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Design of a Bioluminescent Assay Platform for Quantitative Measurement of Histone Acetyltransferase Enzymatic Activity

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Protein acetylation and acylation are widespread post-translational modifications (PTMs) in eukaryotic and prokaryotic organisms. Histone acetyltransferase (HATs) enzymes catalyze the addition of short-chain acyl moieties to lysine residues on cellular proteins. Many HAT members are found to be dysregulated in human diseases, especially oncological processes. Screening potent and selective HAT inhibitors has promising application for therapeutic innovation. A biochemical assay for quantification of HAT activity utilizing luminescent output is highly desirable to improve upon limitations associated with the classic radiometric assay formats. Here we report the design of a bioluminescent technological platform for robust and sensitive quantification of HAT activity. This platform utilizes the metabolic enzyme acetyl-CoA synthetase 1 (ACS1) for a coupled reaction with firefly luciferase to generate luminescent signal relative to the HAT-catalyzed acetylation reaction. The biochemical assay was implemented in microtiter plate format and our results showed this assay sensitively detected catalytic activity of HAT enzyme p300, accurately measured its steady-state kinetic parameters of histone acetylation and measured the inhibitory potency of HAT inhibitor. This platform demonstrated excellent robustness, reproducibility, and signal-to-background ratios, with a screening window $Z' = 0.79$. Our new bioluminescent design provides an alternative means for HAT enzymatic activity quantitation and HAT inhibitor screening.

Introduction

Histone acetyltransferase (HAT) enzymes predominantly catalyze the addition of acetyl moieties to the ε-amino group of lysine residues utilizing the cosubstrate, acetyl-coenzyme A (AcCoA, Ac-CoA, acetyl-CoA), as an acyl donor for the reaction.[1] This phenomenon has been extensively observed on the lysine rich N-terminal tails of histone proteins found in the nucleus of eukaryotic cells.[2] Several families of HATs have been identified in humans and other eukaryotic species including: GCN5/PCAF, MYST, and CBP/p300 families.[3,4] Significant sequence differences and substrate preferences relegate the HAT members to separate families; however, all of them still maintain the ability to catalyze acetyl transfer. HATs serve as critical epigenetic regulatory enzymes that affect many DNA-templated processes by the addition of acetyl groups which function to alter charge states of histones and chromatin structure. Acetyllysine may also serve as biochemical marks for acetyllysine recognizing reader proteins to bind and drive downstream processes by the recruitment of functional enzymes or complexes.[5] Predominantly, lysine acetylation by HATs serve to drive increased gene transcription, but processes such as DNA repair and cell proliferation have been identified to be under the influence of HATs. Because HATs function to regulate gene transcription and several important cellular processes, dysfunction of these enzymes has considerable deleterious effects on the cell or whole organism. Dysregulation of HAT activity has been implicated in cardiovascular disease like hypertension, hypertrophy, and several epithelial cancers.[6–8] For this reason, efforts to discover novel activity modulating drugs of HAT activity has become of considerable importance.[9] Accordingly, robust, and sensitive assay methods to accurately measure HAT enzymatic activity in high throughput manner are demanded to facilitate the investigation of these enzymes and the screening of potential inhibitory compounds.

Currently, several HAT assays exist that can detect the formation of the acetylated peptide/protein product or the thiol-containing CoA side-product utilizing immunosorbent, radiometric, fluorescent, and spectrophotometric output.[10] The commonly used method, the scintillation proximity assay (SPA), is a radiometric assay that has considerable drawbacks relating to the use of radioisotope labeling and the cost-prohibitive nature of these cofactors.[11] The fluorescent assay utilizing the thiol-reactive probe, 7-Diethylamino-3-(4′-Maleimidylphenyl)-4-Methylcoumarin (CPM) suffers from background fluorescence and compound-mediated interference.[12] To overcome limitations associated with other assay methods and to expand the toolbox for studying HAT enzymatic activity, the development of a luminescence-based HAT assay is desirable. Robust luminescent assays have been adopted for a considerable number of enzymes due to their

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high sensitivity and minimal compound interference. The use of firefly luciferase as a luminescent reporter has been applied for almost 40 years and with that time, advancements have been made to improve the stability and lifetime of luminescent signal from this enzyme providing researchers with a plethora of options for specific needs.[5,13,14]

In this work, we sought the development of a luciferase-based bioluminescent assay platform for quantifying HAT enzymatic activity and for HAT inhibitor discovery and characterization. Our hope is that this study provides a new biochemical method for screening HAT inhibitors in high-throughput capacity and meanwhile overcome some limitations met in the previous HAT assays. Our detailed characterization results demonstrate that this assay format has great detection sensitivity, quantitative accuracy, readout stability, and robustness.

