

Sandra Bartoli¹, Alessio Stefanini¹, Filippo Rambaldi¹, Serena Mannucci¹, Francesco Corcella² and Antonella Petri³

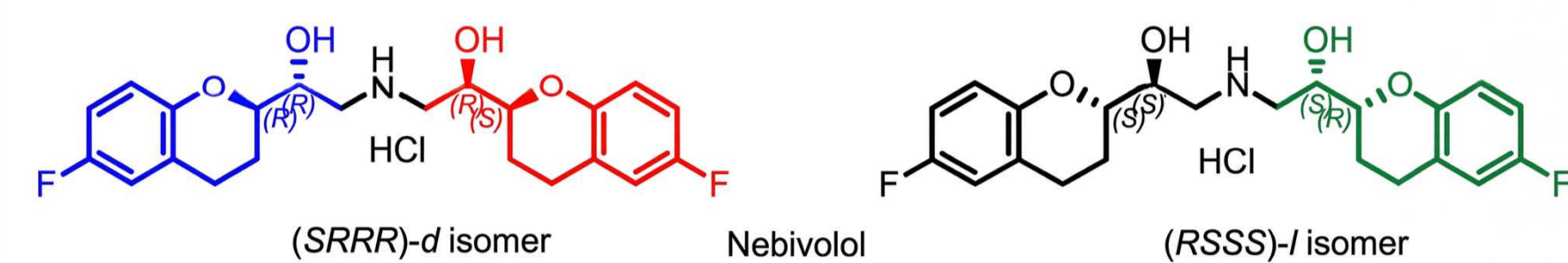
¹ Lusochimica spa, via Livornese 897, 56122 La Vettola (PI), Italy

² Lusochimica spa, via Giotto 9, 23871 Lomagna (LC), Italy

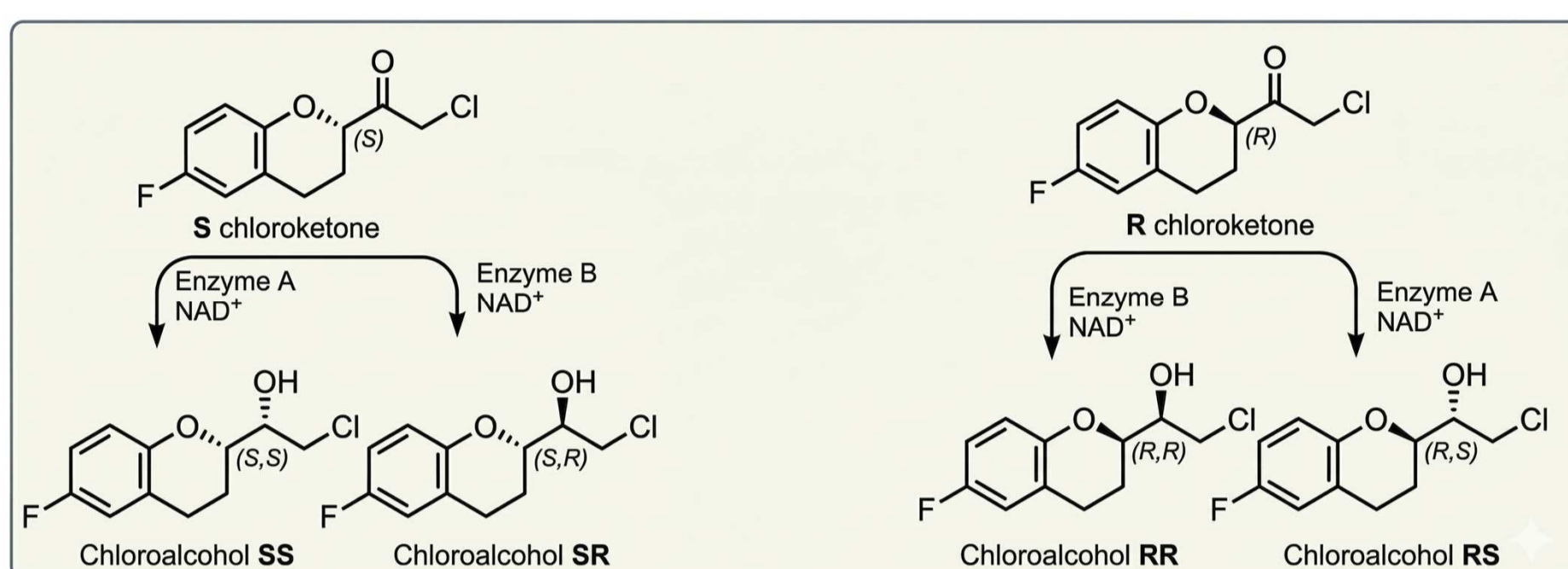
³ Department of Chemistry/Industrial Chemistry, University of Pisa, Italy

