Protein post-translational modifications (PTM) regulate many processes, including catalytic activity, subcellular localisation and protein interactions. Lysine acetylation is a reversible PTM which was first characterised in histones where it performs a crucial function regulating gene expression. Lysine acetylation has since been discovered to be a despread modification with many functions including signal transduction where it is essential for activation of p53 [REF].

Recently, further lysine acylation modifications have been uncovered [REFS]. Lysine malonylation, succinylation and glutarylation involve the addition of a doubly negatively charged moiety with 3, 4 or 5 carbons, respectively. These PTMs thus induce a charge change in lysine from +1 to -1 at physiological pH which might reasonably be expected to have a large impact on protein activity and/or function. To date, there have a small number of studies of these lysine acylations [REFS]. These suggest that the acylation events compete for the same lysine residues and that succinylation is predominantly restricted to mitonchondrial proteins. The later 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 the competition between these succinylation and malonylation/glutarylation may regulate protein activity in response to metabolic fluxes. Our bioinformatic analysis using publically available data also indicates that succinylation and malonylation are enriched in proteins which interact directly or indirectly with E-cadherin (unpublished) and metabolic proteins. This raises the intriguing possibility that lysine acylation may integrate signals from cell-cell adhesion and metabolism.

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 it is not at all clear what the true sub-cellular distributions of these modifications are and whether they compete for the same residues at the same localisations. We have pioneered the development sub-cellular proteomics techniques, utilising TMT tags to quantify peptide profiles over cellular fractions and machine-learning methods to classify proteins to discrete organelles and complexes [REF]. Quantification is critical to our technique since we need to accurately quantify the abundance of each peptide over the fractions in order to accurately define its localisation. Combining our sub-cellular technique, hyperLOPIT, with PTM enrichment 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.