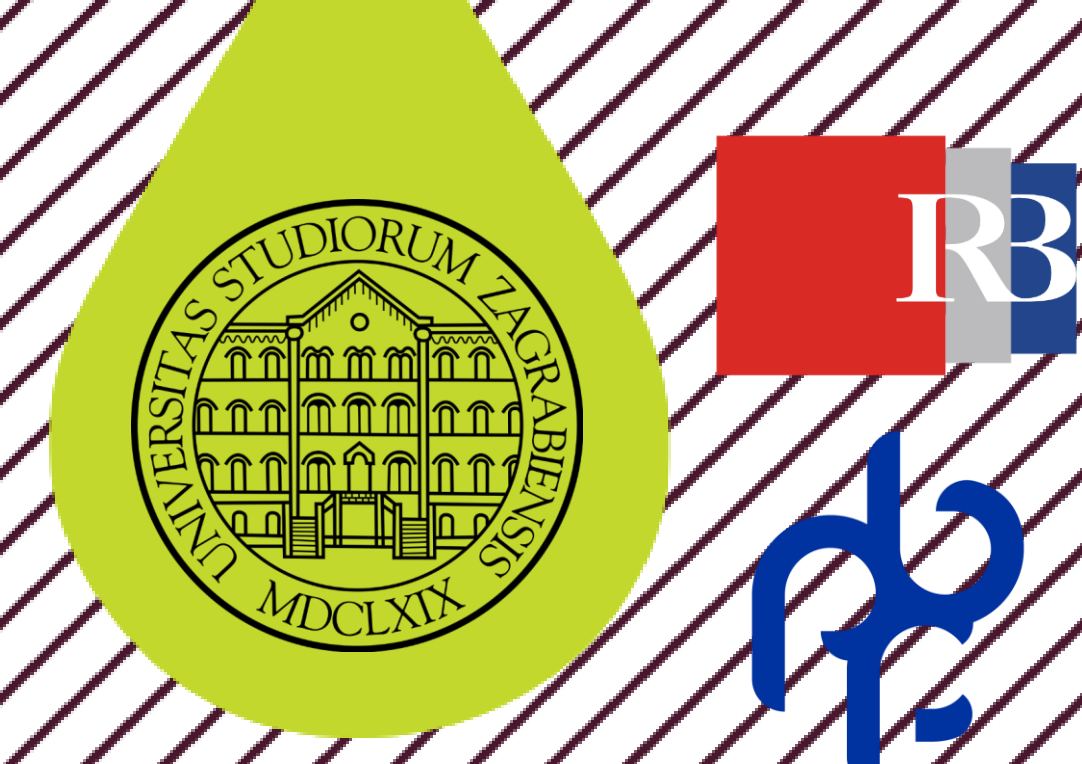


# STABILISING NICOTINAMIDE COFACTORS IN DEEP EUTECTIC SOLVENTS

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## 1 INTRODUCTION & EXPERIMENTAL

**Cofactors** are an indispensable part of oxidoreductase-catalysed reactions, with nicotinamide cofactors NAD<sup>+</sup> and NADH being most frequently present. 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 NAD cofactors in oxidoreductive biocatalytic reactions and minimize their degradation during storage is of great significance.