Results and Discussion

Design the Bioluminescent Assay Platform for Acetyl-CoA Measurements

To quantitatively measure HAT acetyltransferase activity via bioluminescent readouts, we designed an assay platform that integrates three enzymatic components: HAT, acetyl-CoA synthetase 1 (ACS1/ACSS2), and firefly luciferase (Figure 1). This system quantifies HAT enzymatic activity by detecting the consumption of AcCoA during the HAT reaction. We attempt to implement this assay system in a mix-and-measure type manner with a luminescent cocktail containing firefly luciferase, ACS1, adenosine monophosphate (AMP), and pyrophosphate (PPi). Following a HAT reaction of predetermined duration, the luminescent cocktail is applied to the reaction mixture to produce luminescence proportional to the amount of AcCoA in the reaction mixture. This is accomplished by ACS1's reverse reaction catalyzing the formation of ATP from AcCoA, AMP, and PPi. The generated ATP is consumed by the luciferase reaction to produce a luminescent signal proportional to the amount of AcCoA

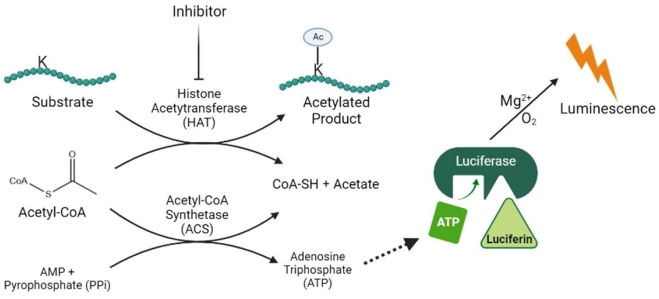

Figure 1. Schematic depiction of the bioluminescent assay platform to quantify HAT enzymatic activity. The pool of AcCoA post-acetylation is converted by ACS1 to an equivalent amount of ATP. Firefly luciferase converts ATP to a proportional amount of light that can be captured by a luminometer. Introduction of a HAT inhibitor into the system will affect the available pool of AcCoA post-reaction and will produce a proportional change in the luminescent signal allowing for quantification.

unconsumed in the HAT reaction mix. Substrate depletion can be measured by comparing the luminescent signal obtained from a HAT reaction to a HAT-negative control allowing for accurate determination of the amount of AcCoA consumed in a given time. AMP and PPi are held in a significant excess compared to the other components in this platform to ensure that the ACS1 reaction continues to proceed in the desired ATP-producing direction. Additionally, AMP and PPi are regenerated *in-situ* by the firefly luciferase reaction. Because there is no source of free acetate in this system ACS1 is ensured to only proceed in the ATP-producing direction.

We sought to determine whether this system would function with the *M. musculus* ACS1 (Gene ID: 60525) as a representative acetyl-CoA synthetase. This enzyme was expressed and purified by affinity chromatography using plasmids available in a commercial repository (Addgene Plasmid #13746). Briefly, a pQE80 vector containing the ACS1 gene with a 6xhis-tag was transformed into BL21(DE3) *E. coli* and the expressed protein was purified by Ni-NTA affinity chromatography. Initial tests with this enzyme were carried out by adding varied concentrations to the luminescent cocktail described above and applying the cocktail to 20 μM AcCoA in a 96-well plate. Luminescence readings were measured with a CLARIOstar® Plus Microplate reader. This test, however, yielded weak activity as demonstrated by the low luminescence signal obtained. Additionally, the enzyme rapidly lost activity and most activity was diminished after 10 minutes (Supplementary Figure S1). However, with the inclusion of a stabilizing buffer containing Triton X-100 and TCEP to the luminescent cocktail, the system's response was greatly improved as demonstrated in the next set of experiments. Varying concentrations of ACS1 in the luminescent cocktail, we demonstrated the system produced luminescent signals proportional to ACS1 concentrations when detecting a fixed concentration of 20 μM AcCoA (Figure 2a). Having found that *M. musculus* ACS1 functions well in this system to produce luminescence from AcCoA, we next determined the linearity of the system's response to AcCoA concentrations for the purpose of generating standard curves. With AcCoA concentrations increasing from 0–20 μM, we found the luciferase signals produced a considerably linear response across the concentrations tested ($R^2 = 0.988$) (Figure 2b and c). We observed that the linearity of this system diminishes with concentrations that exceed 20 μM due to the rate limiting conversion of AcCoA to ATP by ACS1. These results demonstrate that *M. musculus* ACS1 coupled with firefly luciferase was effective to accurately quantify AcCoA as low as 39 nM with a signal to background ratio of 11 : 1.

