Site-Specific, Bioorthogonal Protein Labeling by Tetrazine Ligation using Endogenous β-Amino Acid Dienophiles Derived from Natural Product Biosynthesis

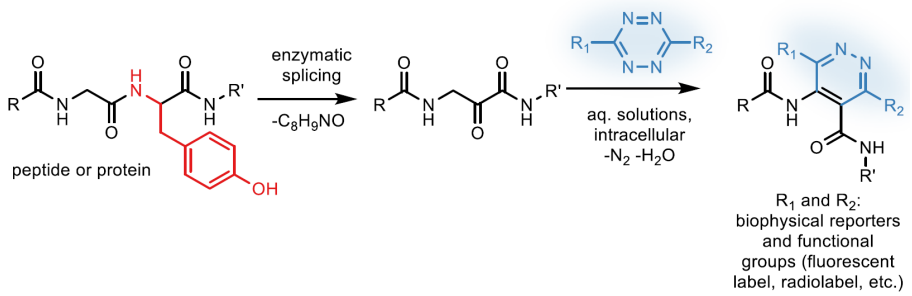
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Recent advancements in bioorthogonal chemistry have enabled the study of proteins inside living cells. Selective modification of a specific protein in the complex mixture of proteins, macromolecules and other metabolites in living cells allows for visualization and modification of proteins involved in diseases. Derivatization with fluorescent dyes enables tracking proteins of interest by confocal laser scanning microscopy. Intracellular labeling enables investigation of proteins involved in disease and subsequent modification to cause physiological changes.

However, bioorthogonal reactions pose a few challenging requirements to be met. Reactions need to proceed selectively, fast, and in aqueous environments. Common approaches today use unnatural amino acids installed in proteins by amber codon suppression or enzymatic ligations. These approaches can suffer from low yields, metabolic changes and interferences in natural pathways which is why novel approaches are needed.

Here we leveraged an enzymatic reaction naturally occurring in the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs) for use in bioorthogonal labelling of proteins. By utilizing the excision of tyramine from protein backbones by a radical *S*-adenosyl methione (rSAM) enzyme,[1-3] we have incorporated a bioorthogonal aminopyruvate residue in ribosomally produced proteins. This building block does not naturally occur in common model organisms and reacts with commercially available tetrazine derivatives in a fast and selective manner. We have characterized and studied this reaction and shown that the reaction proceeds in aqueous media and intracellularly in *Escherichia coli (E. coli)*. We have demonstrated the utility of this method by producing and purifying a Her2-binding Affibody, which we characterized to be fully functional after conjugation. Additionally, we have labeled the bacterial cell division protein FtsZ with a fluorescent dye to investigate the localization of the protein in *E. coli.* We anticipate that the method will be useful for future *in vivo* studies of cellular proteins and as a new method for the engineering of protein conjugates.



[1] Brandon Morinaka et al. *Science,* **2018**, 359, 779-782.
[2] Thomas Scott et al. *Chem*, **2022**, 8, 2659-2677.
[3] Edgars Lakis et al. *Angewandte Chemie International Edition*, **2022**, 61, E202202695.