Incorporation of non-canonical amino acids toward the engineering of artificial metalloenzymes

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Metals are present in almost half of all enzymes to fulfill structural and/or catalytic functions. Metalloenzymes have been successfully engineered for biocatalysis, often by modifying the substrate or metal specificity of a natural metalloenzyme [1]. An alternative to these enzymes consists in the insertion of a metal complex into a versatile protein scaffold able to accommodate various substrates and/or reactions [2]. In this study, the goal is to preserve the natural enzyme's substrate specificity while adapting new-to-nature metal-based reactivity through active site remodelling. However, designing a metal-binding site is not trivial as it often requires a proper positioning of several amino acid residues (usually a histidine brace motif, plus an additional residue) to ensure an efficient coordination of both the metal ion and the *ad hoc* substrate. To circumvent this obstacle, bipyridyl-alanine (BpyA), a metal-chelating non-canonical amino acid (ncAA), was used.

NcAA incorporation can be achieved by reassigning a nonsense codon to a ncAA by the use of an orthogonal amino-acyl tRNA synthetase (aaRS)/tRNA pair [3]. Despite some achievements over the last 15 years, this approach still suffers from highly variable and often low incorporation efficiencies, which can be deterrent.

Based on the historical and widely used pEVOL incorporation system [4], we developed the pINS systems by altering the tRNA and aaRS expression levels. Several amino acid positions identified by molecular modelling were selected to incorporate BpyA in the vicinity of the catalytic site of our model enzyme. Using the pINS system, both BpyA incorporation efficiency and protein production were improved with success, reaching production levels comparable to the wild-type enzyme. For each targeted position, the proper incorporation of BpyA was confirmed by mass spectrometry and X-ray crystallography. Interestingly, the reorientation of a neighboring amino acid side chain was observed to obtain a complete metal ion coordination in the absence of a bound substrate. Moreover, the overall 3D structure and thermal stability of the mutants were found to be poorly affected by the introduction of BpyA. Future experiments will investigate the substrate-binding properties of the BpyA mutants and their catalytic potential.

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