The Bioluminescent Platform Quantitatively Measures the HAT Reactions

Having demonstrated the effectiveness of the *M. musculus* ACS1-firefly luciferase coupled system for measuring AcCoA concentrations we next sought to study whether this system

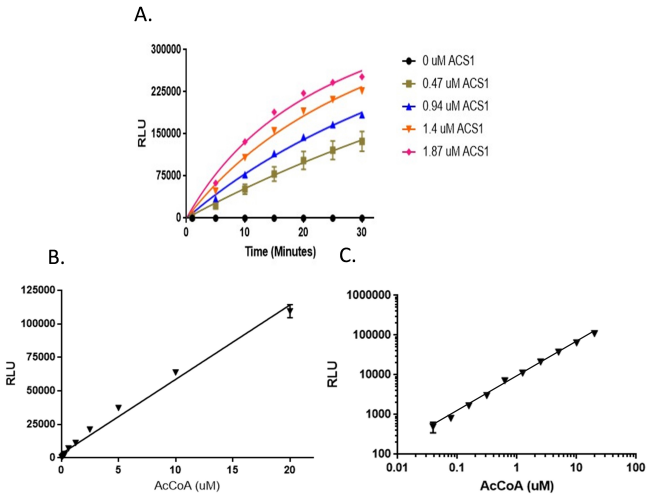

Figure 2. *M. musculus* ACS1 response to Acetyl-CoA. (A) Depicts the luminescent signal (Relative Light Units (RLU)) measured from different amounts of ACS1 (0, 0.47, 0.94, 1.4, and 1.87 μM) in the luminescent cocktail over time with 20 μM AcCoA. (B) Linear response of bioluminescent system to AcCoA. Plotted with linear axes. (C) Linear response plotted with log axes.

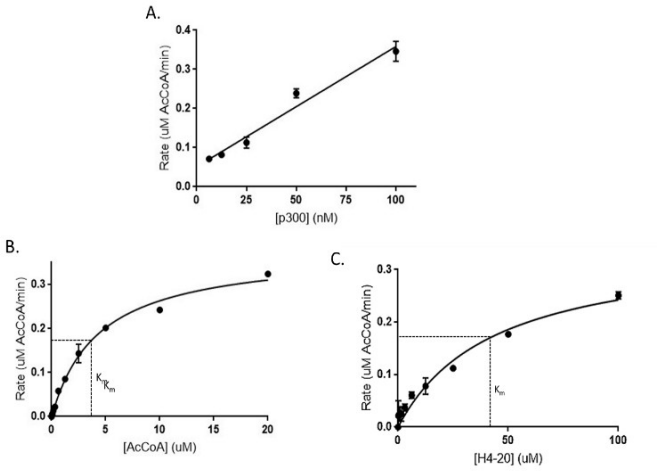

Figure 3. Steady-state kinetic measurements obtained with the bioluminescent platform. (A) 0–100 nM p300 was allowed to react with 5 μM AcCoA for 20 minutes and luminescence measured. (B) Michaelis-Menten plot of AcCoA for p300. Reaction containing: 50 nM p300, 50 μM H4-20, and varied AcCoA allowed to react 20 minutes. Rates were calculated using the difference from the standard curve to determine AcCoA consumption divided by the reaction time. Rate vs. [AcCoA] was plotted and fit to Michaelis-Menten. (C) Michaelis-Menten plot of H4-20 for p300. Reactions containing: 50 nM p300, 5 μM AcCoA, and 0–100 μM H4-20 allowed to react 20 minutes. Plotted as described above. N = 2.

