Multi-enzymatic cascade lignin nanoreactors are privileged scaffolds for the one-pot synthesis of bioactive compounds.

E. Capecchi, E. Tomaino, V. Ubertini, D. Piccinino and R. Saladino[a]

[a]Department of Biological and Ecological Sciences, University of Tuscia, 01100 Viterbo, Italy. Correspondence: e.capecchi@unitus.it

Lignin-based nanoreactors are efficient tools for the transformation of organics in wastes from pulp and paper and bio-refinery industries into high-value bioactive compounds. These renewable and low-cost nanoplatforms exhibit remarkable physicochemical and biological properties, including enhanced electron-transfer behaviours as consequence of optimal supramolecular organization of the aromatic subunits in the polymer. [1] Recently, we reported that lignin electroactive nanoplatforms efficiently improve the activity and stability of redox enzymes even in the case of multi-enzymatic cascades, in which the product of the first transformation works as a substrate for the next-one. In this latter case, the oriented immobilization of enzymes was obtained using Concanavalin A (Con A) as spatial orienting agent able to selectively bind the carbohydrate region of glycoenzymes, thus favouring the correct spatial correlation between the active sites. Example of application of this strategy for both oxidase/peroxidase and lipase/oxidoreductases cascade systems are described. The oxidase/peroxidase lignin-based nanoreactor showed high catalytic activity and reusability in the synthesis of gluconolactone and polydopamine under favourable heterogeneous conditions. [2] Similarly, lipase/ oxidoreductases lignin-based nanoreactor demonstrates substrate channelling effects in the one-pot synthesis of bioactive long-chain esters of hydroxy-tyrosol without the use of extra reducing agents, simplifying purification and work-up procedures and avoiding oxidoreductase inhibition. [3,4] We describe here the possibility to combine multi-component chemistry and lipase/oxidoreductases lignin-based nanoreactors in a unique reactive framework to yield privileged scaffolds useful in the synthesis of bioactive compounds, such as benzoxazines and flavonoids. Highly functionalized benzoxazines were synthesized through *in-situ* generation of electrophilic quinone intermediates from appropriate tyrosol esters by cascade lipase and oxidoreductase catalysis, followed by internal nucleophilic 1,6-Michael addition and subsequent intramolecular lactonization and aromatization processes. Likewise, the preparation of flavonoids required unexpected aldolase-like activity by lipase in one-pot intramolecular oxa-Michael addition between phenols from agro-industrial wastes and carbonyl derivatives. The desired products were obtained from moderate to high yield under mild reaction conditions and excellent atom economy and chemo-selectivity conditions. This approach extended the application of multienzymes cascade reaction and multicomponent chemistry in a unique chemical framework providing new entry for sustainable organic synthesis.

[1] Piccinino, D.; Capecchi, E.; Tomaino, E.; Gabellone, S.; Gigli, V.; Avitabile, D.; Saladino, R. Nano-Structured Lignin as Green Antioxidant and UV Shielding Ingredient for Sunscreen Applications. Antioxidants 2021, 10, 274. https://doi.org/10.3390/antiox10020274.

[2] Capecchi, E.; Piccinino, D.; Tomaino E.; Bizzarri B.M.; Polli F.; Antiochia, R.; Saladino R. Lignin nanoparticles are renewable and functional platforms for the concanavalin a oriented immobilization of glucose oxidase–peroxidase in cascade bio-sensing. RSC Adv., 2020, 10, 29031-29042. https://doi.org/10.1039/D0RA04485G.

[3] Tomaino, E.; Capecchi, E.; Piccinino, E.; Saladino, R. Lignin Nanoparticles Support Lipase-Tyrosinase Enzymatic Cascade in the Synthesis of Lipophilic Hydroxytyrosol Ester Derivatives. ChemCatChem 2022,14, e2022003. https://doi.org/10.1002/cctc.202200380.

[4] Piccinino, D.; Ubertini, V.; Capecchi, E.; Tomaino, E.; Gigli, V.; Saladino R. Synthesis of Bioactive Hydroxytyrosol Esters via Multienzyme Cascade on Electroactive Melanin Lignin Nanoparticles: A One-Pot Approach without Extra Reducing Agents. ChemCatChem 2023, 15, e202300533. https://doi.org/10.1002/cctc.202300533.