TITLE
The biology of mitochondria: multiple approaches to study their activity.

Overview of the course
Mitochondria are fundamental organelles required for multiple pivotal processes that include the conversion of energy, the biosynthesis of reactive oxygen species, the control of calcium homeostasis, and the triggering of cell death. The disruption of each of these processes strongly impacts the function of virtually every cell type. Indeed, mitochondrial dysfunction is associated with aging and chronic pathologies including neurodegenerative diseases, cardiovascular diseases and diabetes. In this course, we will provide an overview of mitochondrial function and structure, focusing on the key methodological approaches that are available to assess their function.

Schedule (~6 hours)
The course will include three lessons (~2 hours each), as it follows.

Lesson one (Dr. Antonella Izzo, DMMBM): April 4, Monday, 3:00 p.m.
Seminar room 4th floor Torre Biologica
"Fundamentals of mitochondrial biology. Methods for assessing mitochondrial quality control mechanisms."
The fundamental aspects of mitochondrial structure-function will be discussed, together with the tools commonly used for their evaluation. We will focus on mitophagy, the mechanism responsible for the removal of damaged mitochondria. Defective mitochondria, if not removed, can be a source of oxidative stress and contribute to compromised tissues and organs function. Mitophagy is a specialized autophagic pathway that mediates the lysosome-dependent clearance of damaged mitochondria. We will describe standard methods to monitor mitophagy in mammalian cells and the recent pH-dependent fluorescence assay, based on the mtKeima protein, which has been increasingly used to measure mitophagy *in vitro* and *in vivo*.
References:

1. Redmann M et al. "Methods for assessing mitochondrial quality control mechanisms and cellular consequences in cell culture". Redox Biol. 2018 Jul;17:59-69.
2. Sun N et al. "A fluorescence-based imaging method to measure in vitro and in vivo mitophagy using mt-Keima." Nat Protoc. 2017 Aug;12(8):1576-1587.

Lesson two (Dr. Claudio Procaccini, IEOS): April 5, Tuesday, 3:00 p.m.
Seminar room 4th floor Torre Biologica
"Methodologies for the study of intracellular metabolism."
Mitochondria play a number of vital roles in the eukaryotic cell, among which the most important is the production of ATP during oxidative phosphorylation (OXPHOS). The mechanisms of mitochondrial bioenergetics will be described as well as the experimental methodologies to measure the OXPHOS-related metabolic parameters using Flux analyzer (Seahorse Technology). Moreover, OXPHOS generates superoxide, the predominant reactive oxygen species (ROS) in mitochondria and ROS altered production has been observed in several pathological conditions. Therefore, the use of flow cytometry to measure ROS production (Mitosox) or Mitochondrial Membrane Potential (TMRE) will be also discussed, given their recent growing popularity in this field of research.
References:

3. Gu X et al. "Measurement of mitochondrial respiration in adherent cells by Seahorse XF96 Cell Mito Stress Test". STAR Protoc. 2020 Dec 30;2(1):100245.

4. Kauffman ME et al. “MitoSOX-Based Flow Cytometry for Detecting Mitochondrial ROS”. React Oxyg Species (Apex). 2016;2(5):361-370.

Lesson three (Dr. Danilo Swann Matassa, DMMBM): April 6, Wednesday, 3:00 p.m.
Seminar room 4th floor Torre Biologica
“From protein synthesis to respiration and back. The MitoRibosome profiling technique for the quantitative analysis of mitochondrial mRNA translation”.
Human respiratory complexes are encoded on both nuclear and mitochondrial DNA. In yeast cells, there is striking correlation between the cytosolic and mitochondrial translation rates of OXPHOS subunits across the respiratory complexes. Additionally, mitochondrial encoded proteins are co-translationally inserted into the inner mitochondrial membrane for the assembly of respiratory complexes, with ribosome-associated proteins assisting such processes, making biosynthetic and bioenergetic processes strictly interdependent. The human mitoribosome is unique, as it contains twice as much protein as RNA and associates with the inner membrane; for these and other reasons translation in the mitochondria is regulated by mechanisms distinct from those acting in the cytosol and in bacteria, yet precise methods for investigating it have lagged behind. A very sensitive approach has been developed recently: the mitochondrial ribosome (mitoribosome) profiling, to quantitatively monitor mitochondrial translation with high temporal and spatial resolution. Mitoribosomes are immunoprecipitated from whole-cell lysate and the protected mRNA fragments are isolated. These fragments are then converted to sequencing libraries or analyzed by Northern blot hybridization to reveal the distribution of mitoribosomes across the mitochondrial transcriptome. Application of mitoribosome profiling can reveal mechanisms of mitochondrial translational control that were not previously possible to uncover, with yet unexplored applications to metabolic diseases.
References:
5. Couvillion MT, Soto IC, Shipkovenska G, Churchman LS. Synchronized mitochondrial and cytosolic translation programs. Nature. 2016 May 26;533(7604):499-503. doi: 10.1038/nature18015.
6. Pearce SF, Cipullo M, Chung B, Brierley I, Rorbach J. Mitoribosome Profiling from Human Cell Culture: A High-Resolution View of Mitochondrial Translation. Methods Mol Biol. 2021;2192:183-196. doi: 10.1007/978-1-0716-0834-0_14.