can accurately quantify the enzymatic activity of a HAT enzyme which uses AcCoA as a substrate in an acetylation reaction. As a proof of concept, we chose to focus on the HAT, p300, as this enzyme catalyzes a significant portion of total lysine acetylation within the cell and has been identified as an important target for potential cancer drug development.[15–17] In the experiment the p300 catalyzed reaction was conducted by incubating recombinant p300 with AcCoA and synthetic histone H4 1–20 for a predetermined duration. Following the HAT reaction, the luminescent cocktail containing firefly luciferase, ACS1, AMP1 and PPi was added to the HAT reaction mixture and luminescent readings were measured. Using the standard curve we generated with varied AcCoA concentrations, the changes in luminescent signal were used to calculate the amount of AcCoA consumed by the reaction. Initial tests to assess p300 enzymatic activity with the bioluminescent system demonstrated that the system could detect AcCoA consumption across a range of p300 concentrations with a linear relationship as a function of p300 concentration (Figure 3a). The lowest p300 concentration we tested was 6.25 nM and we found that the system could monitor the HAT reaction with low nanomolar concentrations of p300. We then measured p300 activity with varied AcCoA concentrations (0–20 μM) at a fixed H4-20 peptide concentration at 50 μM. By taking the difference in luminescence (ΔRLU) from this experiment and the standard curve, we calculated the AcCoA consumed by the p300 reaction to determine rate of the HAT reaction. Fitting the data to the Michaelis-Menten equation yielded $K_m = 4.3 \pm 0.37$ μM, $k_{cat} = 0.126 \pm 0.004$ s^{-1}, and $V_{max} = 0.3776 \pm 0.01$ μM/min (Figure 3b). We also measured p300 activity at varying H4-20 peptide concentrations (0–100 μM) at the fixed AcCoA concentration of 5 μM. Fitting this data to the Michaelis-Menten equation yielded $K_m = 43.6 \pm 9.8$ μM, $K_{cat} = 0.116$ s^{-1}, and $V_{max} = 0.348 \pm 0.04$ μM/min (Figure 3c).

Generally, in enzymatic assays the use of a quenching reagent to halt the reaction's progress at predetermined time points is required to ensure measurements obtained are accurately time controlled. In the bioluminescent system described here, we predicted that the luminescent cocktail used to quantify AcCoA could serve as an effective quencher for the HAT reaction due to its high concentrations of PPi, AMP, and other solutes. We performed the radiometric filter-binding assay to quantify the acetylated peptide product from a p300 reaction when conducted in the presence of the luminescent cocktail (Supplementary Figure S2). Reactions containing 50 nM p300, 1 μM ^{3}H-AcCoA, and 50 μM biotin-H4-20 were allowed to proceed for 20 minutes in the presence of luminescent cocktail or an equivalent amount of buffer. Results from this experiment showed that in the presence of the luminescent cocktail the amount of acetylated biotin-H4-20 from the p300 reaction was reduced to background levels, indicating that the cocktail components effectively inhibited the HAT's activity. Taken together with the previous experiments, these data prove the viability of utilizing this bioluminescent system for quantification of HAT reactions.

The Bioluminescent Platform Can Be Used for Quantitative HAT Inhibitor Evaluation

Because p300 and other HAT enzymes have been implicated in the pathogenesis of a variety of disease states, these

enzymes remain promising targets for discovery of new small molecule drugs. We envision that the bioluminescent platform can be utilized to screen and identify potential inhibitors of HATs like p300. To test whether this platform can be used for inhibitor study we tested the concentration-response of the p300 inhibitor, anacardic acid (Figure 4a) to measure the IC_{50} of this compound.[18] The HAT reaction contained: 5 μM AcCoA, 50 μM H4-20 peptide, and 50 nM p300 with varied concentrations of anacardic acid. These reactions were allowed to run for 20 minutes at room temperature before the luminescent cocktail was added and luminescent readings taken. Plotting the relative activity of p300 at each tested anacardic acid concentration and fitting to the Hill equation yielded a measured $IC_{50} = 15.1 \pm 1$ μM (Figure 4b). To validate the obtained IC_{50} from the bioluminescent platform we tested p300 inhibition by the same concentration range of inhibitor with the established radiometric scintillation proximity assay (SPA) for HATs.[11] Substrate and enzyme concentrations were kept the same as with the bioluminescent experiment, however, AcCoA was replaced by ^{3}H-AcCoA, and H4-20 was replaced by biotin-H4-20 in this format. Results from the SPA exhibited an $IC_{50} = 14.8 \pm 0.8$ μM (Figure 4b), which was close in range to the one obtained with the ACS1-Luciferase bioluminescent assay. Thus, we demonstrated that the bioluminescent assay produces considerably similar results to the established assay format and validate its use for performing inhibition studies.

