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,N333(3)20a674 1.13QW,M

THE ROYAL SOCIETY
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utilization of acyl-CoAs as rate-limiting cofactors in enzymatic protein modifications catalysed by lysine acyl-transferase (KAT) and histone acyltransfease (HAT) enzymes, or through non-enzymatic acylation reactions that can be heavily influenced by the electrophilicity of individual acyl-CoA species [2]. A physiochemically diverse set of acylations have been detected on histone lysine residues, including not only the predominant acetylation but also succinylation, propionylation, (iso)butyrylation, crotonylation, malonylation and several others [3]. Lysine acylations generally correlate closely with the abundance of corresponding acyl-CoA [4].

Recently, Zhang *et al.* [5] reported a new addition to this emerging class of PTMs, known as lysine lactoylation. Applying antibody-based enrichment, this PTM was mapped to histone in M1 polarized macrophages and shown to correlate with altered gene expression during immune activation. Furthermore, lysine lactoylation within *in vitro* transcription assays was found to be dependent on both the presence of a KAT enzyme (p300) as well as lactoyl-CoA, consistent with

25 mM ammonium acetate. Samples were also extracted by addition of 1 ml –

In both cases, lactoyl-CoA was linear ($R^2 > 0.998$) and observed values within 25% of expected values from 0.03 pmol per sample (3 fmol on column) to the highest range tested at 500 pmol per sample (50 fmol on column). Lactoyl-CoA was detectable down to 0.002 pmol per sample (0.2 fmol on column), but quantification was unstable across runs over 24 h. Re-injection of samples from both purified standards, and purified standards spiked into cellular extract were stable (±5%) over 24 h in a chilled autosampler. Unfortunately, as noted, our method of producing internal standards in yeast did not generate a lactoyl-CoA under the conditions we used. Thus, we investigated using surrogate internal standards including $^{13}C_3^{15}N_1$-acetyl-, $^{13}C_3^{15}N_1$-succinyl- and $^{13}C_3^{15}N_1$-HMG-CoA. All produced linear calibration curves with high R^2 values (greater than 0.998) when plotting the ratios of analyte / each internal standard as the dependent variable and similar deviation of calibration points from observed versus expected values.

3.3. La - ab a a a - a a -C A

We quantified lactoyl-CoA via relative response factor to other short-chain acyl-CoAs for retrospective sample analysis. This allowed us to rank the molar amount of lactoyl-CoA versus other acyl-CoAs detected in cells. We calculated proportionality constants using known amounts of lactoyl-CoA across a calibration curve of serial dilutions of cell and tissue relevant concentrations of other short-chain acyl-CoAs. Proportionality constants on a molar basis were calculated as

$$k \text{ ¼ } \frac{[\mathrm{AUC(lactoyl\text{-}CoA)=pmol(lactoyl\text{-}CoA)}]}{[\mathrm{AUC(acyl\text{-}CoA)=pmol(acyl\text{-}CoA)}]}:$$

Propionyl-CoA and succinyl-CoA had the response factors that most closely approximated 1, where the same molar amount of lactoyl-CoA and other acyl-CoAs generated the same