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Global Profiling of Acetyltransferase Feedback Regulation

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Supporting Information

ABSTRACT: Lysine acetyltransferases (KATs) are key mediators of cell signaling. Methods capable of providing new insights into their regulation thus constitute an important goal. Here we report an optimized platform for profiling KAT–ligand interactions in complex proteomes using inhibitor-functionalized capture resins. This approach greatly expands the scope of KATs, KAT complexes, and CoA-dependent enzymes accessible to chemoproteomic methods. This enhanced profiling platform is then applied in the most comprehensive analysis to date of KAT inhibition by the feedback metabolite CoA. Our studies reveal that members of the KAT superfamily possess a spectrum of sensitivity to CoA and highlight NAT10 as a novel KAT that may be susceptible to metabolic feedback inhibition. This platform provides a powerful tool to define the potency and selectivity of reversible stimuli, such as small molecules and metabolites, that regulate KAT-dependent signaling.

Lysine acetyltransferases (KATs) catalyze protein acetylation, a post-translational modification that plays a key role in regulation of enzyme function and epigenetic signaling.[1] Aberrant protein acetylation is observed in many diseases, including cancer.[2] However, deciphering how KAT activity is regulated by reversible stimuli, including small molecules and metabolites, remains a significant challenge.[3] Toward this goal, our laboratory has pioneered the application of chemical proteomic[4] approaches to profile cellular KAT activity. Previously, we have demonstrated that KAT bisubstrate inhibitors[5] can be converted into chemical proteomic probes via incorporation of photo-cross-linking[6] and affinity handles.[7] These probes allow active-site-dependent enrichment of KATs from cellular samples and were recently applied to study the metabolic regulation of GCN5L2. However, a limitation of these first-generation approaches is their ability to report on the activity of only a small number of cellular KATs. The development of methods enabling global insights into the regulation of the KAT superfamily thus remains a critical goal.

To devise a general chemical proteomic platform for KAT profiling, we built on two observations from previous studies: (1) Capture agents derived from the minimal KAT bisubstrate Lys-CoA are relatively promiscuous.[6] (2) Resin-immobilized bisubstrate inhibitors can sensitively enrich endogenous KATs without photo-cross-linking.[7] Combining these insights led us to explore the utility of Lys-CoA-functionalized capture resins for KAT chemoproteomic profiling (Figure 1). Biotinylated and

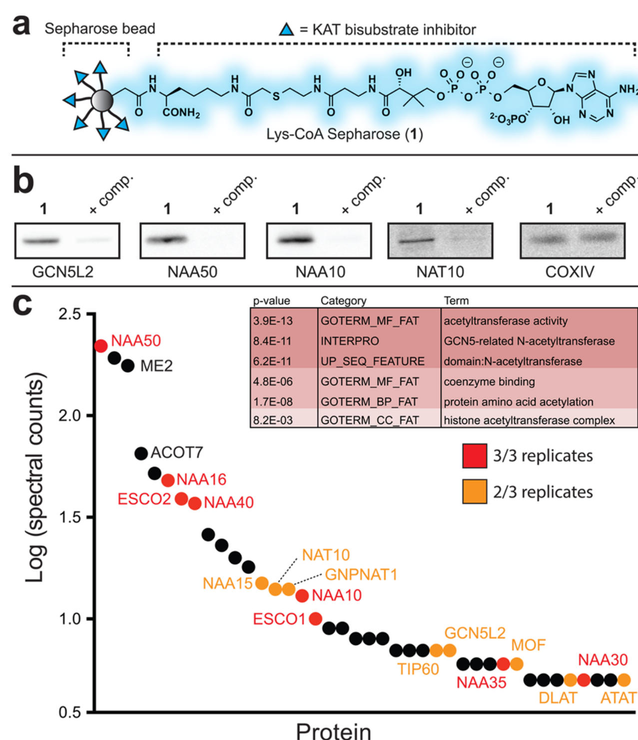

Figure 1. Expanding the scope of KAT chemoproteomic probes. (a) Structure of Lys-CoA Sepharose (**1**). (b) Affinity-based capture of endogenous cellular KATs by **1**. Right lanes ("**1** + comp") denote capture experiments performed in the presence of 300 μM free Lys-CoA. (c) LC-MS/MS analysis of proteins enriched by **1** (>5 spectral counts, >3-fold enriched, data from replicate #1, Table S2). Acyltransferases are colored according to the replicate LC-MS/MS identifications (red = 3; orange = 2). Triplicate MS data sets and gene ontology analysis are provided in Tables S1–S5.