### Nicotinamide cofactors:

①

NAD<sup>+</sup>

Oxidized form

②

NADH

Reduced form

### Stabilising medium screening:

Reference buffers

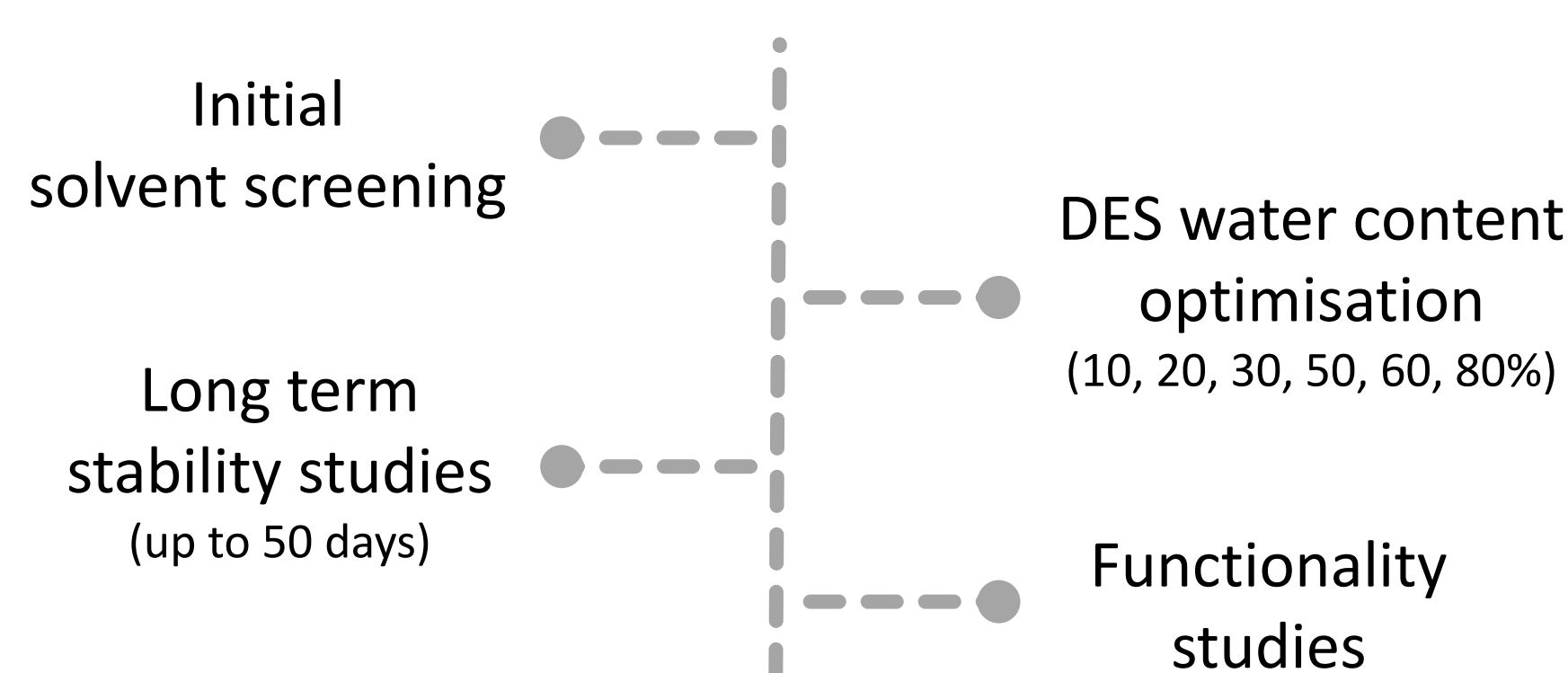
Betaine-based DES

Choline-chloride based DES

Total: 20 solvents

**Deep eutectic solvents (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, NAD<sup>+</sup> as an oxidized and NADH as a reduced form.

