

# In search of the perfect environment: The quest to maximize macromolecule stability

Mia Radović<sup>1\*</sup>, Wolfgang Kroutil<sup>2</sup>, Tamara Reiter<sup>2</sup>, Ivana Radojčić Redovniković<sup>1</sup>, Marina Cvjetko Bubalo<sup>1</sup>

<sup>1</sup> University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, Zagreb

<sup>2</sup> University of Graz, Institute Of Chemistry, Heinrichstrasse 28 / II, Graz

\*mradovic@pbf.hr



## DES [Deep Eutectic Solvents]

### DES

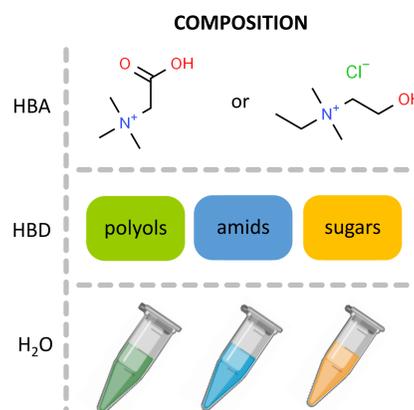
Stabilisation medium

- ✓ substitutes for harmful industrial solvents
- ✓ fully meet the principles of green chemistry
- ✓ designed solvents
  - ✓ modeled structure and physicochemical properties
  - ✓ natural origin

**HYPOTHESIS:** DES can mimic the macromolecule's natural environment

**USE IN BIOCATALYSIS:**

- ✓ Better substrate solubility/loading
- ✓ Improved cofactor stability
- ✓ Improved enzyme activity and stability



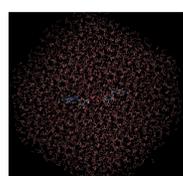
DES structural flexibility offers a possibility for rational solvent design to fulfill specific purposes and industrial requests.

One of their applications has been shown for solubilisation and stabilisation of a wide range of biomolecules. In this work, DES were investigated as a stabilisation medium for nicotinamide cofactors and several oxidoreductive enzymes.

## NICOTINAMIDE COFACTORS

NAD<sup>+</sup> and NADH are an indispensable part of oxidoreductase-catalysed reactions. They are known for their labile nature and short-term stability, so new and fresh stock solutions need to be prepared prior to each reaction. Finding a solvent that could simultaneously stabilise both cofactors forms in biocatalytic reactions and minimize their degradation during storage is of great significance.

Computational approach

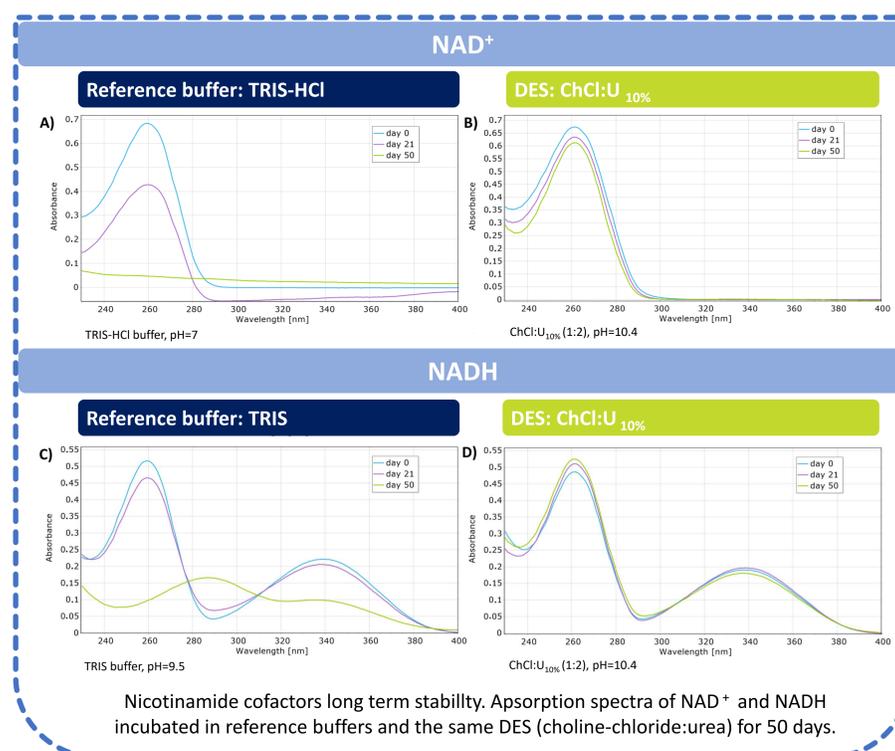


Simulation box (15 Å side length)  
MD [molecular dynamics] simulations  
QM [quantum mechanics] calculations