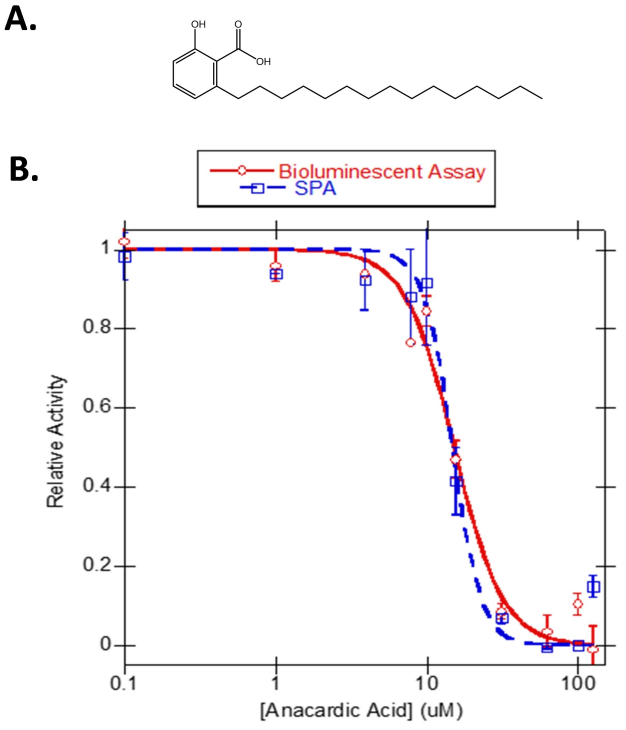

Figure 4. Comparison between the bioluminescent assay and the scintillation proximity assay for assessing p300 inhibition by anacardic acid. (A) Structure of anacardic acid. (B) Bioluminescent assay and SPA inhibitor measurements. p300 reactions containing 50 nM p300, 50 μM H4-20 or BTN-H4-20, and 5 μM AcCoA or 3H-AcCoA were allowed to proceed for 20 minutes at room temperature in the presence of varied anacardic acid concentrations (125, 100, 62.5, 31.25, 15.625, 10, 7.8125, 3.90625, 1, 0.1, and 0 μM). N = 2.

The Bioluminescent System is Amenable to High-Throughput Screening of HAT Inhibitors

Inhibitor screening in the high throughput format is greatly desired for any enzymatic assay allowing for libraries of chemical compounds to be quickly assessed for their anti-enzyme activity. The statistical parameter, screening window Z′-Factor, has been adopted as a measure that can assess the quality and robustness of an assay for high-throughput screening (HTS) suitability based on the signal window between positive and negative controls and the variations of sampling.[19] To measure Z′-factor, 24 p300-positive reactions and 24 p300-negative reactions containing 5 μM AcCoA and 50 μM H4-20 were carried out simultaneously for 20 minutes and luminescence measured. The Z′-factor was determined to be 0.79 (Figure 5) clearly demonstrating the signal window and quality of this assay are well suited for HTS application.