### UV-VIS spectroscopy (nicotinamide cofactor stability in DES)



### Experimental approach

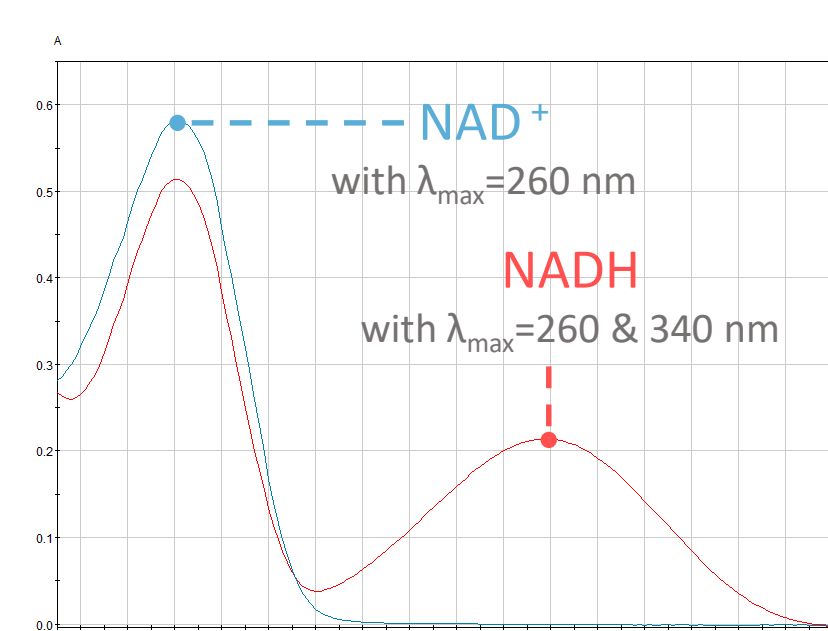


Figure 1. Absorption spectra of NAD<sup>+</sup> and NADH

### Computational approach

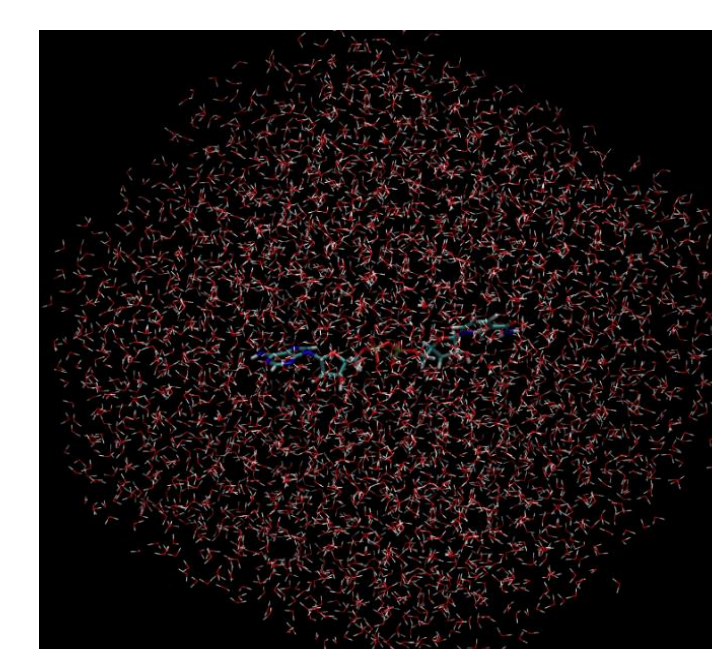


Figure 2. Simulation box (15 Å side length)

### MD [molecular dynamics] simulations QM [quantum mechanics] calculations

Defining simulation systems and simulation box

Cofactor CONFORMATION analysis

Solvent-cofactor INTERACTION analysis

## 2 RESULTS

Cofactor stabilisation strongly depends on the hydrogen bond donor nature and water content. Choline-based DES with urea and ethylene glycol were identified as stabilisers of both reduced and oxidised NAD coenzyme forms to a much greater extent than reference buffers.

Further, during incubation at 4 °C ChCl:U also preserved the cofactor's biological functionality (electron transfer in reactions).