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amine-functionalized Lys-CoA were synthesized using standard protocols (Figure S2).[5] The latter was directly coupled to NHS-Sepharose, a capture resin that has a higher density surface functionalization than streptavidin-agarose and has been usefully applied in other chemoproteomic platforms.[8,9] To assess KAT capture, Lys-CoA resins were incubated with HeLa cell lysates, followed by mild washing, elution, and anti-KAT immunoblotting (Figures 1b and S1). Neither resin was found to enrich the known Lys-CoA target p300 (vide infra). However, we found that Lys-CoA Sepharose (**1**) but not Lys-CoA biotin efficiently enriched GCN5L2 and several other KATs in an active site-dependent fashion (Figures 1b and S1). Because Lys-CoA is known to interact with GCN5L2 family KATs relatively weakly in vitro ($IC_{50} \approx 200\ \mu M$),[5,10] this suggests that high-density display of bisubstrate inhibitors on Sepharose may increase their avidity for KATs and facilitate global profiling efforts.

To test this hypothesis, we performed unbiased LC-MS/MS analyses of HeLa cell proteomes enriched by **1**. We found that Lys-CoA Sepharose **1** enriches a number of enzymes with KAT and N-terminal α-amino acetyltransferase (NAA) activity as well as members of their cognate multiprotein complexes (Figure 1c and Tables S1−S4). The high enrichment of NAAs suggests these enzymes are direct targets of Lys-CoA Sepharose. Previous studies have implicated NAAs in lysine acetylation.[11,12] Enrichment was competed by free Lys-CoA inhibitor, again indicating its active site-directed nature. Of note, this represents the first time that many of these KAT and NAA activities have been shown to be accessible by chemical proteomic approaches.[6,7] Gene ontology analysis of enzymes captured by **1** (spectral counts ≥ 5, enrichment ratio ≥ 3) found enrichment of several terms related to protein acetylation (Figure 1c and Table S5). These results underscore Lys-CoA Sepharose **1** as a versatile platform for profiling cellular KAT activity.

Previous studies have found that the affinity of bisubstrate inhibitors for specific KAT subfamilies can be tuned by varying their peptide moiety.[5−7] For example, PCAF is inhibited far more potently by H3K14-CoA than Lys-CoA.[5] This led us to evaluate H3K14-CoA Sepharose **2** and H4K16-CoA Sepharose **3** as subfamily-specific KAT capture agents (Figure 2). H3K14-CoA resin **2** afforded enhanced capture of several KATs and members of the HBO1 and the STAGA/TFTC KAT complexes relative to **1** (Figure 2b). Because the catalytic domains of these complexes (GCN5L2, PCAF, and HBO1) are known to acetylate H3K14, this suggests that enrichment of KAT complexes is highly dependent on the binding affinity of resin-immobilized bisubstrates for the parent KAT. Consistent with this, H4K16 Sepharose (**3**) displays superior capture of the H4K16 acetylase MOF and its NSL complex (Figure 2b and Table S6−S7). The improved capture of KAT catalytic domains by **2** and **3** was validated by immunoblot (Figure S3). Resins **1**−**3** all capture TIP60, another H4K16 KAT, although **2** and **3** better enrich the TIP60 complex. Resin **3** also efficiently captures the NatC (NAA30) protein complex and several other KATs relative to other probes (Figure 2b). Overall, these studies specify optimal capture agents for each KAT family member, which are summarized in Figure 2c.

Having expanded the phylogenetic scope of KATs accessible to active site profiling, we next applied these methods to better understand the regulation of KATs by metabolism.[3,13] The most abundant endogenous metabolic inhibitor of KATs is the feedback metabolite CoA. As a competitive inhibitor, CoA is