Introduction and Strategy

Nebivolol, a widely used β -blocker primarily used to treat hypertension and heart failure, is a complex molecule with four stereocenters.



The Challenge: A key step involves the enzymatic reduction of two highly enantiopure chloroketones into four corresponding diastereomeric chloroalcohols which has been proven efficiently catalyzed by two complementarily selective ketoreductases (KREDs).



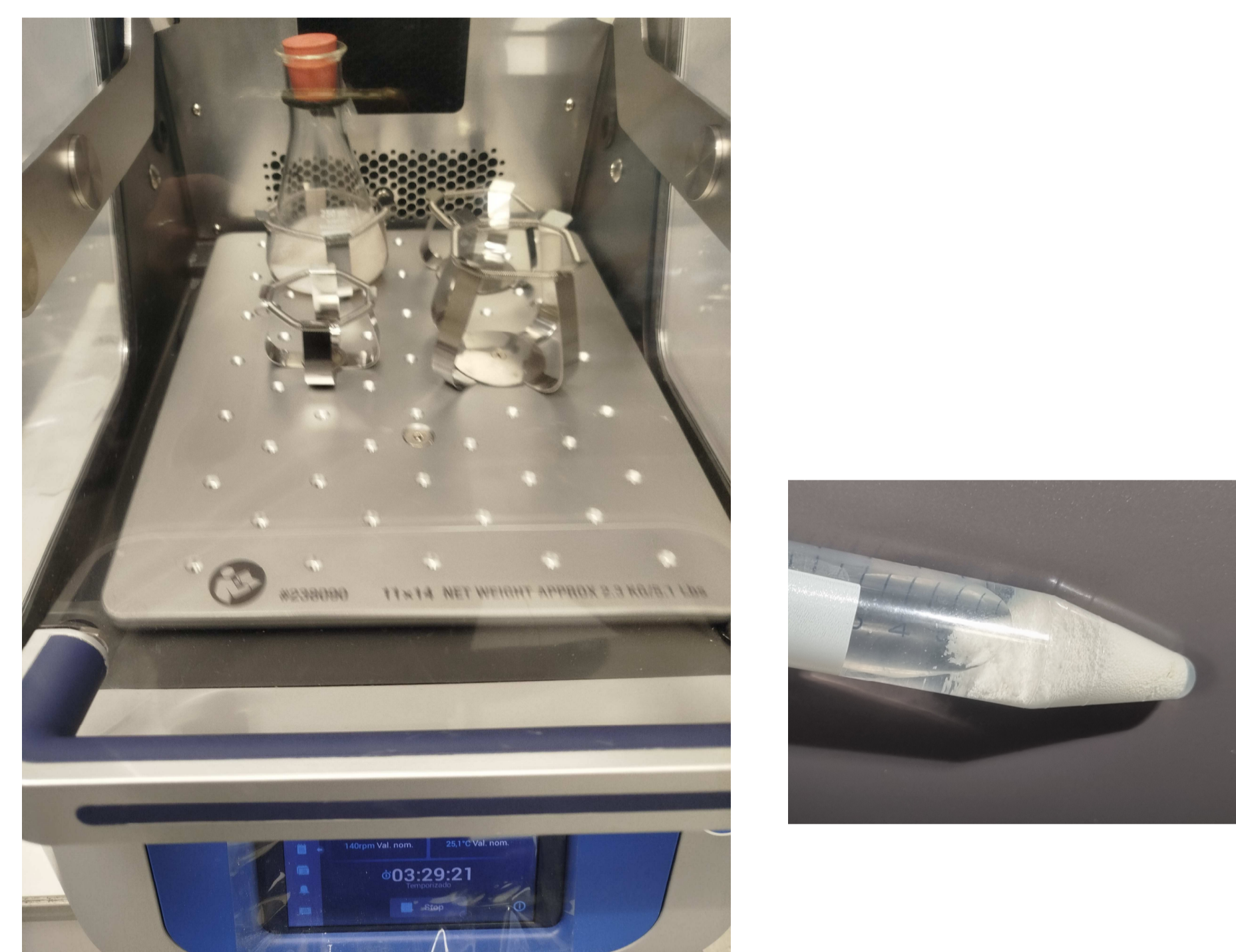
Optimization

Immobilization:

pH: Initial tests at pH 7.0 showed moderate loading. By increasing both pH and buffer concentration, a loading efficiency of ~90% was achieved

Enzyme Loading: While theoretical loads of 50 mg/g were tested, enzyme specific activity was achieved with an optimized enzyme concentration of 5.0 mg/mL during the immobilization phase.

Resin capping: the resin was tested with or without capping with glycine: no difference was noted (see Figure below).

