3-fold reductions in catalytic efficiency ($k_{cat}/K_m$) compared with WT r-SAHH (see TABLE 2).
This reduction in activity is similar to that associated with the individual Lys401 acetylation event, consistent with the hypothesis that disruption of the hydrogen bonding pattern by Lys acetylation perturbs catalysis.

**DISCUSSION**

Protein lysine acetylation is now understood to be a very abundant post-translational modification in the cell, with thousands of proteins and specific sites being recently identified using mass spectrometry (29, 43, 44). A large number of these acetylation events have been linked to metabolic enzymes inside and outside of the mitochondria. The effects of the overwhelming majority of protein acetylation modifications have not yet been explored. In many studies, the effects of these acetyl modifications have been approximated by mutagenesis substitution involving Lys\(^{3}\)Gln (45–49). Although sometimes informative, such Lys → Gln replacements can be misleading because of the structural differences between Gln and acetyl-Lys. In a small number of cases the precise contributions of specific, stoichiometric protein acetylation events have been characterized in detail with the authentic post-translational modification. Prior examples include cyclophilin (50), histone modifications (51–53), p53 (54), and acetyltransferase auto-acetylation (55–57). By using expressed protein ligation on SAHH, we establish here, for the first time to our knowledge, the high resolution structural and functional consequences of acetylation at two independent sites in the same protein.

Although the x-ray structure electron density for the side chains of the acetylated Lys residues was not strong, changes in the electron density for the rotameric conformations of several surrounding residues in acetylated SAHH were clear. Such altered rotameric conformations indicate changing hydrogen bonding patterns in acetylated SAHH. We propose that the reduction in hydrolase activity in acetylated SAHH may be mediated by propagation of these altered hydrogen bond networks to the dynamics of the protein-NAD\(^+\)/H\(^+\) interactions that in turn are transmitted to the catalytic site. A recent theoretical study of SAHH catalysis has highlighted the potential catalytic importance of Asp\(^{293}\) (58), involved in the network of hydrogen bonds to the C terminus as shown in Fig. 8. As the Asn\(^{27}\)
Regulation of S-Adenosylhomocysteine Hydrolase by Acetylation

Asp\textsuperscript{293} hydrogen bond appears altered by Lys\textsuperscript{401} acetylation, it is plausible that this change in interaction may result in reduction of hydrolase activity. In addition, the loss of the water molecule-mediated interaction of Asn\textsuperscript{27} by Lys\textsuperscript{401} acetylation may contribute to catalytic inhibition. The mutagenesis experiments involving Asn\textsuperscript{27} and Asp\textsuperscript{293} reported here confirm the contributions of these residues in catalysis.

The biological consequences of SAHH acetylation have yet to be determined. Identification of the SAHH acetyltransferase(s) and deacetylase(s) and the role of SAHH lysine acetylation potentially affecting the cellular stability and/or protein-protein interactions of SAHH are important topics for future research. However, we are intrigued by the possibility that increased acetylation of SAHH could lead to a global decline in SAM-mediated methyl transfer reactions in the cell. The product of methyltransferase reactions, AdoHcy, is a general competitive inhibitor of AdoMet binding to the active sites of these enzymes (2, 18, 25, 59–63). A crippling of SAHH by acetylation, as identified in our enzymatic data, could lead to a build-up of AdoHcy that in turn would impede most cellular methyltransferases. In this way, SAHH regulation could serve as a key node that facilitates the known connection between increased cellular acetyl-CoA concentrations that are associated with a reduction in histone lysine methylation (64–66). Although the competitive dynamic between lysine acetylation and lysine methylation is a recognized concept (64–66), the potential of the catalytically impaired acetylated SAHH serving as a contributing factor to reduced protein lysine methylation should now be considered. Although the 3–5-fold enzymologic effects of post-translational modification of SAHH are moderate, similar sized effects on other enzymes have been associated with biological consequences (67–69). Future cellular experiments will be needed to investigate the specific role of SAHH acetylation in contributing to this acetylation/methylation dynamic.