Conclusion and Remarks

Histone acetyltransferases are regarded as important new targets for drug discovery. So far there are no bioluminescent assays for HAT activity determination. We investigated the literature to identify methods that can produce ATP using either the substrates or products of a HAT reaction to leverage firefly luciferase for readouts. A platform involving the metabolic enzyme ACS1 has been previously utilized to generate ATP from acetyl-CoA for readout with luciferase.[20] In this report we designed a bioluminescent assay platform to quantify the enzymatic activity of HATs that is demonstrated to be accurate, sensitive, and suitable for HTS. This assay overcomes a few limitations associated with previously developed assay formats. Our bioluminescent assay platform utilizes a coupled reaction between ACS1 and firefly luciferase to detect the consumption of the HAT reaction's cosubstrate, AcCoA, to quantitatively measure the process of

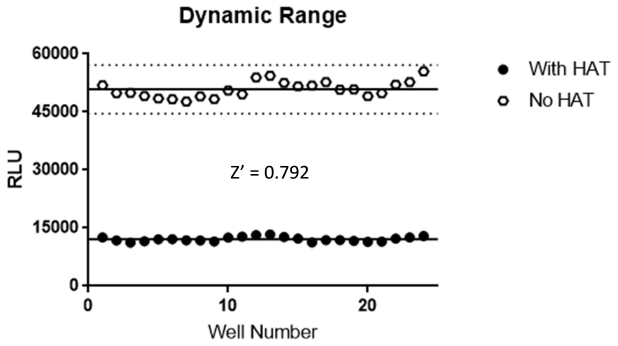

Figure 5. Graphical depiction of Z′-Factor. Solid lines represent averages of each group and dashed lines represent ±3 standard deviations.

a HAT reaction. This platform exhibits a strong linear response to the analyte, AcCoA, in the range of 0–20 μM with a large dynamic range. It allows for sensitive determination of HAT activity in a high throughput setting. This bioluminescent assay platform provides a cheap alternative to current assays. ACS1 can be easily expressed and purified in-house, and the luminescent assay reagents are commonly available (Luciferase, AMP, pyrophosphate) making this assay cost-effective in comparison to radiometric and immuno-based assays. The bioluminescent system eliminates the need for radioactive materials required by radiometric assays, thereby improving operation safety and providing a greener alternative that does not produce radioactive waste. Additionally, this platform provides a new format to screen HAT inhibitors allowing for quick identification of potential therapeutic compounds. Compared to fluorescence-based HAT assays that often are interfered with by photoactive compounds, the new bioluminescent assay will be resistant to fluorescent interference, thus lowering fluorescent artifacts. Of note, because this assay relies on ACS1 for AcCoA detection it is critical to perform batch testing when performing the assay with different batches of enzyme ensuring that a standard curve is generated for each. Furthermore, some consideration is required when performing inhibitor screens with coupled-enzyme assays and luciferase-based assays due to possible inhibition of the reporting enzymes by screened compounds. Inhibition of the reporting enzyme may generate false positives that need to be considered. With this bioluminescent assay, counter-screens should be performed to evaluate false positives and filter out compounds that interfere with the system. We envision that this bioluminescent platform could be adapted for the measurement of acyltransferase activity of HATs with other acyl-CoA cosubstrates besides AcCoA. HATs have acyltransferase activity besides acetyltransferase activity which may be measured by the bioluminescent assay by changing the acyl-CoA synthetase in the coupled reaction to one with substrate specificity for these different acyl-CoA molecules.[21,22] To date, a significant number of acyl-CoA synthetases have been identified with preferences for substrates like propionyl-CoA, acetoacetyl-CoA, and benzoyl-CoA among others which all can serve as a donor for a HAT reaction.[23,24] Each of these acyl-CoA synthetase enzymes may be utilized in the bioluminescent assay platform to assess the corresponding acyltransferase activity of a known or unknown HAT. This would represent a significant development as radiolabeled CoA-analogues are exceedingly cost-prohibitive when attempting to investigate HAT kinetics by radiometric assays.

Experimental Section

Materials

All reagents were obtained from Sigma-Aldrich and ThermoFisher unless stated otherwise. Streptavidin-Coated PVT SPA Beads (Cat. # 50-210-5587) were obtained from PerkinElmer. Kinase-Glo® Luminescent Kinase Assay was obtained from Promega (Cat. # V6711). ^{3}H-AcCoA was obtained from Revvity (Cat. # NEC313050UC). Vivaspin® Turbo 15 PES Centrifugal Concentrators were obtained from Sartorius (Cat. # VS15T02). PMSF was obtained from GoldBio (Cat. # p-470-25). Tris base was obtained from Prima (Cat # KCT60040). Luminescent measurements were taken with a CLARIOstar® Plus Microplate reader.