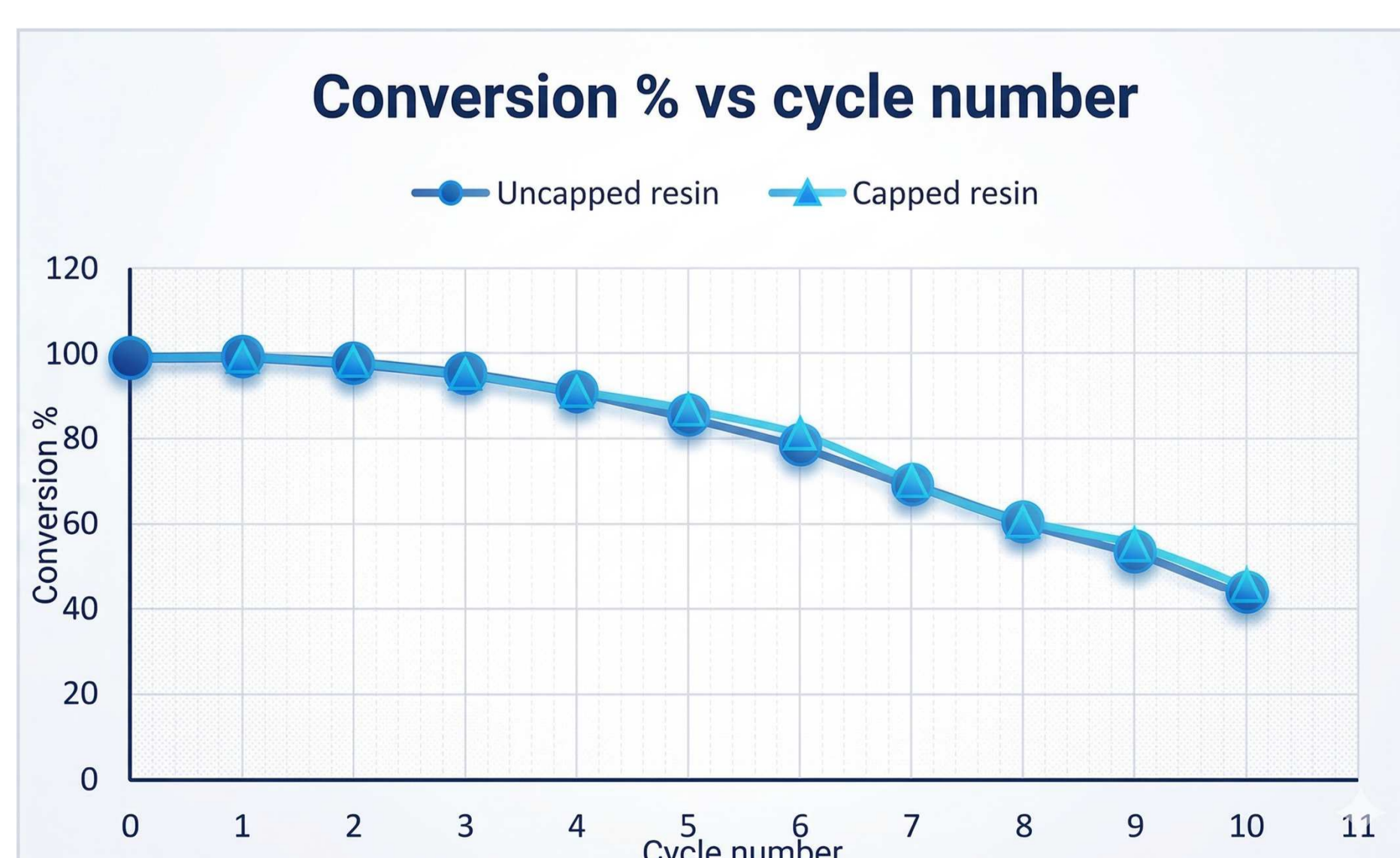


Reduction:

Conversion and Reaction Time: Under optimized batch conditions full conversion was consistently achieved within 1–6 hours on 10 cycles. Tests with or without glycine capping showed no significant difference in performance.

The system was tested in 30 min subsequent cycles. A gradual decrease in catalytic activity was observed as the number of recycles increased. Despite the progressive loss of activity, the system remained functional through the 10th cycle, still delivering a notable conversion of approximately 45% over 30 minutes.

Stereoselectivity: The immobilization process and the recycles did not alter the enzymes' intrinsic selectivity with HPLC analysis confirming a diastereomeric excess (d.e.) of $\geq 99.7\%$ on all cycles.



Main goals and objectives

Enzyme Optimization: Successfully immobilize biocatalysts while maintaining high activity and selectivity.

Process Innovation: Achieve efficient enzyme immobilization on solid supports to transition from batch to continuous flow systems

