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Title: Global substitution of hemeproteins with noncanonical amino acids in *Escherichia coli* with intact cofactor maturation machinery

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Global substitution of hemeproteins with noncanonical amino acids in *Escherichia coli* with intact cofactor maturation machinery

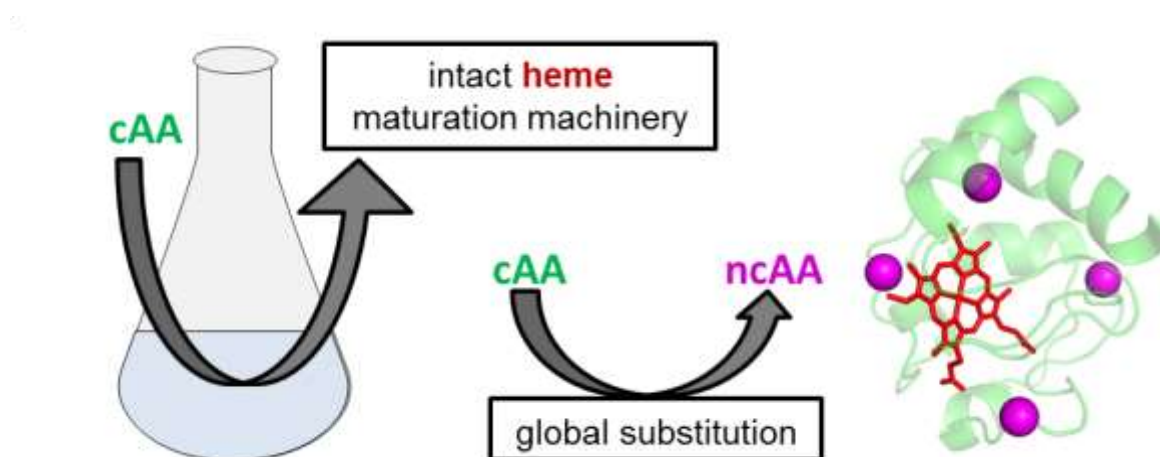
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Graphical Abstract



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Highlights

- We developed an effective SPI method for incorporation of ncAAs into hemeproteins
- The designed experimental setup allows temporal control of heme production
- This guarantees intact cofactor maturation while the protein is engineered
- Our approach is probably applicable for other cofactor-containing proteins
- Presented strategy is valuable for biotechnological production of novel biocatalysts

Abstract: Global substitution of canonical amino acids (cAAs) with noncanonical (ncAAs) counterparts in proteins whose function is dependent on post-translational events such as cofactor binding is still a methodically challenging and difficult task as ncAA insertion generally interferes with the cofactor biosynthesis machinery. Here, we report a technology for the expression of fully substituted and functionally active cofactor-containing hemeproteins. The maturation process which yields an intact cofactor is timely separated from cAA→ncAA substitutions. This is achieved by an optimised expression and fermentation procedure which includes pre-induction of the heme cofactor biosynthesis followed by an incorporation experiment at multiple positions in the protein sequence. This simple strategy can be potentially applied for engineering of other cofactor-containing enzymes.

Introduction

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