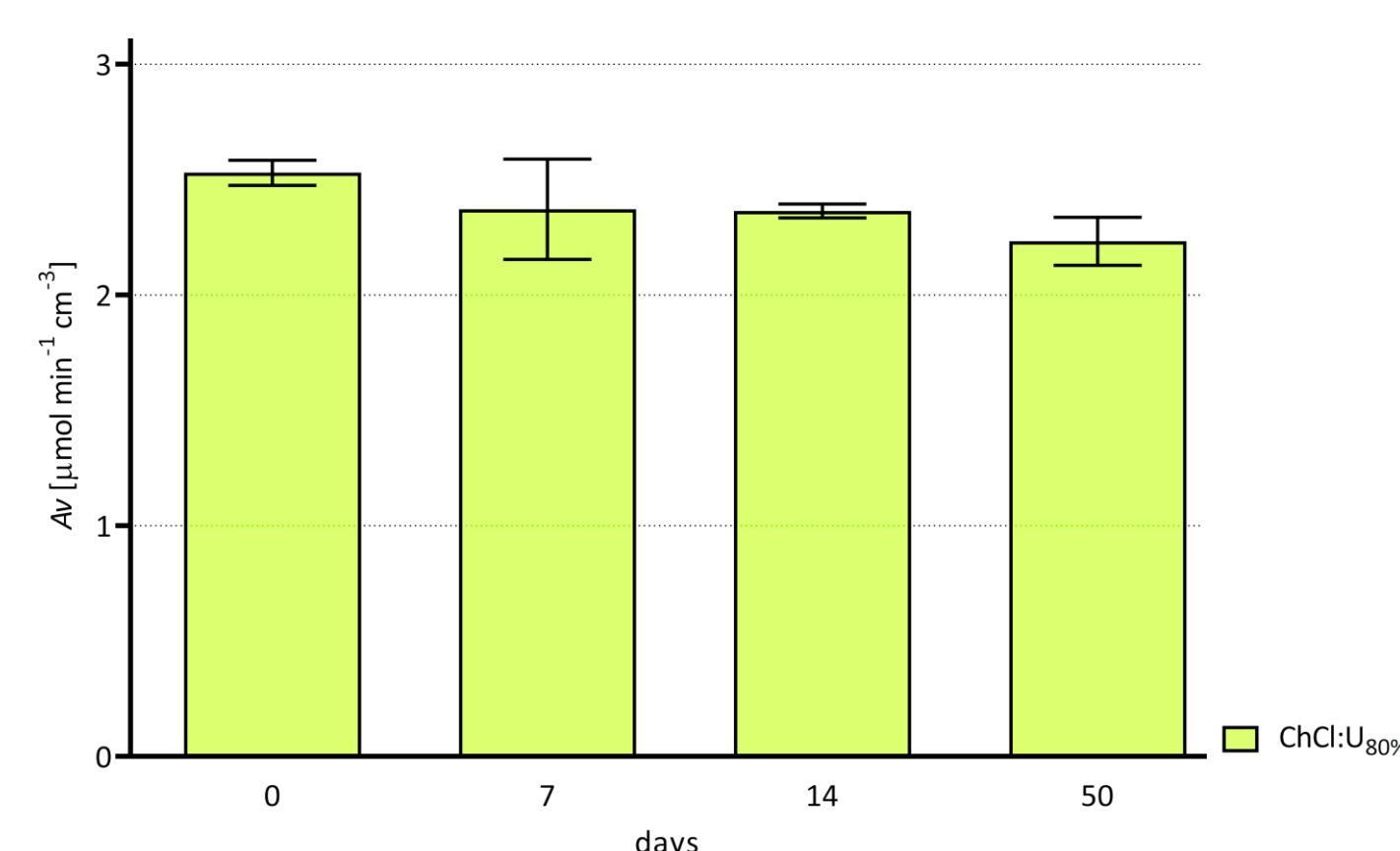
