Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Design of membrane models mimicking the inner nuclear membrane in healthy and premature aging

Synopsis:

Aging acts as a major risk factor for many human pathologies, including cancer, neurodegenerative disorders, diabetes and cardiovascular diseases [1]. Nonetheless, information on the determinants of biological deterioration is still required if we aim at providing a better quality of life for aging individuals. In our Lab, we just started a new project focused on understanding why human cell nuclei tend to lose structural integrity as age progresses, ultimately leading to functional impairment. In other words, we aim at explaining why aged individuals are more prone to certain diseases and how does it relate with the loss of nuclear structural and mechanical properties.

In mammalian cells, the nuclear structural integrity is thought to be safeguarded by the nuclear envelope (NE), in particular the nuclear lamina (NL). The NL is mainly composed of lamin proteins that assemble into filaments and bind both heterochromatin and the inner nuclear membrane (INM) [2]. However, the role of specific INM lipids and lipid-lamin interactions in the nuclear compartment has been mainly disregarded, mostly due to the complexity of the double lipid bilayer and the technical challenges concerning the delivery of labelled lipids to the INM.

At the NSantos Lab, we are directly circumventing these issues by carefully designing simplified model systems with lipid features characteristic of the INM at different stages of healthy age progression and premature aging. INM lipids have been isolated from fibroblast cell lines prepared from healthy individuals of young and old age, and Hutchinson-Gilford Progeria Syndrome patients (premature aging). The age-related INM lipid content is now being assessed by lipidomics analysis through liquid chromatography coupled with mass spectrometry (LC-MS). In this project, the main goals will be as follows:

1. To design membrane model systems mimicking the age-tuned lipid composition of the INM (as seen by LC-MS).
2. To characterize the age-related membrane models using a set of quantitative biophysics tools.

Briefly, small, large and giant unilamellar vesicles will be prepared with age-specific lipid compositions matching the lipidomics results. The biophysical characterization of the models will then be accomplished by a multitude of techniques, including fluorescence spectroscopy and microscopy, dynamic light scattering (DLS) and atomic force microscopy (AFM)-based approaches. Upon characterization, the preparation of more complex systems including lamin proteins will also be attempted.

Ultimately, this project will allow for the first time the implementation of a free-standing nucleus-sized vesicle system (Fig.1) with age-tuned lipid composition (and possibly lamin proteins), **creating a novel platform to study nuclear aging *in vitro*.**

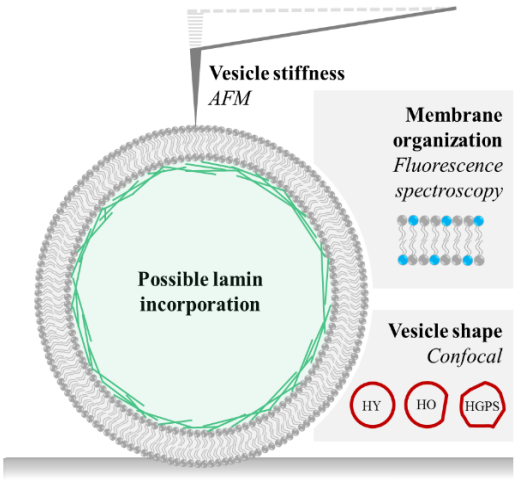

Figure 1. Characterization of lipid-tuned age-specific membrane models

Supervisor: *Maria J. Sarmento, Nuno Santos Lab, maria.sarmento@medicina.ulisboa.pt*
Co-Supervisor: *Nuno C. Santos, Nuno Santos Lab, nsantos@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

1. López-Otín C, Blasco MA, Partridge L, Serrano M & Kroemer G (2013) The hallmarks of aging. *Cell* **153**, 1194–217.
2. de Leeuw R, Gruenbaum Y & Medalia O (2018) Nuclear lamins: thin filaments with major functions. *Trends Cell Biol.* **28**, 34–45.