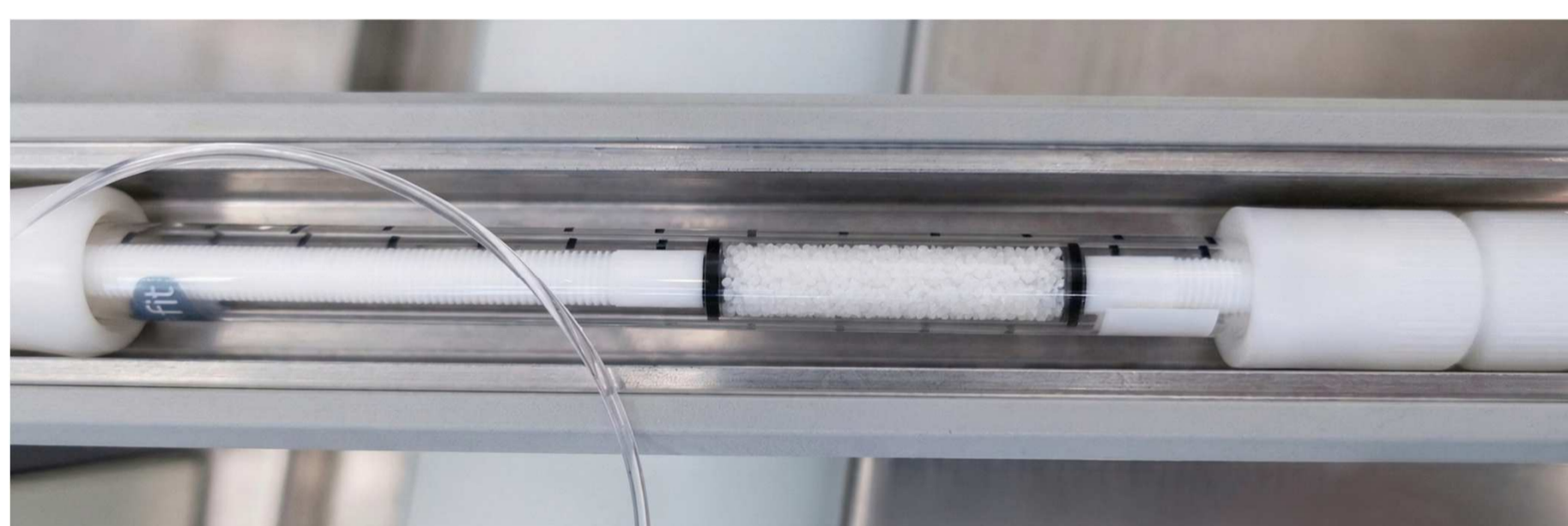
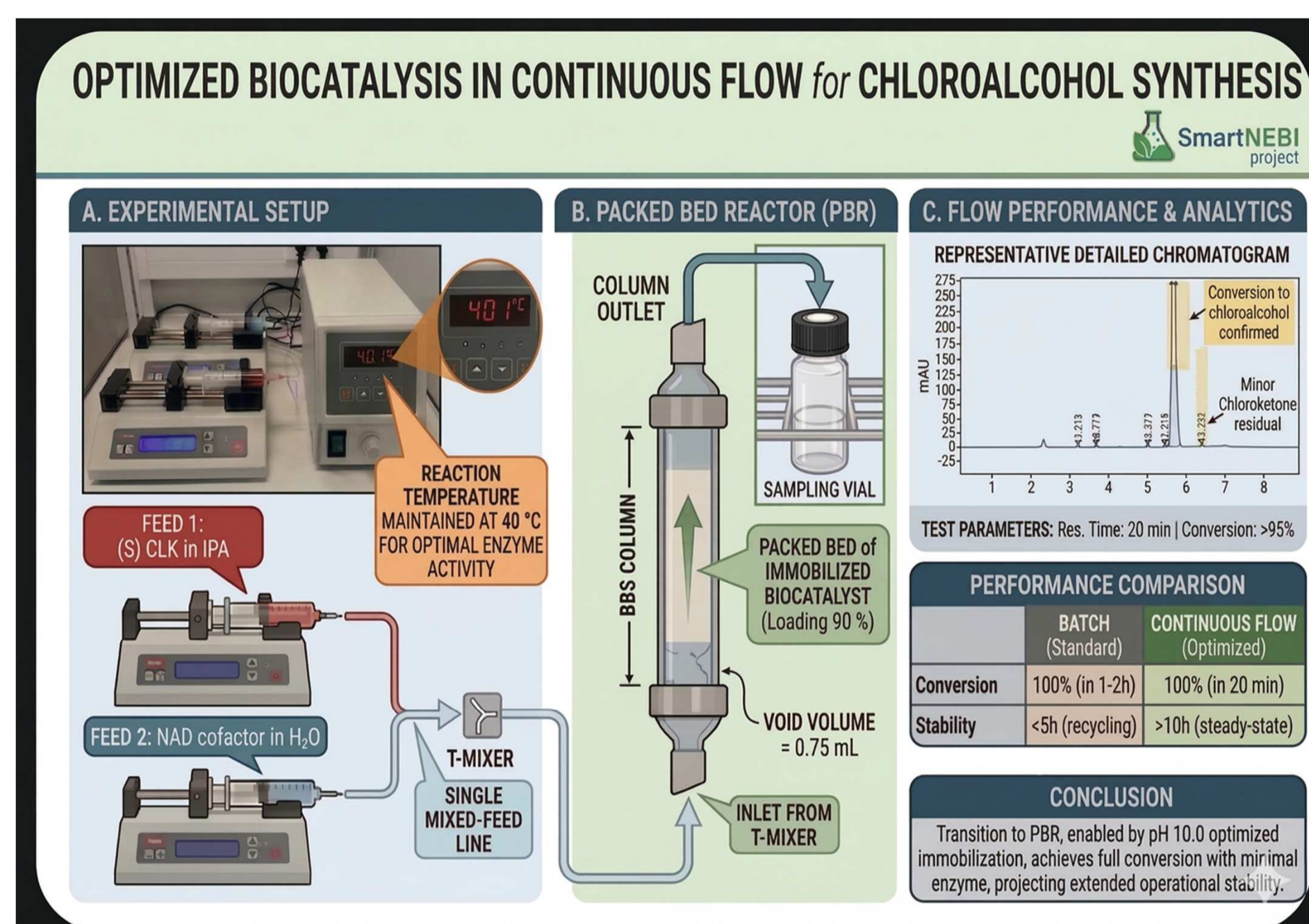
Sustainability: Enhance environmental impact by reducing waste, lowering overall production costs and enabling enzyme recycling.

