2) Abstract of Research Proposal (maximum 3,800 characters)

3) Description of Proposed Project \*   
Background: Please describe your research as it pertains to discovery quantitative proteomics. Please indicate how quantitation is critical for your research area.

Protein post-translational modifications (PTM) regulate many processes, including catalytic activity, subcellular localisation and protein interactions. Lysine acetylation is a reversible PTM that was first characterised in histones where it performs a crucial function regulating gene expression. Lysine acetylation has since been discovered to be a widespread modification with many functions including signal transduction where it is essential for activation of p53 [REF]. Recently, further lysine acylation modifications have been discovered: malonylation, succinylation and glutarylation [REFS]. These involve the addition of a doubly negatively charged moiety, thus inducing a charge change in lysine from +1 to -1 that might reasonably be expected to have a large impact on protein activity and/or function.

Current proteomics analyses suggest these lysine acylations are in competition with one another and enriched in proteins with mitochondrial and metabolic functions [REFS]. Succinylation of mitchondrial proteins may be explained by the production of succinyl-CoA as an intermediate in the mitochondrial citric acid cycle. In contrast, malonyl-CoA and glutaryl-CoA are generated by cytosolic fatty acid pathways, suggesting competition between succinylation, malonylation and glutarylation could regulate protein activity in response to metabolic fluxes. We have also shown that succinylation and malonylation are enriched in proteins which interact directly or indirectly with E-cadherin (unpublished), raising the possibility that lysine acylation may integrate signals from cell-cell adhesion and metabolism.

A crucial requirement to understand the biological function of lysine acylation function is the sub-cellular distributions of these modifications and whether they compete for the same residues at the same localisations. A major caveat from previous studies of lysine acylations is that they have predominantly been conducted using either mouse liver, in which mitochondrial proteins are highly abundant, or very disrupted cell lines such as HeLa [REFS]. Thus the true sub-cellular distributions are still unclear. We are ideally placed to answer this question as we have pioneered the development of the high-resolution sub-cellular proteomics technique hyperLOPIT. This technique utilises 10-plex TMT tags to quantify peptide profiles over cellular fractions and machine-learning methods to classify proteins to discrete organelles and complexes [REF]. Combining hyperLOPIT with PTM enrichment, TMT tags and the Orbitrap Lumos, we will generate unprecedented resolution data regarding the localisation of acylated peptides. This will enable us to address fundamental unanswered questions regarding which proteins contain these modifications and their sub-cellular localisation. Highly accurate quantification is critical to our technique since we need to accurately quantify the abundance of each peptide over the fractions in order to correctly define its localisation.

4) Description of Research \*

Background: Please describe the research that will be enabled through the use of TMT tags and commercial MS Reagents. Specifically, indicate which Thermo Scientific™ Reagents will be utilized to support the research, as well as the Thermo Scientific mass spectrometer(s) that will be used for sample analysis.

Description:

The implementation of unbiased chemical tagging such as TMT Labels and reliable, high-performing mass spectrometers like Orbitrap analysers have increased quantitative data yield and quality from ever lower sample concentrations

And the study of PTMs has benefitted greatly from these advancesWe continue to develop our LOPIT methodology, a proteomic approach which enables simultaneous subcellular compartment assignment of hundreds to thousands of proteins from a single sample by means of TMT-labelling different cell-fractionated lysates. With the implementation of TMT-10plex in our new hyperLOPIT approach, combined with SPS-MS3 acquisition on the Orbitrap Fusion Lumos Tribrid Mass Spectrometer, we have generated the highest resolution sub-cellular proteomics map to date. [REF NATURE COMMS].

As described above, we now seek to investigate lysine PTMs in the spatial-proteome. The sensitivity of Orbitrap Lumos alongside the multiplexing capability of TMT labels will provide the ideal platform to analyse PTMs. This will be combined with PTM-specific scoring algorithms to ensure high confidence identification of modified peptide. TMT-labelling will enable us to enrich PTM-carrying peptides from a single TMT-labelled multiplexed sample, reducing the quantifcation noise between fractions and increasing the accuracy of our peptide profiles. Furthermore, multiple kinds of modifications will be enriched simultaneously, allowing us to interrogate the competition between the lysine acylation modifications. Combining our sub-cellular technique, hyperLOPIT, with PTM enrichment, TMT tags and the Orbitrap Lumos, we will be able to generate unprecedented resolution data regarding the localisation of acylated peptides. This will enable us to address fundamental unanswered questions including: Is there competition between lysine acylations at the same sub-ceullar localisation? Are succinylated peptides truly enriched in the mitochondria? Are metabolic proteins acylated differently in different localisations? Answering these questions will further our understanding of these newly discovered PTMs and help direct future research into their potential role as signal transducers.