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# Photoenzymes: method development and applications of non-canonical amino acids as versatile tools in photobiocatalysis

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## PURPOSE OF THE ABSTRACT

Enzymes play a fundamental role in catalysing a wide spectrum of biotransformations in living systems. Scientists have long harnessed this natural diversity in various fields and applications. However, natural enzymes often possess limitations like lack of stability or narrow substrate scope, which hamper their utilization in industrial processes. Directed evolution has emerged as a powerful tool to overcome such limitations, by replicating evolutionary processes on a lab scale. In recent years, a variety of methods, such as the development of Artificial Metalloenzymes (ArM), have emerged as innovative approaches to broaden the scope of enzymatic reactions and modulate enzyme activities.

Among these techniques, non-canonical amino acids (ncAAs) have been utilized to expand the repertoire of the 20 canonical amino acids with new chemical and physical properties. Thus, ncAAs enable the development of novel enzymes with enhanced or entirely new-to-nature functionalities. This expansion of the genetic code relies on the use of an orthogonal tRNA/aminoacyl-tRNA synthetase (aaRS) pair to incorporate ncAAs at specific and defined positions, by recoding a stop codon. The resulting enzymes possess improved or unique functions, allowing them to catalyze abiological reactions or outperform their natural counterparts.

Our research is focused on incorporating photosensitive ncAAs into protein scaffolds to establish a versatile platform for stereo- and enantioselective photobiocatalysis. By taking advantage of light as an abundant and cost-effective energy source, we aim to implement this technology as a sustainable and promising alternative to existing methods in photoredox catalysis.

We have assessed the feasibility of our approach using the well-established ncAA Benzoyl-Phenylalanine (Bpa). Following its incorporation into various protein scaffolds, we evaluated their performance in a diverse range of reactions, including radical addition through hydrogen atom transfer (HAT) or decarboxylation and energy transfer reactions (EnT). However, this strategy posed significant challenges due to limited control over the generated radical species during the reactions, as well as the occurrence of undesired side reactions, such as crosslinking.

To overcome these limitations, we are focusing on the development of novel ncAAs to expand the range of genetically encoded photosensitizers with improved characteristics, such as thioxanthone and anthraquinone moieties. Here, I will present our efforts on the development and strategies for engineering novel aaRS variants. This includes principles of GoldenGate cloning and different PCR techniques to generate large libraries. With this approach, we enable fast saturation mutagenesis of multiple residues in an aaRS, with the potential for general applicability in various protein scaffolds. In combination with different screening and selection methods, we are able to identify optimized variants in an efficient manner.

By elucidating the underlying principles of ncAA incorporation and expanding the toolbox of genetically encoded photosensitizers, we aim to establish light-mediated enzymatic reactions in the field of biocatalysis.

## FIGURES

### FIGURE 1

### FIGURE 2


## KEYWORDS

Photobiocatalysis | Non-canonical amino acids | Aminoacyl-tRNA synthetase variants | Saturation mutagenesis


## BIBLIOGRAPHY