Transition in flow

Stress Reduction: Unlike batch processes, a Packed Bed Reactor (PBR) should eliminate mechanical shear (agitation/filtration) and concentration shocks.

Steady-State Environment: The biocatalyst operates in a gentle environment, enhancing operational stability.

Based on optimized batch resilience, we aim to be able to achieve several hour of continuous operation at 100% conversion by adjusting slightly, if necessary, the residence time.



References

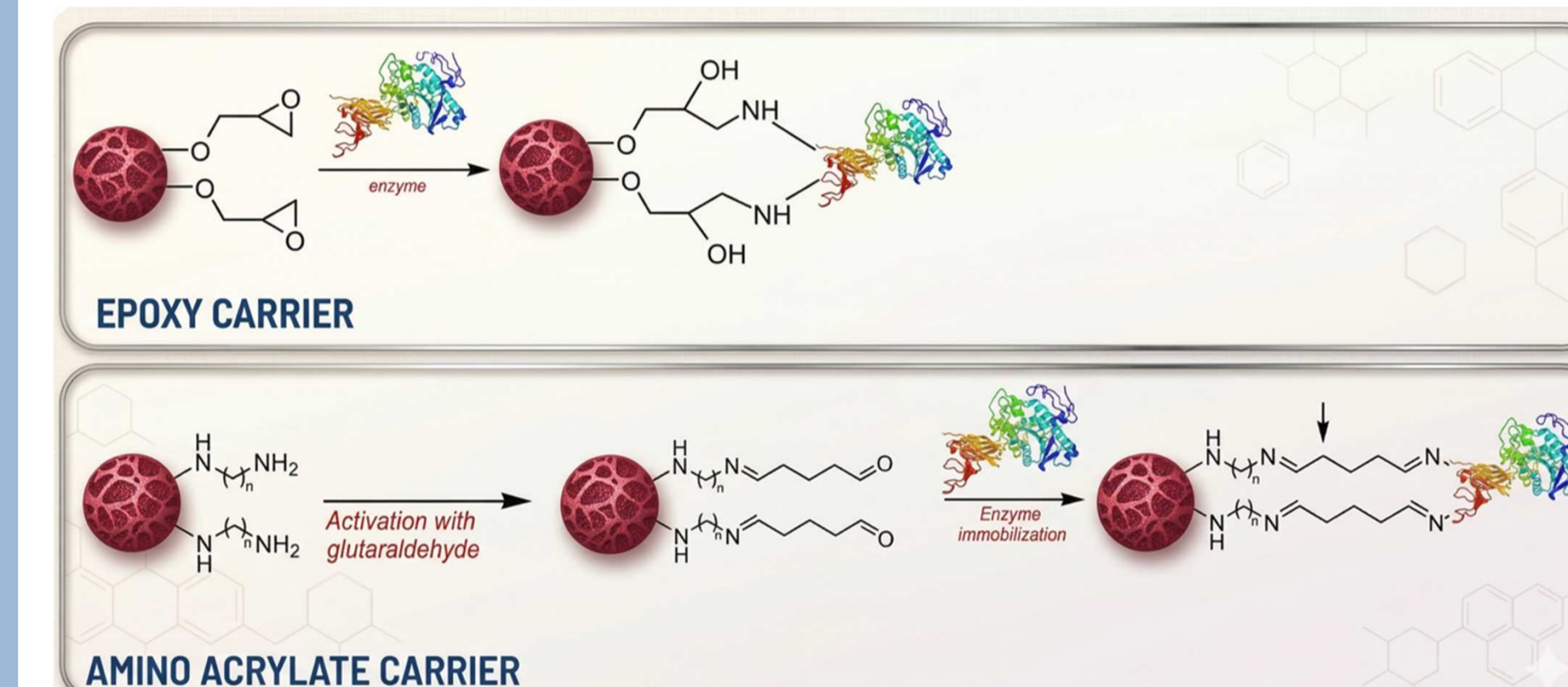
- On Nebivolol synthesis:**
Sandra Bartoli, Serena Mannucci, Alessio Griselli; Alessio Stefanini «Process for the synthesis of intermediates of Nebivolol», EP3405467.
- On covalent and ADH Immobilization:**
Hongmei Li, Johannah Moncecchi, Matthew D. Truppo «Development of an Improved Immobilized Ketoreductase for the Enantioselective Reduction of Ketones» *Org. Process Res. Dev.* 2015, 19, 7, 695–700;
Theja Prabhakar, Jacopo Giaretta, Riccardo Zulli, Ronil J. Rath, Syamak Farajikhan, «Covalent immobilization: A review from an enzyme perspective» *Chem. Eng. J.* 2025, 158054–158072.
- On Flow Biocatalysis:**
Jing Bai, Yunting Liu, Xiaobing Zheng, Jianqiao Liu, Liya Zhou, Jinlong Liu, Yanjun Jiang «Integrating biocatalysis with continuous flow: current status, challenges, and future perspectives» *Journal of Advanced Research* 2026, 613–656;
Thompson, M. P., Itziar Peñafiel, Sebastian C. Cosgrove, Nicholas J. Turner «Biocatalysis Using Immobilized Enzymes in Continuous Flow for the Synthesis of Fine Chemicals» *Org. Process Res. Dev.* 2019, 23, 1, 9–18;
D. Armani, O. Piccolo, A. Petri «Biocatalytic asymmetric reduction of (S)-1-[3,5-bis(trifluoromethyl)phenyl]ethanol by an immobilized KRED in Batch and Flow Conditions» *ChemCatChem* 2023, e202300809
Micol Santi, Luca Sancineto, Vanessa Nascimento, Juliano Braun Azeredo, Erika V. M. Orozco, Leandro H. Andrade, Harald Gröger and Claudio Santi «Flow Biocatalysis: A Challenging Alternative for the Synthesis of APIs and Natural Compounds» *Int. J. Mol. Sci.* 2021, 22, 990-1021.
Tamborini, L.; Fernandes, P.; Paradisi, F.; Molinari, F. «Flow Bioreactors as Complementary Tools for Biocatalytic Process Intensification» *Trends Biotechnol.* 2018, 36, 73–88

