BioISI - Biosystems & Integrative Sciences Institute

Title: Restoring truncated protein function by protein complementation in rare neurodegenerative disorders

Place of work/: Cell Structure and Dynamics Laboratory, Dep. Quimica e Bioquimica, Faculdade de Ciências, Universidade de Lisboa.

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Abstract / MSc thesis project proposal

Protein-truncating mutations are frequently a cause of human disorders. ARSACS and SPAX8 are two rare ataxias diagnosed in new-borns caused by nonsense and frameshift mutations that lead to truncated, dysfunctional sacsin and NKX6-2 proteins. We are currently studying a phenomenon known as Protein Complementation (PC), which in theory could restore the function of truncated proteins like sacsin or NKX6-2. PC is a property observed in many proteins, such as ubiquitin, the green fluorescent protein family, thymidine kinase or luciferase. These proteins can be split in two or more fragments that are not functional, but that recover their function when they are brought back together by non-covalent bonds. The aim of this project is to establish whether the function of truncated forms of sacsin or NKX6-2 found in patients could be restored by PC with the fragments that are missing. We will design constructs carrying the truncated forms of the gene or the missing parts of the gene for each mutation. We will combine them in cell lines without endogenous sacsin or NKX6-2 and compare their behaviour and function with full length proteins. We expect these experiments to be a Proof-of-Concept for future design of gene therapy and other therapeutic approaches for NKX6-2-related ataxias.

The student will learn advanced molecular and cellular biology methods, including cloning, site-directed mutagenesis, western blotting, luciferase assays, mammalian cell cultures, flow cytometry and fluorescence microscopy, among others. He will also get training on soft skills related to scientific research. He will present his advances regularly in lab meetings, and discuss his results in individual meetings with his supervisor. We intend to encourage him to present his work in at least one national conference, as a poster communication, and he will probably present his work in internal BioISI seminars. At FCUL, there are regular seminars in various fields related to biochemistry of health and disease, and he will be strongly encouraged to attend periodically. Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.