Title: Improving the downstream processing of interferon α-2b using alternative purification platforms based on ionic liquids

Authors: Leonor S. Castro[1], Guilherme S. Lobo[1], Patrícia Pereira[2], Márcia C. Neves[1], Mara G. Freire[1], Augusto Q. Pedro[1]

Affilitation :[1] CICECO–Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[2] University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Chemical Engineering, Rua Sílvio Lima Polo II, 3030-790 Coimbra, Portugal

Abstract (250): Improvements on human life expectancy and the lack of effective therapies has led to an increment of chronic diseases, being the application of biopharmaceuticals an efficient strategy to mitigate this scenario. Among the current available biopharmaceuticals, the role of interferon α-2b (IFNα-2b) should be highlighted, as it has been marketed over 30 years with a considerable impact on the global therapeutic proteins market (Castro *et al,* Vaccines, 2021). IFN manufacturing requires the use of the recombinant DNA technology, involving two main stages, the upstream and downstream stages. The first includes recombinant protein production in a suitable host microorganism, such as *Escherichia coli* (Castro *et al,* Sep. Purif. Technol., 2020), while the second comprises protein recovery, isolation, purification and polishing. Due to the high demands of the pharmaceutical industry for products with high purity and biological activity, the downstream stage is responsible for the majority of the production costs of biopharmaceuticals (50–90%), often including time-consuming and multi-step processes. Therefore, there is an immediate need to develop more efficient, cost-effective, and sustainable protein purification methodologies. In this work, two ionic-liquid-(IL)-based strategies were investigated for the purification of IFNα-2b recombinantly produced from *E. coli* fermentation broth, namely as adjuvants in aqueous biphasic systems or as chromatographic ligands immobilized in solid materials. Overall, the obtained results demonstrate that by tailoring IL's chemical structures, improved protein purification processes are obtained and that the secondary structure of proteins is preserved.

Acknowledgements: The authors acknowledge the funding by FEDER through COMPETE 2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES from the project "IL2BioPro" – PTDC/BII-BBF/30840/2017. This work was developed within the scope of the project CICECO – Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, and CEMMPRE project UID/EMS/00285/2020, financed by national funds through FCT/MCTES and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. Márcia C. Neves acknowledges the research contract CEECIND/00383/2017, and Leonor S. Castro acknowledges FCT for her Ph.D. grant 2020/05090/BD.