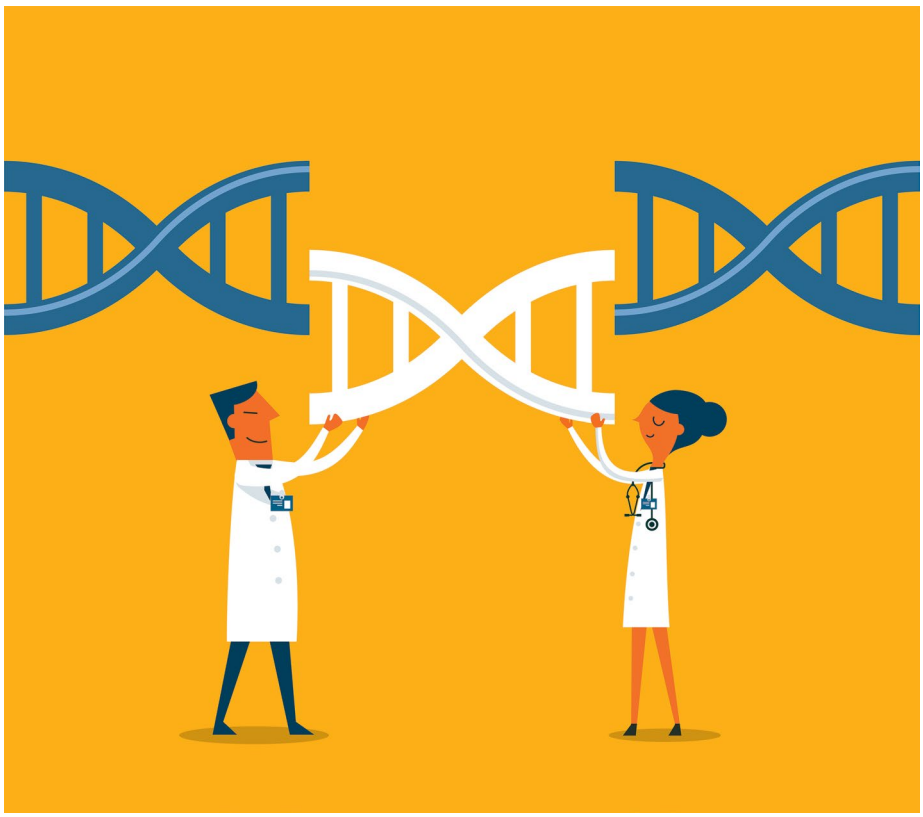
Tools of the trade

Expanding the genetic code for site-specific lysine lactylation



Post-translational modifications (PTMs) are pivotal regulators of protein function, influencing processes such as enzymatic activity, stability and molecular interactions, yet their precise study has been hampered by the lack of tools to produce proteins with site-specific and homogeneous modifications. Traditional approaches rely on enzyme-based modulation, chemical synthesis or site-directed mutagenesis to mimic modifications. However, these methods are often indirect and lack precision, as the enzymes used in these approaches exhibit broad substrate specificity and mutagenesis cannot fully replicate the functional properties of the native modification.

To address these challenges, we utilized genetic code expansion (GCE) to incorporate lysine lactylation — a recently identified PTM — at specific sites in proteins of interest. GCE enables the site-specific incorporation of unnatural amino acids into proteins using orthogonal aminoacyl-tRNA synthetase (aaRS)–tRNA pairs. By replacing natural amino acids with unnatural amino acids that carry the desired chemical modification, GCE offers a powerful and precise approach to directly study PTMs in target proteins.

By constructing and optimizing orthogonal aaRS–tRNA pairs for efficient amber codon suppression in bacterial and mammalian cells, this approach enabled the incorporation of lysine lactylation at defined sites in target proteins. Using this method, we successfully introduced lactylation into p53 at two lysine residues in its DNA-binding domain and systematically investigated the functional impact on p53 activity both in vitro and in vivo. By circumventing the ambiguity associated with mutation-based or enzyme-based approaches, this system provides a direct and reliable tool for studying lysine lactylation and related acylations. Additionally, the orthogonal nature of the system ensures minimal interference with native cellular machinery, allowing robust and physiologically relevant experiments.

Our work highlights the versatility of GCE in exploring the functional diversity of PTMs, particularly acylations beyond acetylation, which often share enzymes and pathways. This method can be extended to dissect complex PTM networks, revealing novel regulatory mechanisms and interactions. By enabling precise chemical modification of proteins in living systems, GCE bridges a critical gap in molecular biology, offering insights that were previously inaccessible.

Future applications could integrate GCE with ultra-sensitive proteomics to uncover the roles of PTM crosstalk in complex cellular systems, enhancing our understanding of protein regulation in health and disease. Moreover, the scalability of this approach positions it as a transformative tool for developing therapeutic proteins with tailored properties, paving the way for advances in synthetic biology and precision medicine.

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Competing interests
The author declares no competing interests.

Related article: Zong, Z. et al. Alanyl-tRNA synthetase, AARS1, is a lactate sensor and lactyltransferase that lactylates p53 and contributes to tumorigenesis. *Cell* **187**, 2375–2392.e33 (2024)