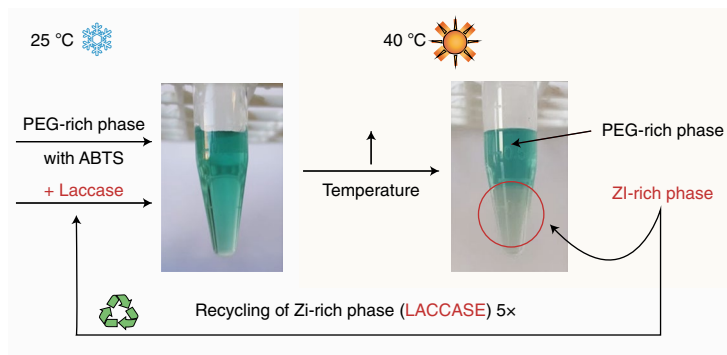


## ENZYME RECYCLING

## Small temperature changes matter

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Credit: Royal Society of Chemistry

Recycling of biocatalyst and solvent provides an opportunity to reduce operational costs in industrial chemical reactions. A common strategy to recycle enzymes is their immobilization on solid supports. However, the associated extra costs and workload — as well as possible mass transfer limitations and often-reduced catalytic activity — have hindered a wide use of this approach in industrial processes so far. Integrated reaction-separation processes provide an alternative recycling approach without the need for biocatalyst modification.

Now, a team led by Mara Freire and João Coutinho at the University of Aveiro investigated and designed aqueous biphasic systems (ABS) for bioreaction-separation processes. The designed ABS systems allow a reaction in a homogeneous medium and separation of reactants and enzyme by switching to a two-phase system induced by a small change in temperature. This process is reversible allowing the repeated use of biocatalyst and solvent.

The thermoreversible ABSs presented in this work are composed of ammonium-based zwitterions (ZIs) and polyethylene glycol (PEG) polymers in an aqueous environment. These particular systems are in general compatible with enzymes,

because their phase transition occurs at mild temperatures and their main component is water.

In a model reaction (pictured), the enzyme laccase oxidized the substrate 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) in homogeneous medium at 25 °C. Subsequently, an increase in temperature to 40 °C induced the formation of two phases resulting in the complete separation of the enzyme (ZI-rich phase) from the products (PEG-rich phase). The separation process depends on the hydrophobic and hydrophilic character of the phases and the reactants. As enzymes in general tend to migrate to hydrophilic phases, a high efficiency of this separation process can be achieved if the products are of hydrophobic nature.

Tailoring the properties of reversible mono/biphasic aqueous biphasic systems for particular biocatalytic processes is a key to achieve favourable enzyme kinetics and stability, low energy consumption, optimal separation efficiency and waste reduction.

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