Mechanisms of protein dysfunction in mitochondrial leukodystrophies – studies on glutamyl-tRNA synthetase (EARS2)

Place of work: Protein Misfolding and Amyloids in Biomedicine laboratory (Lab 8.5.56),
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MSc Research Plan

Mitochondrial aminoacyl-tRNA synthetase-related neurological diseases are a heterogeneous group of rare mitochondrial disorders (MDs), that lead to a wide diversity of phenotypes commonly affecting mitochondrial morphology and bioenergetics. Among them are the rare leukodystrophies, causing abnormal development or destruction of the white matter of the brain, such as LBTL (leukoencephalopathy with thalamus and brain stem involvement and high lactate) caused by mutations on glutamyl-tRNA synthetase (EARS2).

In the recent years over 30 mutations on EARS2 gene have been identified, however the molecular basis for leukodystrophies associated to EARS2 mutations remains to be established. It has been suggested that the location of missense mutations in certain domains might influence disease phenotype, but the structure of these enzyme remains poorly characterized which is a major gap towards effective therapies. Here, we propose to investigate the molecular pathophysiology associated to defects on glutamyl-tRNA synthetase by comprehensively characterize EARS2, wild-type and variants associated to LBTL, using biochemical and biophysical methodologies.

Specifically, this work will involve the following tasks:

- Optimization of recombinant protein expression (EARS2 disease-related variants, EARS2-p. Gly110Ser, EARS2-p. Asp349Asn and EARS2-p. Arg489Gln);
- Purification EARS2 variants using a combination of chromatographic methodologies (his-tag affinity or ion exchange columns and gel filtration columns);
- Characterize the variants regarding structure and conformation stability using circular dichroism (CD), Fourier Transform Infrared Spectroscopy (FTIR), fluorescence spectroscopy;
- Establish protocols for EARS2 enzymatic activity determination.

Research at the Protein Misfolding and Amyloids in Biomedicine laboratory takes place in a highly multidisciplinary and collaborative environment, at the national and international levels. We seek candidates which are highly motivated to tackle a challenging research activity, ability to work independently, and to undertake intensive learning and training in multiple methodologies, with an excellent academic track record and communication skills.

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).