Methodology: Biocatalysis & Immobilization

Immobilization Strategy:

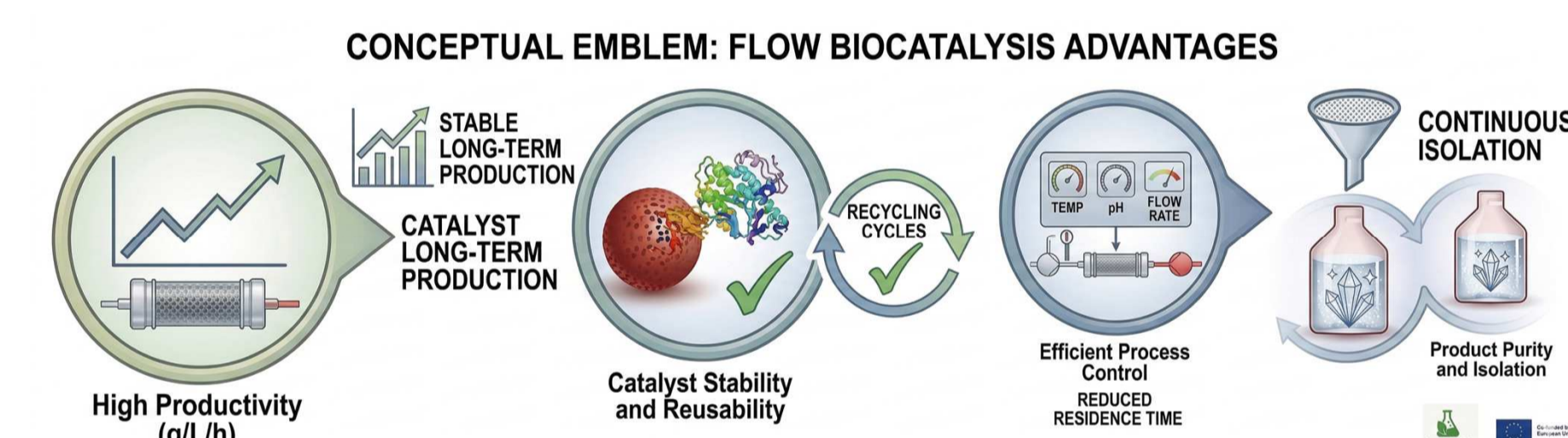
Support selection: Covalent attachment of KREDs onto solid supports using two differently functionalized resins: Epoxy and Amino Acrylate

