Exploring electric fields towards enhanced control of protein crystallization

F. Castro[1,2*], R. Pereira[1,2*], I. Gomes[3*], A. Soares[3*] and J.A. Teixeira[1,2]

[1]CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710-057 Braga, Portugal, [2]LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal, [3]University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

filipa.castro@ceb.uminho.pt

**contributed equally to the present work*

Advances in protein crystallization methods are attracting increasing attention from formulation scientists, expanding protein delivery applications beyond purification and structural analysis [1]. Electric fields have emerged as a green processing technology, offering a scalable and energy-efficient approach that enables precise control over protein architecture, thus supporting the development of functional systems and biotechnological applications [2]. This study aims to develop a comprehensive understanding of how electrical protocols can be tailored to modulate the chemical environment during crystallization process, including induction time, crystal size distribution (CSD), orientation, and the selective recovery of crystalline compounds from multicomponent solutions. Electric fields of varying intensities (0.1 V/cm–2 kV/cm), electrical frequencies (50 Hz to 20 kHz), and waveforms (sinusoidal and pulsed) were designed and applied to solutions of pure β-lactoglobulin (BLG) at room temperature (~22 °C). Following electrical treatment, BLG solutions were subjected to controlled crystallization conditions (Figure 1). For this, microbatch crystallization experiments were carried out by mixing with 1:1 volume ratio a treated BLG solution (2.5 to 0.75 mg/mL) with a zinc chloride solution (0.1 mg/mL) under fixed temperature and pH. The crystallization experiments were monitored on-line by turbidity measurements (induction time) [3] and off-line by optical microscopy (CSD) and UV-Vis spectroscopy (protein concentration). Circular dichroism spectroscopy was employed to assess potential changes in the secondary and tertiary structure of BLG. The results provided valuable insights into the mechanisms by which applied electric field protocols influence nucleation and crystal growth of BLG, as well as a systematic understanding of generalized principles that may be applied to other protein systems. In particular, the study highlights the potential of electric fields to enhance selective crystallization for purification purposes, offering a promising alternative to conventional separation / purification techniques.

Figure 1. Optical microscopy images of typical BLG crystals produced: A) X10X20; B) X10X40.

[1] Yan, E. K., Zhao F-Z, Zhang, C-Y., et al. (2018) Cryst Growth, **18** (2), pp. 1090–1100

[2] Leal, R. T., Pereira, R. S., Rodrigues, R. M. and Pereira, R. N. (2024) *Food Research International*, **196**, p. 115033

[3]. Castro, F., Ferreira, A., Teixeira, J.A. and Rocha, F. (2016) Crystal Growth and Design, **16**(7), pp. 3748-3755

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/04469 unit, CrystalWhey project 2023.11352.PEX (DOI: 10.54499/2023.11352.PEX), and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.