This study also highlights the utility of expressed protein ligation in the study of protein acetylation. Unnatural amino acid mutagenesis by nonsense suppression strategies (30, 70), Cys derivatization (31, 32, 71), enzyme-catalyzed acetylation (33, 1806–1816), acid mutagenesis by nonsense suppression strategies (30, 70), and sortase-mediated semisynthesis (75) are viable strategies for site-specific introduction of acetyl-Lys or close mimics into proteins. Expressed protein ligation is especially attractive, however, when these modifications occur near the C terminus of proteins of interest (42, 55, 67–69, 76–78). This is particularly relevant when multiple acetylation sites are clustered as is the case with SAHH.

REFERENCES

1. De La Haba, G., and Cantoni, G. L. (1959) The enzymatic synthesis of S-adenosyl-L-homocysteine from adenosine and homocysteine. J. Biol. Chem. 234, 603–608
2. S. Clarke, Banfield, K. (2001) in Homocysteine in Health and Disease (Carmel, R., and Jackobsen, D. W., eds) Cambridge University Press, Cambridge, United Kingdom
3. Miranda, T. B., Cortez, C. C., Yoo, C. B., Liang, G., Abe, M., Kelly, T. K., Marquez, V. E., and Jones, P. A. (2009) DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. Mol. Cancer Ther. 8, 1579–1588
4. Zhou, J., Bi, C., Cheong, L. L., Mahara, S., Liu, S. C., Tay, K. G., Koh, T. L., Yu, Q., and Chng, W. J. (2011) The histone methyltransferase inhibitor, DZNep, up-regulates TXNIP, increases ROS production, and targets leukemia cells in AML. Blood 118, 2830–2839
5. Teng, Y. W., Mehedint, M. G., Garrow, T. A., and Zeisel, S. H. (2011) Deletion of betaine-homocysteine S-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. J. Biol. Chem. 286, 36258–36267
6. Baric, I. (2009) Inherited disorders in the conversion of methionine to homocysteine. J. Inherit. Metab. Dis 32, 459–471
7. Bjursell, M. K., Blom, H. J., Cayuela, J. A., Engvall, M. L., Lesko, N., Balasubramanian, S., Brandenburg, G., Halldin, M., Falkenberg, M., Jakobs, C., Smith, D., Struys, E., von Dobeln, U., Gustafsson, C. M., Lundeberg, J., and Wedell, A. (2011) Adenosine kinase deficiency disrupts the methionine cycle and causes hypermethioninemia, encephalopathy, and abnormal liver function. Am. J. Hum. Genet. 89, 507–515
8. Dayal, S., Bottiglieri, T., Arning, E., Maeda, N., Malinow, M. R., Sigmund, C. D., Heistad, D. D., Faraci, F. M., and Lentz, S. R. (2001) Endothelial dysfunction and elevation of S-adenosylhomocysteine in cystathionine \( \beta \)-synthase-deficient mice. Circ. Res. 88, 1203–1209
9. Loother, F. M., Tschöp, M., Angst, C. P., Litynski, P., Jäger, K., Fowler, B., and Hafeli, W. E. (2001) Disturbed ratio of erythrocyte and plasma S-adenosylmethionine/S-adenosylhomocysteine in peripheral arterial occlusive disease. Atherosclerosis 154, 147–154
10. Herrmann, W., Schorr, H., Obeid, R., Makowski, J., Fowler, B., and Kuhlmann, M. K. (2005) Disturbed homocysteine and methionine cycle intermediates S-adenosylhomocysteine and S-adenosylmethionine are related to degree of renal insufficiency in type 2 diabetes. Clin. Chem. 51, 891–897