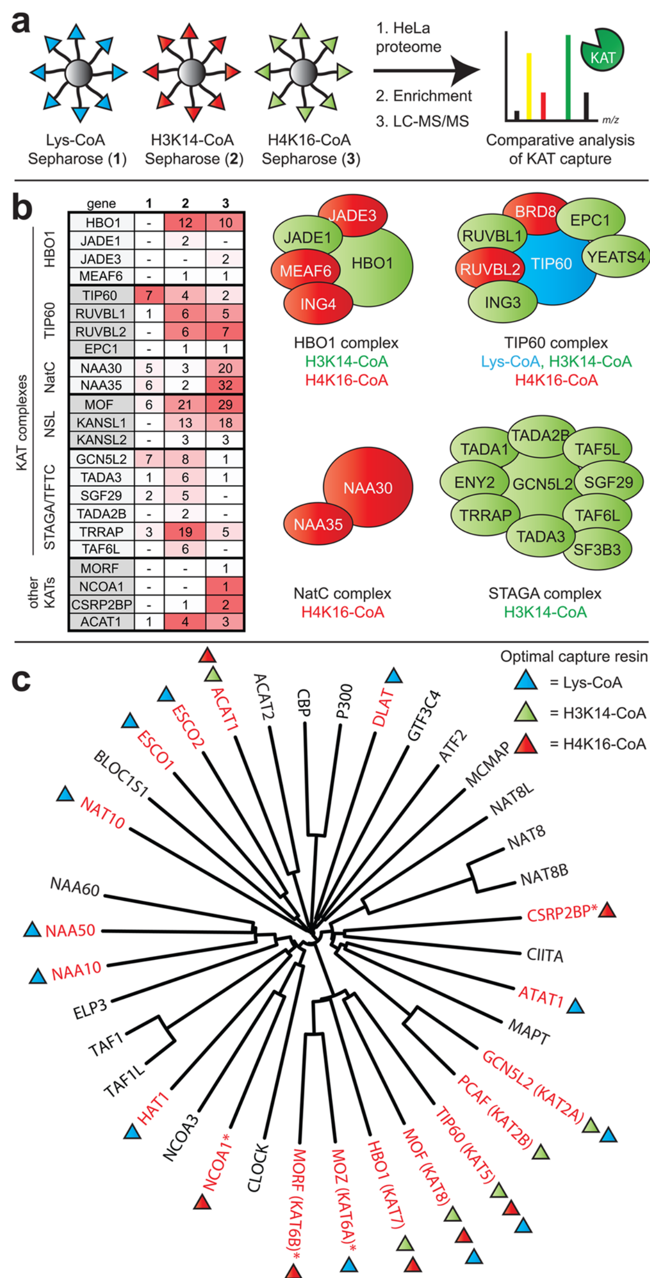

Figure 2. (a) Comparison of KAT capture by Lys-CoA (**1**), H3K14-CoA (**2**), and H4K16-CoA (**3**) Sepharose. (b) Left: LC-MS/MS data comparing capture of KATs and KAT complexes by **1**−**3**. Full unicate data in Tables S2, S7, and S8. Right: KAT complexes with members colored according to optimal enrichment probe. (c) Phylogenetic tree depicting KATs and optimal capture probes for chemoproteomic analysis. Asterisks (*) denote KATs observed as weakly enriched by LC-MS/MS (1−2 spectral counts, Tables S6−S8) that were not verified by immunoblot.

thought to preferentially inhibit KATs that bind it and acetyl-CoA with similar affinities. However, although GCN5L2 has been implicated as one target of CoA feedback inhibition, no study has yet examined the superfamily wide selectivity of this metabolic mechanism of epigenetic regulation. To assess KAT−CoA interactions, we developed immuno-assisted competitive chemoproteomic methods to analyze diverse KATs (GCN5L2, PCAF, MOF, HAT1, ESCO2, NAT10, and DLAT) as well as two NAAs with putative KAT activity (NAA10 and NAA50).[14] Comparative analysis of cancer cell

