ANNEX I

VERÃO COM CIÊNCIA 2022 | BioISI Internship Projects

BioISI's research encompasses the collaborative work of scientists from different areas and is implemented along 5 thematic lines: Biomedicine, Biotechnology, Biological Chemistry, Computational Biology and Biophysics.

These are the projects available for trainees in a computational approach, which represent four of the five BioISI's thematic lines, namely, Biomedicine, Computational Biology, Biophysics and Biotechnology[1]:

VC22_1) A natural polyphenol as a promising compound for cancer treatment | Supervisor: Bruno L. Victor (blvictor@fc.ul.pt)| 1 trainee | Aquaporins (AQPs) are transmembrane protein channels essential in fluid homeostasis and the body's energy metabolism that facilitate the transport of water, glycerol, and small solutes. However, AQP1 and AQP3 appear aberrantly expressed in a wide range of clinical disorders including cancer and therefore their inhibition in tumor cells has been suggested as a novel therapeutic strategy. Most AQPs modulators reported so far are non-selective and toxic, which makes them difficult to be used in in vivo experiments. Using an innovative computational approach, we were able to identify a polyphenol compound with proven drug-like properties, which strongly inhibits AQP3 glycerol permeability (IC50 = 6.02 ± 0.13 µM) and significantly reduces AQP1 water permeability (IC50 = 22.83 ± 0.01 µM). Considering the role of these proteins in tumorigenesis, our goal is to use molecular docking and molecular dynamics simulations to understand the structural details regulating the observed inhibition, and further boost the development of other derivatives with improved selective inhibition of AQP1 and AQP3.

VC22_2) An up-to-date C(pH,E)MD method for tackling heme-containing proteins | Supervisor: Paulo J. Costa (pjcosta@ciencias.ulisboa.pt) & Co-Supervisor: Miguel Machuqueiro (mamachuqueiro@fc.ul.pt)| 1 trainee | Heme and heme-resembling groups are very common cofactors in proteins such as haemoglobin, myoglobin, cytochrome P450, cytochrome c/c3, and are essential for life. The use of in silico methods capable of accurately describing and studying the redox characteristics of these systems is very scarce. The first method developed to describe redox processes at constant-pH [C(pH,E)MD] was developed in 2009 suffering the last update in 2013. Therefore, an up-to-date and free-to-use implementation of the C(pH,E)MD method would be of great value to investigate the redox effects of porphyrinic systems in chemical and biochemical processes. In this project, we will start to update, calibrate,

[1] All these topics are integrated in on-going funded research projects.

and benchmark a C(pH,E)MD method which, in the future, will be applied to increasingly complex porphyrinic systems.

VC22_3) Is the aggregation-prone intermediate of human b2m protein conserved across homologs? Supervisor: Patrícia Faísca (pffaisca@fc.ul.pt) & Co-Supervisor: Miguel Machuqueiro (mamachuqueiro@fc.ul.pt) | 1 trainee | We used molecular simulations to predict that the intermediate state, which triggers the amyloid pathway in several human variants of protein b2m (hb2m), displays a conserved core and two unstructured termini. The so-called 'termini' intermediate has been confirmed by solid-state NMR for the D76N mutant. We will investigate whether this species is conserved across b2m homologs or exclusively populated by hb2m. We will use an integrative approach that samples conformations with MC simulations and isolates the intermediate states using structural clustering. The student will be involved in the structural clustering task of murine b2m. After intermediate identification, we will posteriorly investigate their structural stability using classical MD and establish a relationship with the in vitro aggregation potential. This work will clarify the role of the 'termini' intermediate as a potential therapeutic target for b2m amyloidosis.

VC22_4) The role of protein knotting role in complex stability and enzyme catalysis | Supervisor: Miguel Machuqueiro (mamachuqueiro@fc.ul.pt) & Co-Supervisor: Patrícia Faísca (pffaisca@fc.ul.pt) | 1 trainee | Ubiquitin C-terminal hydrolases (UCHs) are papain-like cysteine proteases that hydrolyze the ubiquitin adduct, countering ubiquitination in proteins. Besides its important role in this proteolytic pathway, UCH-L1 is also highly abundant in the brain and forms one of the most complicated 3D knotted structures yet discovered. There are five crossings of the polypeptide backbone forming a '5 2' or 'Gordian' knot. We have used MD simulations to study several truncated versions of UCH-L1 in complex with its ubuquitin partner. With this protocol, we promote the unknotting process and study its impact on the UCH-L1:Ubiquitin complex stability. In this project, we will calculate the binding affinities of the different truncated complexes using an MM-PBSA methodology (PyBindE) developed in our research unit. The results will provide key evidence of the protein knotting role in complex stability and enzyme catalysis.

Additionally, for this Summer Programme, there are also experimental research topics available for trainees to get involved in scientific research. The following topics are available:

VC22_5) Molecular characterization of individuals with Clinical diagnosis of Familial Hypercholesterolemia| Supervisor: Mafalda Bourbon (mafalda.bourbon@insa.min-saude.pt) & Co-Supervisor: Ana Catarina Alves (catarina.alves@insa.min-saude.pt) | 1 trainee | To perform the Molecular diagnosis of index and relatives cases of familial hypercholesterolemia (FH) and to establish phenotype/genotype relationships using PCR and sanger sequencing.

VC22_6) Functional characterization of variants in the LDLR and APOB genes found in the Portuguese FH study | Supervisor: Mafalda Bourbon (mafalda.bourbon@insa.min-saude.pt) & Co-Supervisor: Ana Catarina Alves (catarina.alves@insa.min-saude.pt) | 1 trainee | To perform functional study of variants of uncertain significance found in genes associated of FH. To study the variants in the LDLR, a directed mutagenesis will be carried out, the plasmid will be transfected into heterologous cells (involves cell culture) and then by flow cytometry the complete cycle of the LDLR will be evaluated. To study the variants in the APOB gene, a cell line that depends on cholesterol to grow will be used, which will be supplemented with LDL from patients and the LDLR cycle will also be studied by cytometry.

VC22_7) Visualization of proteins involved in neurodegenerative diseases by biochemical and bioimaging techniques | Supervisor: Federico Herrera (fherrera@fc.ul.pt) | 3 trainees | The basic mechanisms of life and human pathologies can be studied in cells in culture, minimizing the number of animals killed in the laboratory during our investigation, and making our studies more sustainable and respectful towards living organisms. Our laboratory specializes in developing cellular models of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease or various ataxias. We study the structure and dynamics of several proteins related to these human pathologies by a combination of bioimaging and biochemical approaches. To this end, we produce our own molecular and cellular tools. Students will perform a technique for detecting some of these proteins in cells in culture so that they can be visualized under a fluorescence microscope and, in parallel, a biochemical analysis of their expression by immunoblotting techniques. We will compare the phenotypes of cells under normal conditions with cells subjected to pathological circumstances. Alternatively, students can create a new molecular tool for the study of this type of proteins by means of cloning or site-directed mutagenesis methods. At the end of the internship, students will have to present a brief report of what they have done, including the images obtained.