Enzyme Selection: Identified two complementarily selective (*R* and *S*) ketoreductases (KREDs) through extensive screening. Through an initial screening of several ketoreductases (KREDs), two specific candidates were selected for their high activity and complementary diastereoselectivity.

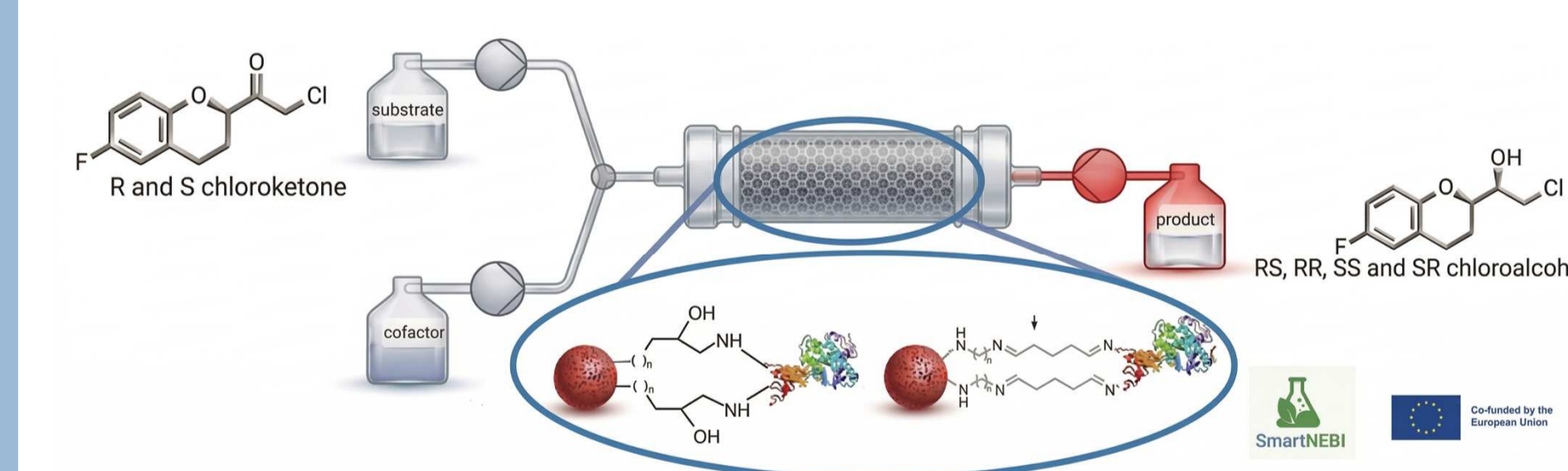


Conclusions and future outlook

The optimized immobilization protocol on acrylic supports provides a robust foundation for enantiopure Nebivolol synthesis. The transition to continuous flow represents a significant leap in productivity and process intensification.



Future Work: Focus on long-term industrial durability and on further optimization of immobilization and flow parameters.



Acknowledgements

The authors wish to express their sincere gratitude to Roberto Falorni, Daniela Fattori, Elisabetta Falchi, Gianluca Spagnolo, Barbara Svabikova, Piergiuliano Bugada, the entire Lusochimica R&D Group and Lusochimica Pisa for their contribution to the success of the project.

Special thanks to the European Commission for the SmartNEBI project recognition and funding.

Contact Information

Sandra Bartoli

sbartoli@lusochimica.it

Alessio Stefanini

astefanini@lusochimica.it