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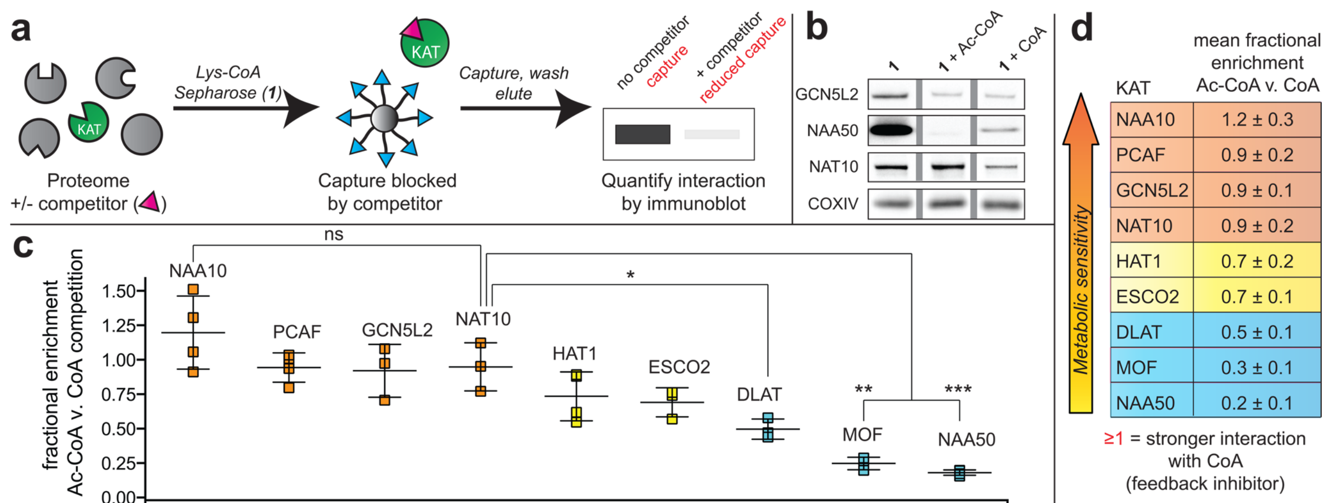


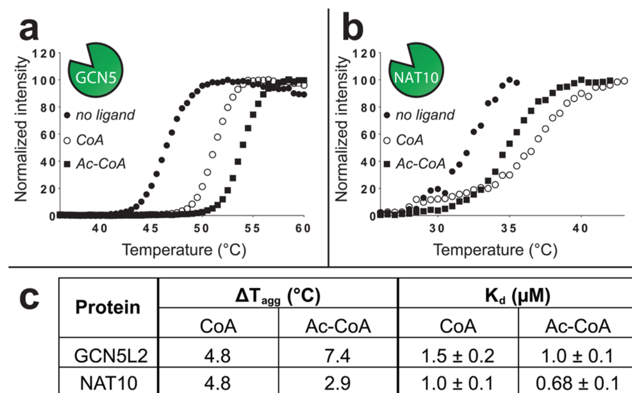
KAT	mean fractional enrichment Ac-CoA v. CoA
NAA10	1.2 ± 0.3
PCAF	0.9 ± 0.2
GCN5L2	0.9 ± 0.1
NAT10	0.9 ± 0.2
HAT1	0.7 ± 0.2
ESCO2	0.7 ± 0.1
DLAT	0.5 ± 0.1
MOF	0.3 ± 0.1
NAA50	0.2 ± 0.1

Figure 3. Applying KAT capture to profile metabolic feedback regulation. (a) Schematic of competitive immunoaffinity profiling. (b) Competitive profiling data for enrichment of KATs in the presence of acetyl-CoA (Ac-CoA) and CoA. Fractional enrichment is calculated by comparing competitor to competitor-free enrichment values. (c) Relative fractional enrichment of KATs by Lys-CoA Sepharose (**1**) in proteomes treated with Ac-CoA versus CoA (30 μM). Values greater than 1 indicate better competition by CoA; values less than 1 indicate better competition by Ac-CoA. Values represent ≥3 replicates, analyzed by two-tailed Student's t test (ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$). Additional data are provided in the Supporting Information: full blot data (Figure S5), calculations (Figure S6), and fractional enrichment values (Table S9). (d) Tabulated fractional enrichments.

lines revealed activity for the majority of these KATs in HeLa lysates, providing an abundant proteome source for competitive profiling studies (Figure S4 and Table S8).

To assess the susceptibility of KATs to feedback inhibition, lysates were pre-incubated individually with each cofactor (acetyl-CoA or CoA), enriched using Lys-CoA Sepharose **1**, and quantified via gel densitometry analysis of anti-KAT immunoblots (Figures 3b and S5). Fractional enrichment in the presence of each competitor was calculated by comparison to an untreated control. Relative fractional enrichment in acetyl-CoA versus CoA samples was used as a measure of competition, with values greater than 1 indicating stronger interaction with CoA (Figures 3c and S6). For most KATs, capture was partially competed at 30 μM acetyl-CoA/CoA (Figure S5), which lies close to cellular acetyl-CoA levels.[15] Consistent with previous studies, GCN5L2[16] and PCAF[17] display near equal competition by acetyl-CoA and CoA, signifying a capacity for feedback regulation (Figure 3c). Similarly, CoA preferentially inhibits enrichment of NAA10, whose metabolic regulation has been proposed to mediate apoptosis.[18] In contrast, MOF and NAA50 bind CoA less strongly than acetyl-CoA, indicating that these KAT activities are unlikely to be sensitive to feedback regulation.