11. Jabs, K., Koury, M. J., Dupont, W. D., and Wagner, C. (2006) Relationship between plasma S-adenosylhomocysteine concentration and glomerular filtration rate in children. Metabolism 55, 252–257
12. Poirier, L. A., Brown, A. T., Fink, L. M., Wise, C. K., Randolph, C. J., Delongchamp, R. R., and Fonseca, V. A. (2001) Blood S-adenosylmethionine concentrations and lymphocyte methylenetetrahydrofolate reductase activity in diabetes mellitus and diabetic nephropathy. Metabolism 50, 1014–1018
13. Leal, J. F., Ferrer, I., Blanco-Aparicio, C., Hernández-Losa, J., Ramón Y Cajal, S., Carnero, A., and Leonart, M. E. (2008) S-Adenosylhomocysteine hydrolase downregulation contributes to tumorigenesis. Carcinogenesis 29, 2089–2095
14. Miller, M. W., Duhl, D. M., Winkes, B. M., Arredondo-Vega, F., Saxon, P. J., Wolff, G. L., Epstein, C. J., Harrisfield, M. S., and Barsh, G. S. (1994) The mouse lethal nonagonist (a(x)) mutation deletes the S-adenosylhomocysteine hydrolase (Achy) gene. EMBO J. 13, 1806–1816
15. Baric, I., Fumič, K., Glenn, B., Cuk, M., Schulze, A., Finkenstein, J. D., James, S. J., Mejskaj-Bosnjak, V., Pazarin, L., Pogriviny, I. P., Rados, M., Sarnavka, V., Scukanec-Spoljar, M., Allen, R. H., Stabler, S., Uzelac, L., Vugrek, O., Wagner, C., Zeisel, S., and Mudd, S. H. (2004) S-Adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. Proc. Natl. Acad. Sci. U.S.A. 101, 4234–4239
16. Baric, I., Cuk, M., Fumič, K., Vugrek, O., Allen, R. H., Glenn, B., Maradin, M., Pazarin, L., Pogriviny, I., Rados, M., Sarnavka, V., Schulze, A., Stabler, S., Wagner, C., Zeisel, S. H., and Mudd, S. H. (2005) S-Adenosylhomocysteine hydrolase deficiency: a second patient, the younger brother of the index patient, and outcomes during therapy. J. Inherit. Metab. Dis. 28, 885–902
17. Buist, N. R., Glenn, B., Vugrek, O., Wagner, C., Stabler, S., Allen, R. H., Pogriviny, I., Schulze, A., Zeisel, S. H., Baric, I., and Mudd, S. H. (2006) S-Adenosylhomocysteine hydrolase deficiency in a 26-year-old man. J. Inherit. Metab. Dis. 29, 538–545
18. Turner, M. A., Yang, X., Yin, D., Kuczerka, K., Borchardt, R. T., and Howell, P. L. (2000) Structure and function of S-adenosylhomocysteine hydrolase. Cell. Biochem. Biophys. 33, 101–125
19. Lee, K. M., Choi, W. J., Lee, Y., Lee, H. J., Zhao, L. X., Lee, H. W., Park, J. G., Kim, H. O., Hwang, K. Y., Heo, Y. S., Choi, S., and Jeong, L. S. (2011) X-ray crystal structure and binding mode analysis of human S-adenosylhomocysteine hydrolase complexed with novel mechanism-based inhibitors, haloneplanocin A analogues. J. Med. Chem. 54, 930–938
20. Cai, S., Fang, J., Li, Q. S., Borchardt, R. T., Kuczerka, K., Middaugh, C. R., and Schoven, R. L. (2010) Comparative kinetics of cofactor association
and dissociation for the human and trypanosomal S-adenosylhomocysteine hydrolase: 3. role of jysl and tyrosyl residues of the C-terminal extension. Biochemistry 49, 8434 –8441

21. Turner, M. A., Yuan, C. S., Borchardt, R. T., Hershfield, M. S., Smith, G. D., and Howell, P. L. (1998) Structure determination of selenomethionyl S-adenosylhomocysteine hydrolase using data at a single wavelength. Nat. Struct. Biol. 5, 369 –376

