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

Authors: Jan-Stefan Völler, Tuyet Mai Thi To, Hernan Biava, Beate Koksch, Nediljko Budisa

PII: S0141-0229(17)30124-2
DOI: http://dx.doi.org/doi:10.1016/j.enzmictec.2017.06.014
Reference: EMT 9098

To appear in: *Enzyme and Microbial Technology*

Received date: 16-3-2017
Revised date: 23-6-2017
Accepted date: 28-6-2017

Please cite this article as: Völler Jan-Stefan, Thi To Tuyet Mai, Biava Hernan, Koksch Beate, Budisa Nediljko.Global substitution of hemeproteins with noncanonical amino acids in Escherichia coli with intact cofactor maturation machinery.*Enzyme and Microbial Technology* http://dx.doi.org/10.1016/j.enzmictec.2017.06.014

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

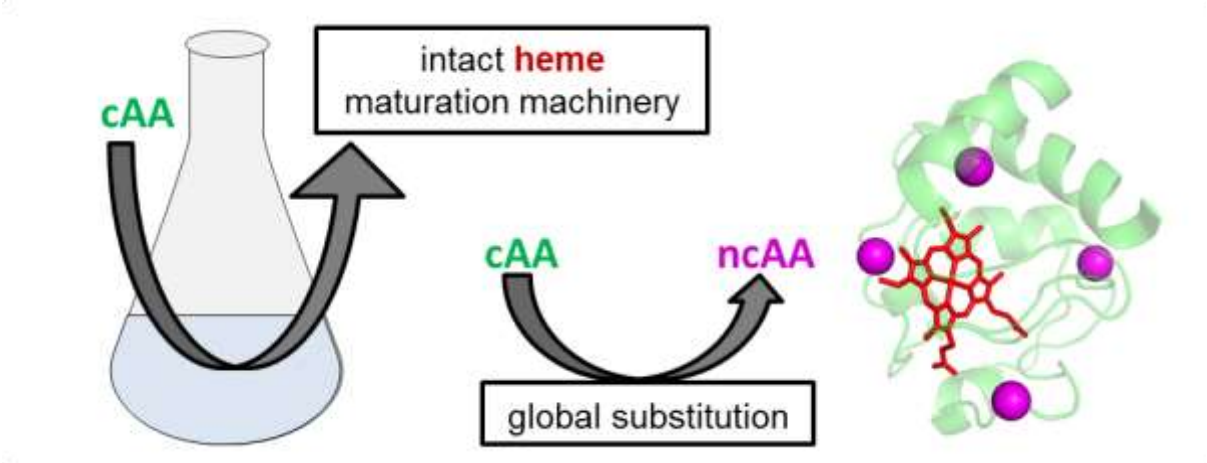
Jan-Stefan Völler[a][b]§, Tuyet Mai Thi To[a][b]§, Hernan Biava[a], Beate Koksch[b], Nediljko Budisa[a]*

[a]Department of Chemistry, Technische Universität Berlin, Müller-Breslau-Strasse 10, 10623 Berlin, Germany.

[b]Institute of Chemistry and Biochemistry – Organic Chemistry, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany.

*Corresponding author: Nediljko Budisa, Email: nediljko.budisa@tu-berlin.de

Graphical Abstract



<InlineImage1>

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