An unexpected KAT showing a competition profile suggestive of sensitivity to metabolic feedback inhibition was NAT10. NAT10 is a recently characterized acetyltransferase that has been implicated in modification of histones, tubulin, and RNA.[19] Cellular studies of NAT10's yeast homologue found it to be sensitive to changes in acetyl-CoA biosynthesis;[20] however, the molecular basis for this effect was not established. To expand on our competitive profiling results, we expressed full-length recombinant NAT10 and performed biophysical analyses of CoA and acetyl-CoA binding. Differential static light scattering (DSLS) and surface plasmon resonance (SPR) were used to compare directly cofactor-mediated stabilization of NAT10 with that of GCN5L2, a known feedback-regulated KAT. DSLS indicates that both enzymes bind CoA with similar affinity but that NAT10 interacts more weakly with the acetyl-CoA cofactor (Figure 4a). Markedly stronger NAT10/acetyl-CoA binding is observed by SPR; however, overall the cofactor



Protein	ΔT_{agg} (°C)		K_d (μM)	
	CoA	Ac-CoA	CoA	Ac-CoA
GCN5L2	4.8	7.4	1.5 ± 0.2	1.0 ± 0.1
NAT10	4.8	2.9	1.0 ± 0.1	0.68 ± 0.1

Figure 4. Comparing the cofactor-binding properties of KATs. DSLS data for (a) GCN5L2 and (b) NAT10. (c) Ligand binding properties calculated from DSLS and SPR.

binding profiles of NAT10 and GCN5L2 are strikingly similar (Figures 4b and S7). Recombinant NAT10 exhibits little histone KAT activity, suggesting that histones may not be a physiological substrate. However, the detectable activity that can be observed is inhibited by CoA (Figure S8). Overall these results suggest a molecular mechanism for metabolic regulation of NAT10 activity and illustrate the power of chemical proteomics to fuel biological hypothesis generation.

Here we have described a platform for profiling KAT−ligand interactions in complex proteomes. Next-generation capture resins significantly expand the scope of KATs accessible to chemoproteomic methods. Applying this approach to study KAT−CoA interactions led to the finding that NAT10 has a binding profile indicative of susceptibility to metabolic feedback inhibition. Future studies will be necessary to determine whether this mechanism may be exploited to limit pathological NAT10 activity in cancer and progeria.[21] In addition to

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feedback inhibition, our preliminary results indicate that Lys-CoA Sepharose **1** can be used to study a wide range of KAT−metabolite interactions (Figure S9). Such studies may be useful in understanding the ability of acyl-CoA metabolites to function as inhibitors or alternative cofactors for KATs (Figure S7).[7,19] Finally, we note some limitations and unexplored features of our current method. First, our platform was unable to sample the activity of p300/CBP family KATs at either endogenous or ectopic expression levels (Figure S10).[5] This may indicate that p300's unique active site, in which the cofactor and substrate sites are linked by a narrow tunnel, cannot accommodate bulky Sepharose-linked bisubstrates.[2] Addressing this limitation will require complementary methods to monitor cellular p300/CBP activity.[22] Second, our immunoblot-based method capably identified overall trends, but can be laborious and have trouble differentiating KAT−cofactor interactions similar in magnitude (i.e., NAT10 and HAT1, Figure 3c). Both the throughput and precision of this method will benefit from integration of our probes with quantitative proteomic platforms. Finally, we note that the capture agents reported here also enrich several cofactor-dependent enzymes that do not harbor annotated KAT or NAA activity in an active site-dependent fashion (Tables S1−S4). This raises the possibility that in addition to their uses in the study of KATs, CoA-based probes may have broad applications in profiling disease pathologies driven by aberrant cofactor-dependent enzyme activity.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03036.

Protocols, additional figures, and extended acknowledgment information. (PDF)

Spectral count data, Sepharose pull down data, gene ontology analysis, additional fractional enrichment values, and background contaminant data. (XLSX)

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Notes

The authors declare no competing financial interest.

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