1) Calcium, mitochondria and neurodegeneration.

Supervisor: Prof. Marisa Brini, e-mail: marisa.brini@unipd.it

Defects in mitochondrial function, endoplasmic reticulum-mitochondria crosstalk and Ca^{2+} signalling are associated with a range of neurodegenerative diseases and play a primary role in the pathogenesis of Parkinson's disease (PD). The effects of these alterations on cellular function are complex. The identification of mutations on genes that cause familial PD has improved our understanding of the pathophysiology that may occur in both mendelian and sporadic forms of PD. Our research aim is to investigate the molecular details of these aspects, focusing on role of the PD-related proteins alfa-synuclein, PINK1, Parkin and DJ-1 in mitochondrial function with a special attention to the crosstalk between signalling pathways such as those mediated by Ca^{2+} ions, cAMP and ROS.

Most of our work involves live cell fluorescence and confocal microscopy and imaging to monitor cellular parameters (intracellular Ca^{2+}, ATP, mitochondrial potential and morphology, autophagy, apoptosis etc.) using targeted probes, GFP tagged proteins, etc. A broad range of cellular, molecular and biochemical techniques are also currently employed, including the development of primary neuronal cultures from mice models.

2) New weapons in the war against bacteria resistant to antibiotics: structural and functional approaches.

Supervisor: Dr Laura Cendron, e-mail: laura.cendron@unipd.it

Aim of the research project is the discovery of new compounds able to counteract bacterial resistance and recover antibiotics efficacy. Within the routes that bacteria exploit to acquire resistance, the production of β-lactamases (BLs) represents the most widespread mechanism adopted by bacteria to contrast β-lactam antibiotics treatments. In this project, we will approach the bacterial resistance mechanisms by two lines of action: one with the aim of counteracting β-lactamases hydrolytic activity by developing β-lactamases inhibitors, the other exploring the chance to keep SOS bacterial pathways repressed by inhibiting the proteolytic activity of LexA repressor. The study will be mainly focused on the structural aspects of protein-inhibitors interaction and will be developed in collaboration with microbiologists and drug developers.

3) Assessing the integration of ROS and calcium signatures in mediating tolerance to multiple stresses in rice.

Supervisor: Prof. Fiorella Lo Schiavo, e-mail: fiorella.loschiavo@unipd.it

In our laboratory, recent advances in the understanding of salt tolerance in the Baldo rice variety highlighted the role of calcium and ROS in determining effective root-to-shoot signalling and plant resilience. Our results also suggest that calcium and ROS interplay at the level of the root tip, can promote multiple-stress resilience.
Despite the recent development of new techniques in rice, methods used so far didn't allow the observation of calcium and H2O2 waves from cell to cell, in each root layer.
This project aims to introduce genetically encoded probes for calcium (YC3.6) and H2O2 (HyPer), already available in our laboratory, in specific cell layers of the root tip in rice. Constructs with rice cell-specific promoters are available at the laboratory of Prof. J. Bailey-Serres at the Center for Plant Cell Biology (Riverside, California, USA), with which we collaborate.
The final goal is to exploit imaging techniques to determine the shape of calcium and H2O2 signatures in response to salt, drought and submergence, in order to figure out a common response to different stresses that can be used in improvement of rice resilience in a challenging environment.

4) Investigation of the role of respiration in plants metabolism.

Supervisor: Prof. Tomas Morosinotto, e-mail: tomas.morosinotto@unipd.it

Photosynthetic eukaryotes convert light into chemical energy thanks to reactions localized in an organelle called chloroplast. Respiration within mitochondria, however, plays a seminal influence on bioenergetics metabolism also in photosynthetic organisms. In unicellular algae this idea is supported by an increasing body of evidence showing the crosstalk between the two organelles and the influence of respiration also on photosynthetic reactions.
In plants the full investigation of this major biological problem has been until now limited by the inability to generate mutants, since respiratory activity is indispensable for some tissues or developmental stages. We recently developed a strategy to overcome this limitation and generate mutants with impaired respiration in the early plant *Physcomitrella patens*. These plants will allow assessing for the first time the impact of respiration inactivation in a multicellular plant.

5) Exploring the role of FIS1 in angiogenesis

Supervisor: Prof. Luca Scorrano, e-mail: luca.scorrano@unipd.it

Angiogenesis is the process of new blood vessel formation. Endothelial cells retain the ability to proliferate, and their rapid division can be induced in some pathophysiological conditions, ranging from wound healing, to diabetic retinopathy and growth and metastatic spread of tumors. Processes which involve remodelling and new tissue production, like angiogenesis, require high levels of ATP, mostly synthetized in mitochondria. Recent studies suggest that mitochondrial dynamics is relevant to endothelial cell function. For example, inhibition of mitochondrial fusion reduces angiogenic response to VEGF. We propose to investigate the contribution of mitochondrial fission protein FIS1 to angiogenic potential of endothelial cells, using two animal model systems (mouse and zebrafish) which will enable investigation of FIS1 function at the system level, in vivo. Human endothelial cells will be used to study the molecular mechanism by which FIS1 controls angiogenesis.

