Developing genetic code expansion tools to study acidic protein post-translational modifications (PTMs)

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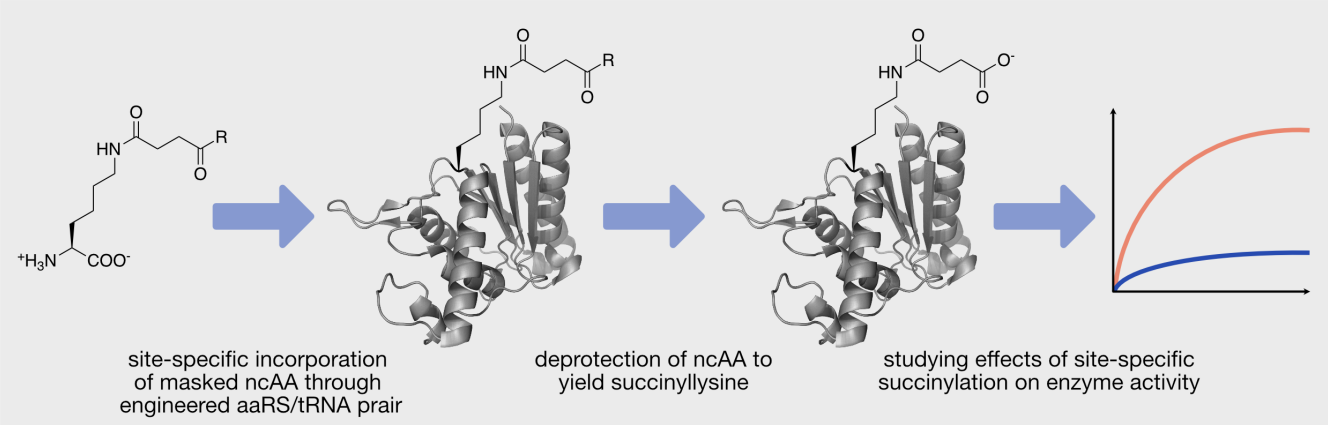
While only being comprised of 20 different amino acids, proteins carry out a myriad of different functions. This repertoire is increased even further by the post-translational installation of small chemical moieties on specific amino acid residues – (PTMs). A prominent and versatile group of PTMs consist of acylations of lysine residues, which generally neutralise its positive charge, affecting i.a. protein-protein interaction, protein structure, and protein localisation. Rather recently a new type of lysine-acylation was discovered, which does not neutralise the charge of lysine but inverts it by covalently attaching negatively charged groups (e.g. succinyl, glutaryl) to lysine. This inversion of charge in combination with the bulkiness of the modification has been associated with changes in enzyme activity and an increase in transcription, since it is often found on histones where it disrupts binding to the negatively charged DNA.



To investigate the role of succinylation and glutarylation within target proteins, we have developed an orthogonal aminoacyl-tRNA synthetase (aaRS) that charges a masked derivative of succinyllysine or glutaryllysine onto its respective orthogonal tRNA to direct its site-specific incorporation into a protein of interest. We designed the masking group in such a way that it can be removed *in vitro* on the folded proteins. Using this approach, we were able to study site-specific succinylation and glutarylation within several target proteins and could show its influence on enzyme activity, and that the modification can be removed by deacylases.

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