Protein Expression and Purification

The expression of *M. musculus* ACS1 was done following the method developed by Denu's lab with slight modification. Transformation was done with BL21(DE3)-RIL competent cells using heat-shock, spreading on plates containing ampicillin. Colonies were harvested and grown at 37 °C in 8 mL, then inoculated to 1 L culture of 2XYT media containing ampicillin. Cultures were allowed to grow to OD600 0.5–0.7. Protein expression was induced with 1 mL IPTG per liter and shaken at 250 RPM for 16 hours at 16 °C. Cells collected by centrifugation at 4000 RPM for 30 minutes at 4 °C and resuspended in lysis buffer (50 mM Tris-HCl (pH 8), 250 mM NaCl, 5 mM Imidazole, 1 mM 2-Mercaptoethanol, and 0.1 mM PMSF. The cells were lysed by passing through a microfluidizer (Microfluidics) at 17,000 psi and the supernatant was purified on Ni-NTA His-Bind® Resin. The disrupted cell lysate was allowed to incubate with Ni-NTA resin for 1 hour at 4 °C with gentle agitation. The protein-loaded resin was washed twice with a wash buffer (50 mM Tris-HCl (pH 8.0), 250 mM NaCl, and 1 mM 2-mercaptoethanol). ACS1 was eluted from resin by sequential washes with a 0–200 mM imidazole gradient (0–200 mM Imidazole, 250 mM NaCl, 50 mM Tris-HCl (pH 8.0), and 1 mM 2-Mercaptoethanol). Collected fractions were analyzed with a 15% SDS-PAGE and those with high purity were pooled for further concentration. Pooled fractions were concentrated by centrifugation at 5000 RPM in a Vivaspin® Turbo 15 10,000 MWCO centrifugal concentrator. Fraction was loaded to Snakeskin® Pleated Dialysis Tubing 3500 MWCO and dialyzed for 16 hours in dialysis buffer (25 mM Tris (pH 7.5), 100 mM NaCl, 10% Glycerol, and 5 mM DTT). Repeated dialysis for 3 hours with fresh buffer. Protein was further concentrated by centrifugal concentration as described above. ACS1 was verified with 10% SDS-PAGE. Bradford assay was performed to determine protein concentration and protein was stored at −80 °C.

p300 was expressed and purified as described in a previous publication.[25]

HAT Luminescence Assay

First, to determine the relative activity of *M. musculus* ACS1 with the bioluminescent system different concentrations of ACS1 (0–1.87 μM) in the luminescent cocktail (80 μM Pyrophosphate (PPi), 80 μM Adenosine Monophosphate (AMP), and 0.8X Kinase-Glo®) were tested to generate a 25 μL total volume for the cocktail. Experiments were conducted in Corning/Costar 96-Well Round Opaque Bottom White Polystyrene Microplates with 20 μM AcCoA in reaction buffer (0.01% Triton X-100, 1 mM tris(2-carboxyethyl) phosphine (TCEP), 100 mM NaCl, and 50 mM Na-HEPES (pH 8.0)). To a 25 μL AcCoA solution, 25 μL of luminescent cocktail containing varied ACS1 concentration was added, and luminescent output measured every 5 minutes using a CLARIOstar® Plus Microplate reader with an integration time of one second per well. Luminescent output reported as relative light units (RLU).

To assess the linearity of the bioluminescent system's response to AcCoA, we tested 0–20 μM AcCoA in reaction buffer with

luminescent cocktail described above containing 1.87 μM ACS1. Measurements obtained as described above.

HAT reactions were performed with 50 nM p300 (final concentration) purified as described. Generally, the 25 μL HAT reaction volumes consisted of fixed H4-20 and varied AcCoA concentrations or vice versa to measure Michaelis-Menten kinetics of the HAT reaction. The 25 μL reaction was allowed to proceed for 20 minutes at room temperature followed by the addition of 25 μL luminescent cocktail. Readings were taken every 5 minutes. Michaelis-Menten curves and Km values were generated by taking the difference of the readings post-HAT reaction from the AcCoA standard curve and plotting the difference against AcCoA concentration. The curve was fit using the equation V=Vmax[S]/(Km + [S]) on GraphPad Prism.