Experimental approach

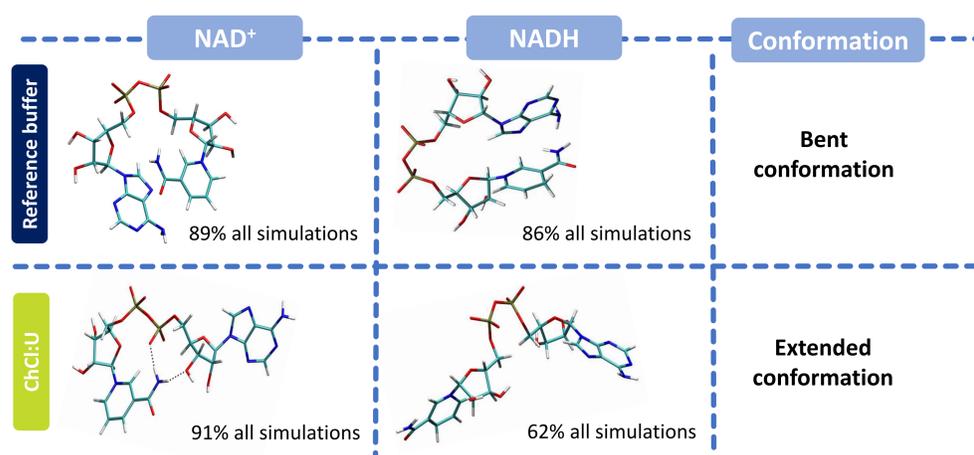
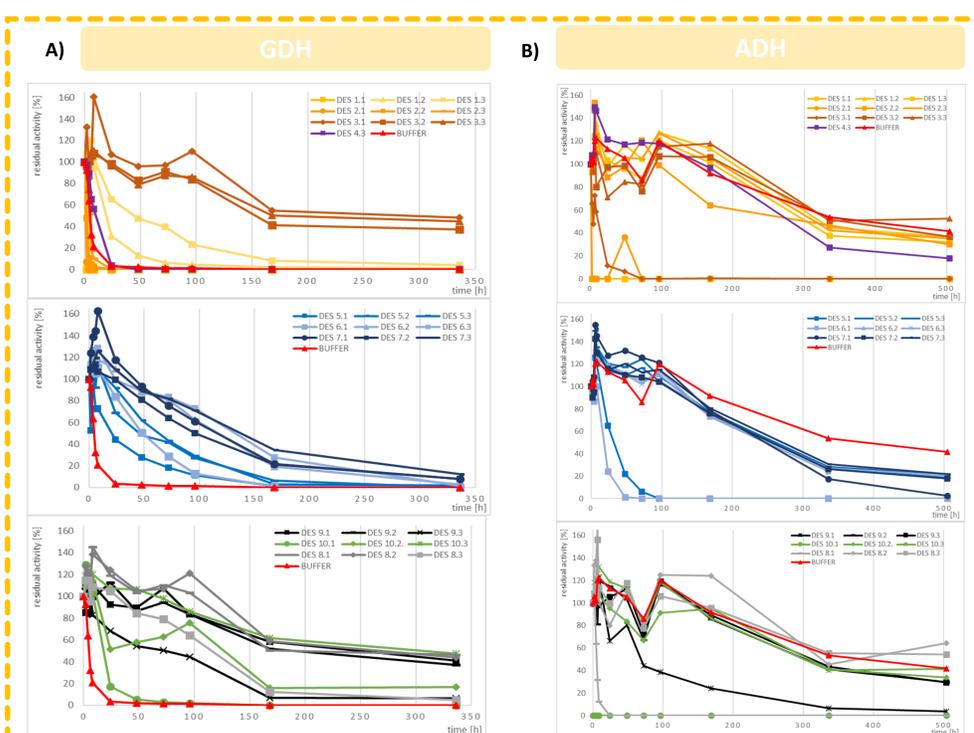


UV-VIS spectroscopy



## OXIDOREDUCTIVE ENZYMES

Several oxidoreductases were tested for stability and activity in 28 different choline-chloride and betaine-based DES. By carefully choosing DES constituents, it is possible to mimic their perfect environment, enhance their activity and prolong stability long-term.



Nicotinamide cofactors conformation and interaction with DES plays a role in their long term stability.

## CONCLUSION

Due to **deep eutectic solvents (DES)** natural origin, they can mimic the macromolecule's natural environment more effectively. This study presents the use of betaine and choline chloride-based DES as a medium for several alcohol dehydrogenases and nicotinamide cofactors long-term stabilization. Macromolecule stabilization is a promising area of research with potential applications in various fields, including biotechnology and pharmaceuticals.

## LITERATURE

1. M. Radović et al., *Green Chem.*, **2022**, 24, 7661–7674.
2. A. Paiva et al., *ACS Sustain. Chem. Eng.*, **2014**, 2, 1063–1071.
3. M. Panić et al., *J. Chem. Technol. Biotechnol.*, **2021**, 96, 14–30.