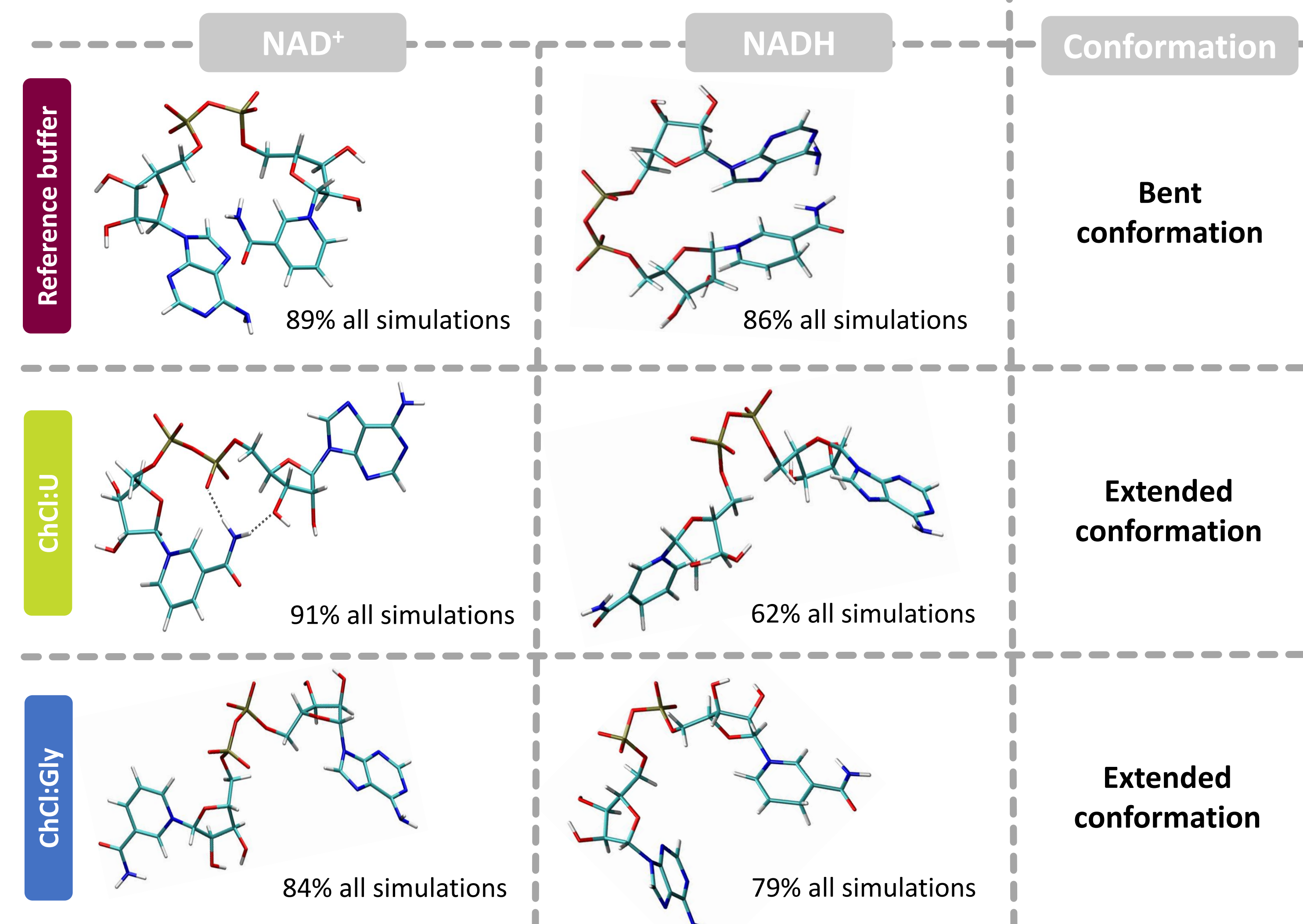


