Project ASYMMETRIC - Supervisor: Federico Herrera, BioISI | Co-supervisor: Veit Schwämmle (University of Southern Denmark)

Title: Asymmetric post-translational modifications (PTMs) as a new regulatory mechanism in self-associating signaling proteins

Objectives: Identify the possible existence and functional consequences of PTMs occurring in only one monomer within homodimers of the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3), and use it as a proof of concept for new regulatory mechanisms in other self-associating proteins.

Methodology: Protein self-association in homodimers and oligomers is very common in nature, playing key roles in both physiological and pathological conditions. Protein homodimers frequently display some structural symmetry and are generally assumed to be formed by identical molecules, not only in terms of amino acid composition, but also in terms of PTMs. This is partly due to the extreme technical difficulties to determine directly non-genetic differences between two monomers within a particular homodimer, such as PTMs. However, a perfect symmetry is very unlikely considering the high number and dynamic nature of the different PTM proteoforms that can co-exist at any given time and for the same protein (i.e. the proteoform stoichiometry). We have recently reported that asymmetrically phosphorylated huntingtin homodimers/oligomers showed a distinct aggregation pattern, with implications for Huntington´s disease; and that the intracellular distribution of STAT3 homodimers changed strikingly when specific PTMs could not occur on only one of the monomers. Based on these results, we launched the hypothesis that PTM asymmetry could constitute a new level of functional regulation for self-associating proteins. To challenge this hypothesis, the student will study the putative role of asymmetric PTMs on the behaviour and function of STAT3 homodimers by means of a multidisciplinary combination of **advanced bioimaging methods in living cells, proteomics and bioinformatics**. We have created a cell model to visualize STAT3 homodimers in living cells. In the first stage, the student will systematically create PTM mutants for the 80 PTMs described for STAT3 by site-directed mutagenesis in this model. By means of immunoprecipitation with STAT3 and GFP antibodies, protein extracts will be enriched in STAT3 proteoforms and subsequently analysed by mass spectrometry to determine the stoichiometry of STAT3 PTM proteoforms and homodimers under different biological conditions, such as stimulation with cytokines. Proteomics data will be then analysed by bioinformatics tools developed by Dr. Schwämmle during a 6-12 month stay in his laboratory. We expect to bring new know-how to BioISI in the analysis of PTM interplay. This project is the basis of my last application to FCT grants, two current projects within FCUL MSc programmes and 4 MSc theses and 1 PhD thesis in my laboratory. We are also gathering an international team and preliminary results to apply for next ERC synergy, Pathfinder and Human Frontiers grants (end of 2021) with an advanced version of this project.

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Type of fellowship
☐ National
☒Mixed (Portugal and abroad: University of Southern Denmark)