22. Ault-Riché, D. B., Yuan, C. S., and Borchardt, R. T. (1994) A single mutation at lysine 426 of human placental S-adenosylhomocysteine hydrolase inactivates the enzyme. J. Biol. Chem. 269, 31472–31478

23. Hoffman, D. R., Marion, D. W., Cornatzer, W. E., and Duerre, J. A. (1980) Strategies in the design of antiviral drugs. J. Biol. Chem. 255, 10822–10827

24. Palmer, J. L., and Abeles, R. H. (1979) The mechanism of action of S-adenosylhomocysteinase. J. Biol. Chem. 254, 1217–1226

25. Liu, S., Wolfe, M. S., and Borchardt, R. T. (1992) Rational approaches to the design of antiviral agents based on S-adenosyl-l-homocysteine hydrolase as a molecular target. Antiviral Res. 19, 247–265

26. De Clercq, E. (2002) Antimalarial activity of a 4’-unsaturated 5’-fluoroadenosine mechanism-based inhibitor of S-adenosyl-l-homocysteine hydrolase. Biochem. Pharmacol. 60, 601–606

27. Bitonti, A. J., Baumann, R. J., Jarvis, E. T., McCarthy, J. R., and McCann, P. P. (1990) Antimalarial activity of a 4’,5’-unsaturated 5’-fluoroadenosine mechanism-based inhibitor of S-adenosyl-l-homocysteine hydrolase. Biochem. Pharmacol. 49, 601–606

28. Wolos, J. A., Frondorf, K. A., and Esser, R. E. (1993) Immunosuppression mediated by an inhibitor of S-adenosyl-l-homocysteine hydrolase. Prevention and treatment of collagen-induced arthritis. J. Immunol. 151, 526–534

29. Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehmam, M., Walther, T. C., Olsen, J. V., and Mann, M. (2009) Lysine acetylation targets cellular complexes and co-regulates major cellular functions. Science 325, 834–840

30. Neumann, H., Peak-Chew, S.Y., and Chin, J.W. (2008) Genetically encoding Nε-acetyl-lysine in recombinant proteins. Nat. Chem. Biol. 4, 232–234

31. Huang, R., Holbert, M. A., Tarrant, M. K., Colquihou, D. R., Dancy, B. M., Dancy, B. C., Hwang, Y., Tang, Y., Meeth, K., Marmostein, R., Cole, R. N., Khochbin, S., and Cole, P. A. (2010) Site-specific introduction of an acetyl-lysine mimic into peptides and proteins by cysteine alkylation. J. Am. Chem. Soc. 132, 9986–9987

32. Li, F., Allalverdi, A., Yang, R., Luo, G. B., Zhang, X., Gu, Y., Korolev, N., De Clercq, E., Yang, C., Zheng, Y. G., Speicher, D. W., Thibault, P., Johnstone, F.B., Berger, S. L., Sternglanz, R., McMahon, S. B., Côte, J., and Chin, J. W. (2009) A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. Mol. Cell 36, 153–163

33. Di Cerbo, V., Mohn, F., Ryan, D. P., Montellier, E., Kacem, S., Tropberger, J., Allen, M. D., Veprintsev, D. B., Robinson, C. P., Keller, M. P., Westphall, M. S., Denu, J. M., Attie, A. D., Coon, J. J., and Chin, J. W. (2009) A general method for protein engineering. J. Biol. Chem. 284, 2126–2132

34. Yuan, H., Rossetto, D., Mellert, H., Dang, W., Srinivasan, M., Johnson, J. C., and Peterman, C. L. (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 311, 884–847

35. Neumann, H., Hancock, S. M., Buning, R., Routh, A., Chapman, L., Somers, J., Owen-Hughes, T., van Noort, J., Rhodes, D., and Chin, J. W. (2009) H3 K56 acetylation regulates cyclophilin A catalysis, immunosuppression and HIV isomerization. Nat. Chem. Biol. 6, 331–337