6) Unveiling the Mitophagy-Apoptosis Inducing factor-Sirtuins Axis

Supervisor: Prof. Luca Scorrano, e-mail: luca.scorrano@unipd.it

Apoptosis Inducing Factor (AIF) is an inner mitochondrial membrane protein capable of triggering parthanatos, a caspases and cytochrome c-independent cell-death pathway. AIF deletion causes embryonic lethality; the Harlequin mutant mice, where AIF levels are reduced of approximately 80%, display severe ataxia and neurodegeneration, like in two rare human diseases characterized by progressive neuropathy and impaired neurological symptoms (Cowchock Syndrome and a form of encephalomyopathy). AIF deletion induces accumulation of large lysosomal vacuoles like those observed upon ablation of PINK1, a master regulator of mitophagy. Further, accumulation of Poly ADP ribose and PARP1 activity control on one side AIF activation, and on the other hand Sirtuin (Sirt) 1 function. We hypothesize that a parthanatos-mitophagy signalling axis exists and that it could contribute to neuronal biology. We propose to address this possibility by studying the interplay between Sirtuin activity and AIF, in AIF and Sirt1 knockout pluripotent stem cells (generated by genome editing techniques) that we will appropriately differentiate in several types of neurons.

7) Understanding how Opa1 controls systemic metabolism

Supervisor: Prof. Luca Scorrano, e-mail: luca.scorrano@unipd.it

Mitochondria-shaping proteins are crucial regulators of bioenergetics, apoptosis, Ca2+ signalling and autophagy. Increasing evidence suggests that their levels correlate with mitochondrial metabolism and are

dysregulated for example in diabetes and obesity, raising the interesting possibility that they are potential targets for novel therapies for type II diabetes and for its main risk factor, obesity. Among the mitochondria-shaping proteins, the inner membrane Optic atrophy 1 (Opa1) was recently found by us to protect multiple tissues from cell damage by regulating mitochondrial respiratory efficiency. We hypothesize that Opa1 improves whole-body glucose metabolism by impinging on UCP1 function through a previously unrecognized signalling pathway. We will study the effects of alterations of Opa1 levels in vivo using mouse models of Opa1 tissue specific deletion and upregulation on systemic glucose and insulin metabolism and we will reveal the underlying molecular mechanisms by genome-wide approaches.

8) Shedding light on the endoplasmic reticulum-mitochondria interface

Supervisor: Prof. Luca Scorrano, e-mail: luca.scorrano@unipd.it

Outer mitochondrial membrane and endoplasmic reticulum (ER) are physically tethered and properly spaced at the Mitochondria-Endoplasmic Reticulum (ER) contacts (MERCs) by proteinaceous bridges whose nature is largely unknown. A genome wide FRET based screening identified 148 gene candidates classified as tethers (i.e., genes whose ablation increases ER-mitochondria distance) and 47 genes as Spacers (i.e., genes whose ablation decreases ER-mitochondria distance). Sub cellular localization analysis of the tethers candidate revealed four proteins predicted to be present in both ER and outer mitochondrial membrane.

9) Stress related inter-organelle communication in plants: role of calcium signaling and inter-organelle contact sites.

Supervisor: Prof. Michela Zottini, e-mail: michela.zottini@unipd.it

Inter-organelle cooperation maintained via controlled retrograde-signaling pathways (RS) is an evolutionary necessity for maintenance of cellular homoeostasis.
One of the earliest events following the perception of environmental changes (temperature, salt stress, drought, pathogen, or herbivore attack) is intracellular variation of free calcium concentrations occurring either simultaneously or after a lag time in a single or several intracellular compartments of the cell (cytosol, nucleus, mitochondria, etc.).
The modulation of intracellular Ca2+ signatures is considered, together with reactive oxygen species, the master switch that initiate molecular and biochemical processes involved in inter- and intracellular communication in response to environmental cues.
The present project will focus on the study of the role of calcium transients as key component of the RS in plants in stress related responses. Genetically-encoded specific calcium probes have been already developed in the laboratory, that allow to investigate in deep this topic. Moreover the existence of physical contacts among intra-cellular compartments and their role in the RS will be explored.

Here we propose to characterize the molecular function of the identified spacer and tether candidates by combining molecular biology, genetics, advanced imaging, electron microscopy, biochemistry and functional measurements in vitro and in vivo.