Thermodynamics vs kinetics of enzymatic activity: DSC investigation on an enantioselective *Bacillus coagulans* carboxylesterase

Francesca Saitta¹, Pietro Cannazza¹, Silvia Donzella¹, Valerio De Vitis¹, Marco Signorelli¹, Diego Romano¹, Francesco Molinari¹, <u>Dimitrios Fessas¹</u>

Università degli Studi di Milano, Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Via Celoria 2, 20133, Milano, Italy, dimitrios.fessas@unimi.it

Keywords: Carboxylesterases, pH influence, Thermodynamic analysis; DSC.

Microbial carboxylesterases are valuable biocatalysts that can selectively hydrolyze a wide spectrum of esters and may find their own applicability at industrial scale.

Among others, a carboxylesterase from *Bacillus coagulans* (BCE) was previously isolated and characterized from a biochemical and structural point of view for its industrial appealing peculiarity to enantioselectively hydrolyze different esters of the chiral alcohol 1,2-O-isopropylideneglycerol (IPG) [1], whose enantiomers are valuable chiral building blocks for the synthesis of β -blockers, prostaglandins and glycerophospholipids [2].

In this study, the influence of different pH conditions (from pH 6 to 9) on BCE thermal stability was investigated through Differential Scanning Calorimetry (DSC). The thermodynamic exploitation of these data permitted to obtain the $\Delta G^{\circ}(T)$ function, that is correlated with the protein functionality as concerns the thermodynamic standpoint, and the correlation with measurements of enzymatic specific activity allowed to dissect the determinants that dictate the BCE functionality in view of an industrial process design. The results reported by this study may be applicable as guidelines to assess enzyme optimal working conditions through calorimetric methods [3].

References

- [1] V. De Vitis, C. Nakhnoukh, A. Pinto, et al. FEBS J., 285 (2018) 903
- [2] J. Jurczac, S. Pikul, T. Bauer, Tetrahedron Asymmetry, 42 (1986) 447
- [3] F. Saitta, P. Cannazza, S. Donzella, et al., Thermochim. Acta, In press (2022)