36. Shogren-Knaak, M., Ishii, H., Sun, J. M., Pazin, M. J., Davies, J. R., and Peterson, C. L. (2006) Histone H4-K16 acetylation controls chromatin structure and other post-translational modifications. Mol. Cell 31, 844–847

37. Yang, X., Lloyd, H., Liao, T., and Heynen, C. (2011) Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription. eLife 3, e01632

38. Arbel, E., Natan, E., Brandt, T., Allen, M. D., Veprintsev, D. B., Robinson, C. V., Chin, J. W., Joerger, A. C., and Fersht, A. R. (2011) Acetylation of lysine 120 of p53 endows DNA-binding specificity at effective physiological salt concentration. Proc. Natl. Acad. Sci. U.S.A. 108, 8251–8256

39. Karukuchi, K. R., Wang, L., Uzasi, L., Manlandro, C. M., Wang, Q., and Cole, P. A. (2010) Analysis of p300/CPB histone acetyltransferase regulation using circular permutation and semisynthesis. J. Am. Chem. Soc. 132, 1222–1223

40. Albaugh, B. N., Arnold, K. M., Lee, S., and Denu, J. M. (2011) Autoacetylation of the histone acetyltransferase Rtt109. J. Biol. Chem. 286, 24694–24701

41. Yuan, H., Rossetto, D., Mellert, H., Dang, W., Srinivasan, M., Johnson, J., Hodawadekar, S., Ding, E. C., Speicher, K., Abshur, N., Perry, R., Wu, J., Yang, C., Zheng, Y. G., Speicher, D. W., Thibault, P., Veerlaat, A., Johnson, F. B., Berger, S. L., Sternglanz, R., McMahan, S. B., Côte, J., and Marmostein, R. (2012) MYST protein acetyltransferase activity requires active site lysine autoacetylation. EMBO J. 31, 58–70

42. Lee, Y., Jeong, L. S., Choi, S., and Hyeon, C. (2011) Link between allosteric signal transduction and functional dynamics in a multisubunit enzyme.
Regulation of S-Adenosylhomocysteine Hydrolase by Acetylation

S-adenosylhomocysteine hydrolase. J. Am. Chem. Soc. 133, 19807–19815

Chiang, P. K., and Cantoni, G. L. (1979) Perturbation of biochemical transmethylation by 3-deazaadenosine in vivo. Biochem. Pharmacol. 28, 1897–1902

Galloway, S. E., and Wertz, G. W. (2008) S-Adenosylhomocysteine-induced hyperpolyadenylation of vesicular stomatitis virus mRNA requires the methyltransferase activity of L protein. J. Virol. 82, 12280–12290

Malanovic, N., Streith, I., Wolinski, H., Rechberger, G., Kohlwein, S. D., and Tehlivets, O. (2008) S-Adenosyl-l-homocysteine hydrolase, key enzyme of methylation metabolism, regulates phosphatidylcholine synthesis and triacylglycerol homeostasis in yeast: implications for homocysteine as a risk factor of atherosclerosis. J. Biol. Chem. 283, 23989–23999

Perna, A. F., Ingrosso, D., Violetti, E., Luciano, M. G., Sepe, I., Lanza, D., Capasso, R., Ascione, E., Raiola, I., Lombardi, C., Stenvinkel, P., Massy, Z., and De Santo, N. G. (2009) Hyperhomocysteinemia in uremia: a red flag in a disrupted circuit. Semin. Dial. 22, 351–356

Tehlivets, O., Hasslacher, M., and Kohlwein, S. D. (2004) S-Adenosyl-l-homocysteine hydrolase in yeast: key enzyme of methylation metabolism and coordinated regulation with phospholipid synthesis. FEBS Lett. 577, 501–506

Katada, S., Imhof, A., and Sassone-Corsi, P. (2012) Connecting threads: epigenetics and metabolism. Cell 148, 24–28