Fig. 3 Volumetric ADH activity depending on NAD<sup>+</sup> incubation time in ChCl:U<sub>80%</sub>

Presumably, observed geometric preference showed in simulations plays an important role in cofactor stabilisation.



DES components dominate in their tendency to solvate NAD<sup>+</sup> and NADH relative to water. Observed stability trends come as a result of an interplay between the component nucleophilicity, responsible for the cleavage of the phosphodiester P–O bond, and its basicity, determining the feasibility to undergo the nicotinamide–ribose N–C bond breaking, the latter being the prevailing degradation route especially for NAD<sup>+</sup>.

## 3 CONCLUSION

Nicotinamide cofactor stabilisation phenomena in DES can open a new chapter in coenzyme recycling and reuse during continuous biocatalytic reactions and most importantly, it could solve cofactor storage shortcomings.

## 4 REFERENCES

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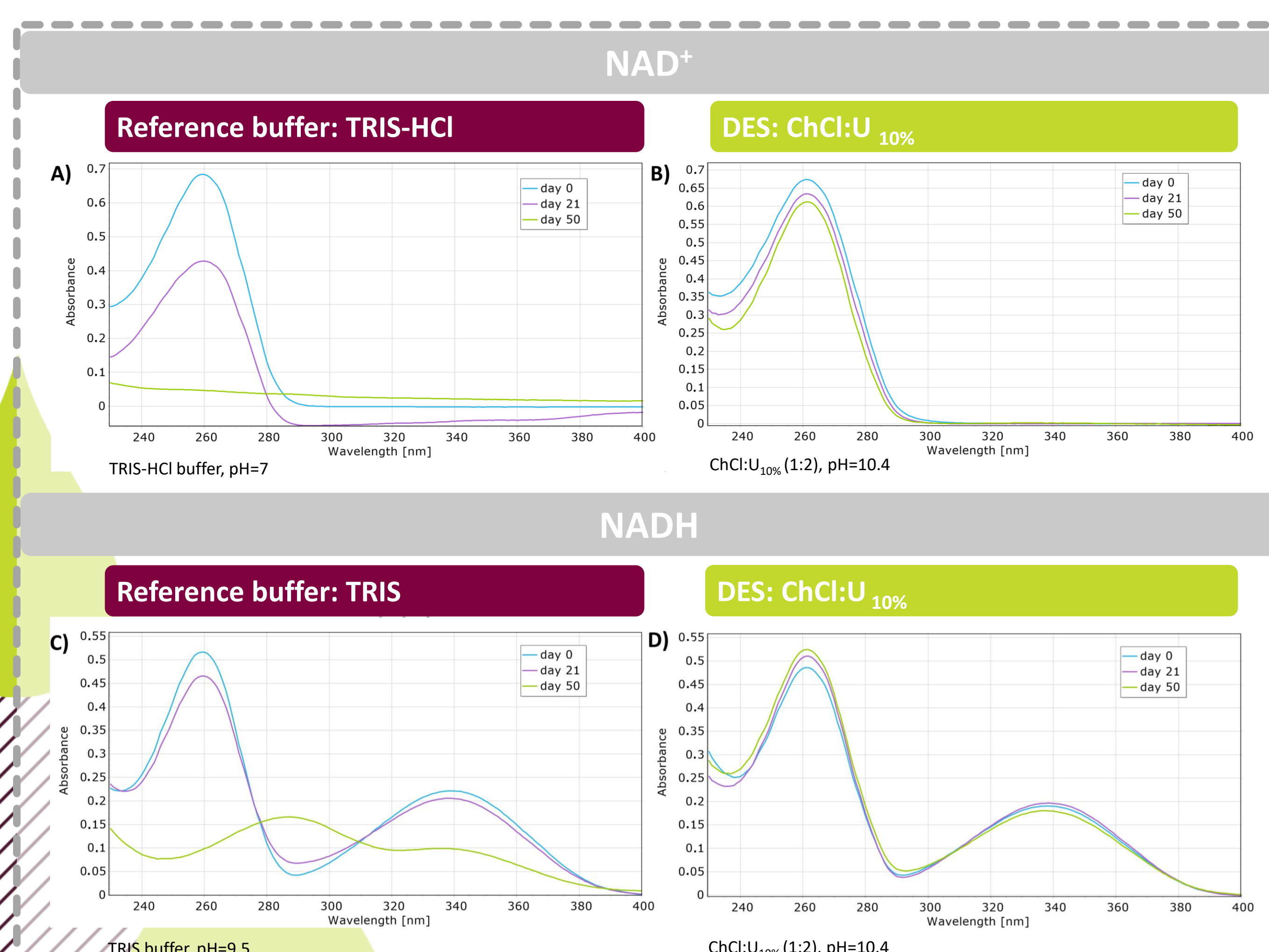


Figure 4. Nicotinamide cofactors long term stability. Absorption spectra of NAD<sup>+</sup> and NADH incubated in reference buffers and the same DES (choline-chloride:urea) for 50 days



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