Biocatalytic reduction of six-membered ring heterocyclic imines in continuous flow

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Heterocyclic amines are a key structural motif in the backbone of medicinal natural products (i.e. antibiotics) as well as pesticides and flavors. For this reason, and the lack of general synthetic routes, many efforts are still ongoing towards using more efficient alternatives [1]. In this regard, imine reductases (IREDs) have recently emerged as highly selective and sustainable biocatalysts for asymmetric reductive aminations [2]. Herein, we have tested 4 known IREDs and 2 newly characterized IREDs for the reduction of heterocyclic imines containing S, O, or N. Most of the IREDs showed the highest catalytic activity towards the newly reported sulfur-containing heterocyclic amine. Since IREDs are NADPH-dependent enzymes, a cofactor-regenerating enzyme was added to the catalytic system with the glucose dehydrogenase from *Bacillus megaterium* (BmGDH). The bi-enzymatic system achieved full conversion at a 10 mM scale of the S-containing heterocyclic imine in less than 2 h and using only 2% w/v of biocatalyst. To further improve the efficiency and sustainability of the system, we have carried out enzyme immobilization on porous microparticles (inSEIT technology). In fact, immobilization of IREDs is very challenging due to the decrease of enzyme activity upon immobilization, thus it has barely been explored until now [3]. By using CapiPy bioinformatic tools,[4] we quickly developed an immobilized biocatalyst that retains up to 90% of its initial activity. This enables the integration of the biocatalytic system into a continuous-flow reactor for process intensification. In this project, the multidisciplinary synergy between academia and industry allowed the development of a synthetic technology platform that gives access to new molecules in a green and efficient manner (Figure 1).

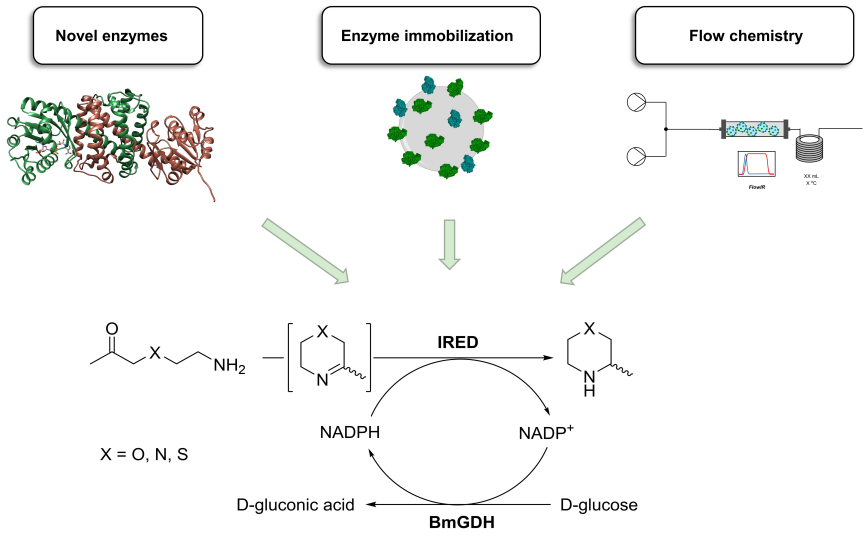


Figure 1. Enzymatic catalysis by IRED towards the synthesis of six-membered ring heterocyclic imines. On the top, the key enabling technologies for this project are highlighted. On the bottom, the reaction scheme including the cofactor recycling system is depicted.

1. Jonathan Barrios-Rivera *et al. Organic Chemistry Frontiers*, **2020**, 7, 3312-3342.
2. Amelia K. Gilio *et al. Chemical Science*, **2022**, 13, 4697-4713.
3. David Roura Padrosa *et al. Green Chemistry,* **2020,** 22, 5310- 5316.
4. David Roura Padrosa *et al. Bioinformatics*, **2021**, 37, 2761-2762.