Nicolas, E., Roumillac, C., and Trouche, D. (2003) Balance between acetylation and methylation of histone H3 lysine 9 on the EZF-responsive dihydrofolate reductase promoter. Mol. Cell. Biol. 23, 1614–1622

Evertts, A. G., Zee, B. M., Dimaggio, P. A., Gonzales-Cope, M., Coller, H. A., Garcia, B. A. (2013) Quantitative dynamics of the link between cellular metabolism and histone acetylation. J. Biol. Chem. 288, 12142–12151

Flavell, R. R., and Muir, T. W. (2009) Expressed protein ligation (EPL) in the study of signal transduction, ion conduction, and chromatin biology. Acc. Chem. Res. 42, 107–116

Balduc, D., Rahdar, M., Tu-Sekine, B., Sivakumaren, S. C., Raben, D., Amzel, L. M., Devreotes, P., Gabelli, S. B., and Cole, P. (2013) Phosphorylation-mediated FTEN conformational closure and deactivation revealed with protein semisynthesis. Elife 2, e00691

Thompson, P. R., Wang, D., Wang, L., Fulco, M., Pediconi, N., Zhang, D., An, W., Ge, Q., Roeder, R. G., Wong, J., Levreto, M., Sartorelli, V., Cotter, R. J., and Cole, P. A. (2004) Regulation of p300 HAT domain via a novel activation loop. Nat. Struct. Mol. Biol. 11, 308–315

Huang, Y., Russell, W. K., Wan, W., Pai, P.-J., Russell, D. H., and Liu, W. (2010) A convenient method for genetic incorporation of multiple noncanonical amino acids into one protein in Escherichia coli. Mol. Biosyst. 6, 683–686

Chalker, J. M., Lercher, L., Rose, N. R., Schofield, C. J., and Davis, B. G. (2012) Conversion of cysteine into dehydroalanine enables access to synthetic histones bearing diverse post-translational modifications. Angew. Chem. Int. Ed. Engl. 51, 1835–1839

Saeed, M., Schwarze, F., Loidl, A., Meraner, J., Lechner, M., and Loidl, P. (2012) In vitro phosphorylation and acetylation of the murine pocket protein Rb/p130. PLoS One 7, e46174

Han, J., Zhou, H., Li, Z., Xu, R. M., and Zhang, Z. (2007) Acetylation of lysine 56 of histone H3 catalyzed by Rtt109 and regulated by Asf1 is required for replisome integrity. J. Biol. Chem. 282, 28587–28596

Tsubota, T., Berendsen, C. E., Erkmann, J. A., Smith, C. L., Yang, L., Freitas, M. A., Denu, J. M., and Kaufman, P. D. (2007) Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. Mol. Cell 25, 703–712

Piotukh, K., Geltinger, B., Heinrich, N., Gerth, F., Beyermann, M., Freund, C., and Schwarzer, D. (2011) Directed evolution of sortase A mutants with altered substrate selectivity profiles. J. Am. Chem. Soc. 133, 17536–17539

Dhall, A., and Chatterjee, C. (2011) Chemical approaches to understand the language of histone modifications. ACS Chem. Biol. 6, 987–999

Tarrant, M. K., Rho, H. S., Xie, Z., Jiang, Y. L., Gross, C., Culhane, J. C., Yan, G., Qian, J., Ichikawa, Y., Matsuoka, T., Zachara, N., Etzkorn, F. A., Hart, G. W., Jeong, J. S., Blackshaw, S., Zhu, H., and Cole, P. A. (2012) Regulation of CK2 by phosphorylation and O-GlcNAcylation revealed by semisynthesis. Nat. Chem. Biol. 8, 262–269

Liu, X., Wang, L., Zhao, K., Thompson, P. R., Hwang, Y., Marmostein, R., and Cole, P. A. (2008) The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. Nature 451, 846–850

Chen, V. B., Arendall, W. B., 3rd, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2010) Molprobity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D Biol. Crystallogr. 66, 12–21