BIOTRANS
LA ROCHELLE 2023

N°1237 / PC
TOPIC(s) : Enzyme discovery and engineering / Artifical intelligence / computational methods

Mapping redox enzymes' active sites for coenzyme versatility

AUTHORS

Alice GUARNERI / LABORATORY OF ORGANIC CHEMISTRY, WAGENINGEN UNIVERSITY, STIPPENENG 4, WAGENINGEN
Georg STEINKELLNER / INNOPHORE GMBH, AM EISERNEN TOR 3, GRAZ
Christian GRUBER / INNOPHORE GMBH, AM EISERNEN TOR 3, GRAZ
Maurice C.R. FRANSSEN / LABORATORY OF ORGANIC CHEMISTRY, WAGENINGEN UNIVERSITY, STIPPENENG 4, WAGENINGEN
Willem J. H. VAN BERKEL / LABORATORY OF FOOD CHEMISTRY, WAGENINGEN UNIVERSITY, BORNSE WEILANDEN 9, WAGENINGEN
Corresponding author : Caroline E. PAUL / C.E.Paul@tudelft.nl

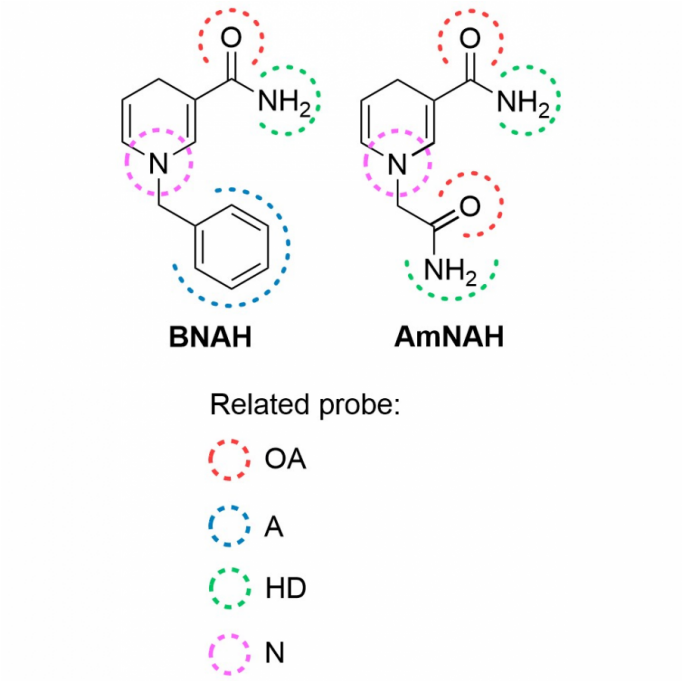
PURPOSE OF THE ABSTRACT

Synthetic nicotinamide coenzyme biomimetics (NCBs) are attractive alternatives to the natural pyridine dinucleotides NAD(H) and NADP(H) for applied biocatalysis, allowing access to more cost-effective processes for enzyme-catalyzed redox reactions.[1] Their use has been hampered by the fact that only a limited number of oxidoreductases are known to accept them as hydride donors or acceptors during catalysis.

To overcome the lack of coenzyme versatility, we developed a bioinformatics approach to create a portfolio of novel NCB-accepting enzymes. To do so, we computationally mapped the coenzyme-binding sites of biocatalysts active with synthetic coenzymes and searched the Protein Data Bank for enzymes presenting an active site with comparable characteristics. Several oxidoreductase candidates were identified and four of them were tested with NCBs, resulting in activity for three enzymes: actinorhodin ketoreductase from Streptomyces coelicor, α-ketoglutarate semialdehyde dehydrogenase from Azospirillum brasilense and isopiperitenone reductase from Mentha piperita. In conclusion, the designed computational approach was able to correctly predict coenzyme versatility. This novel in silico method has the potential to be used as platform for non-natural coenzyme engineering designs in the future.

The results of the biocatalytic conversions, the relative efficiency of synthetic coenzymes compared to the natural NAD(P), and the implications for applied biocatalysis are discussed.

FIGURES



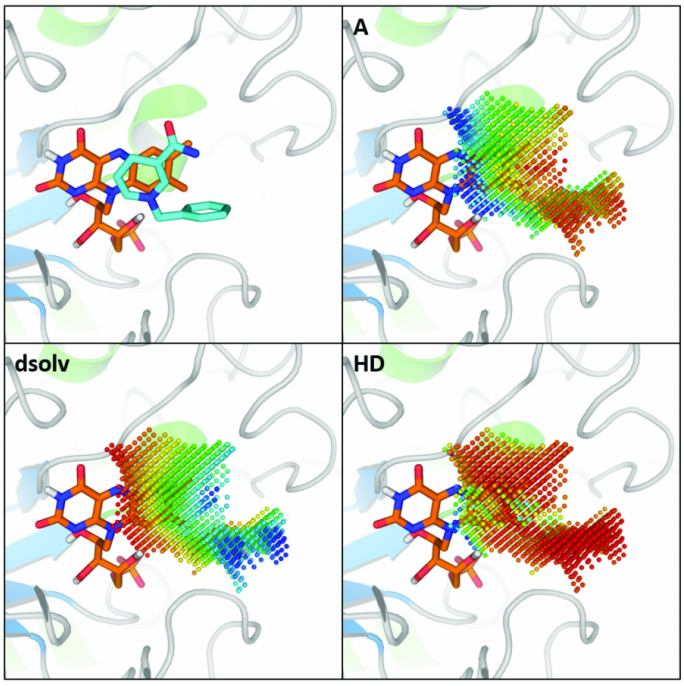


FIGURE 1

NCBs used in this work

Chemical structures of two NCBs (BNAH, AmNAH) and corresponding chemical features (OA: oxygen as H-bond acceptor; A: aromatic functional group; HD: H-bond donor; N: non-hydrogen bonding nitrogen)

FIGURE 2

Exemplary characterization of the BNAH-binding pocket of the Old Yellow Enzyme 1 from Saccharomyces pastorianus

NCB□s docking pose in the upper left; the response of its binding cavity to different chemical (A, HD) and structural (dsolv: desolvation) probes are shown as 3D-point clouds: blue (negative) to red (positive response the physicochemical functionality)

KEYWORDS

biocatalysis | synthetic coenzymes | oxidoreductases | bioinformatics

BIBLIOGRAPHY

[1] Guarneri, A. et al., Curr. Opin. Biotechnol. 2019, 60: 63-71