IC_{50} Measurements with Bioluminescence Assay

IC_{50} measurements were obtained with reactions containing 50 nM p300, 50 μM H4-20, 5 μM AcCoA, and varied anacardic acid concentrations in reaction buffer for 20 minutes at room temperature. Following HAT reaction, 25 μL luminescent cocktail added and readings taken as described above. IC_{50} values were obtained by plotting the relative activity against anacardic acid concentration. Relative Activity was calculated by the equation: $\frac{(No\ p300\ Control\ RLU - Inhibitor\ RLU)}{No\ Inhibitor\ RLU}$. In this system, no p300 controls represent 0% relative activity and the no inhibitor controls represent 100% relative activity. Curves were fit in Kaleidagraph with the equation: $= \frac{1}{(1+\frac{[I]}{IC50})^h}$, where h is hill coefficient, and [I] is the inhibitor concentration.

IC_{50} Measurements with Scintillation Proximity Assay

To measure IC_{50}, 50 nM p300 reactions containing: 50 μM biotin-H4-20, 5 μM ^{3}H-AcCoA, 1x reaction buffer, and varied anacardic acid were prepared in 96-well plate (Isoplate-96; Perkin Elmers) and allowed to react for 20 minutes at room temperature. After 20 minutes, reactions were quenched by the addition of Guanidine HCl to a final concentration of 0.5 M. After quenching, Streptavidin-Coated PVT SPA Beads were added to the solution to a final concentration of 3.33 mg/mL. SPA output measured with MicroBeta2 scintillation counter (Perkin Elmer).

Quenching of p300 Catalyzed Reaction by Luminescent Cocktail

To assess HAT reaction quenching by the luminescent cocktail, a filter-binding assay was performed. 50 uL reactions containing 50 nM p300, 50 μM biotin-H4-20, 1 μM ^{3}H-AcCoA, 1x reaction buffer and 25 uL luminescent cocktail or 25 uL 1x buffer for control were allowed to react for 20 minutes at room temperature. Reaction solutions were then added to Whatman P81 phosphocellulose filter paper and allowed to air dry. Filters were washed 3 times with 50 mM $NaHCO_3$ (pH 9.0) for 30 minutes each time. Allowed filters to air dry overnight. Next day, filters were added to scintillation cocktail and amount of acetylated product measured by scintillation counter.

Z′-Factor for Bioluminescence Assay

Z′-Factor was determined by comparing 24 positive reactions (containing p300) with 24 negative reactions (lacking p300). Reaction mixtures containing 5 μM AcCoA, 50 μM H4-20 peptide, and 1x reaction buffer were prepared, and reactions initiated by the addition of p300 to a final concentration of 50 nM in 25 μL. Negative reactions contained reaction buffer in place of p300. Reactions were allowed to proceed for 20 minutes at room temperature and a 25 μL luminescent cocktail was added to each well. Z′-Factor was calculated using the equation: $Z' = 1 - \frac{(3\sigma_{c+} + 3\sigma_{c-})}{|\mu_{c+} - \mu_{c-}|}$. wherein σ is standard deviation. μ is average.

Supporting Information Summary

Supporting information contains data depicting the response of the luminescent assay without the stabilizing buffer and the results of the SPA experiment to determine the luminescent cocktail's ability to quench a HAT catalyzed reaction.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Histone acetyltransferase · HAT · Luciferase · High-throughput screening · Enzyme assay design

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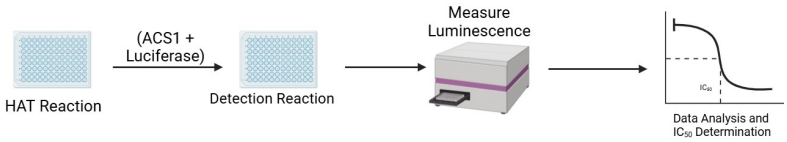

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Design of a Bioluminescent Assay Platform for Quantitative Measurement of Histone Acetyltransferase Enzymatic Activity

A sensitive novel bioluminescent system was developed that can accurately quantify the kinetics of histone acetyltransferase (HAT) catalyzed acylation reactions. This system utilizes acetyl-CoA synthetase 1 and firefly luciferase for a coupled reaction that generates luminescence proportional to acetyl-CoA (AcCoA) concentrations allowing for determination of AcCoA consumption by a HAT catalyzed reaction. This system can be used in high throughput for inhibition studies.