**3rd year report**

Age-related neurodegenerative diseases (NDs) include a number of sporadic, and less frequently genetic, pathological conditions affecting the central or the peripheral nervous system, causing cognitive decline and motor problems. As the lifespan globally increases, NDs they are becoming one of the most pressing medical and societal challenge worldwide. Despite their differences, NDs are associated with a decline in cellular proteostasis and the accumulation of insoluble deposits of misfolded proteins, which aggregate in highly ordered cross-β fibrils named amyloids. Even if amyloid deposits are the main histopathological hallmark of NDs, a series of evidences points to the soluble, prefibrillar oligomers as the ultimate neurotoxic species. Oligomers can induce various cytotoxic effects, eventually leading to neuroinflammation and cognitive impairment. The β-amyloid peptide oligomers (AβOs) are believed to play a central role in the pathogenesis of Alzheimer’s disease (AD), the most common ND, and a therapy specifically targeting AβOs would be desirable. Lately, polyphenolic compounds like flavonoids and flavan-3-ols are gaining attention for their neuroprotective properties, but their host- and gut microbiota-assisted metabolism complicates the identification of the most relevant bioactive species.

During the first years of my Ph.D., I addressed this issue by investigating the ability of a comprehensive set of phenyl-γ-valerolactones (PVLs), the main circulating flavan-3-ol metabolites in humans, to prevent amyloid-oligomer toxicity. For this purpose, I developed a new cellular model for AD in the budding yeast *Saccharomyces cerevisiae*, based on the inducible expression of an artificial peptide (β23) able to form amyloid-like oligomers toxic to yeast cells. Several PVLs, and particularly the monohydroxylated 5-(4’-hydroxyphenyl)-γ-valerolactone metabolite [(4’-OH)-PVL], relived β23 oligomer-induced cell death when screened in this model system or in a human cell line (HEK293T) transiently transfected with β23 coding sequence. (4’-OH)-PVL also interfered with AβO formation *in vitro* and, conversely, it did not show any significant effect on Aβ fibrillization. Importantly, treatment of AβOs with (4’-OH)-PVL prior to brain injection reduced recognition memory deterioration in a mouse model of AβO-induced memory impairment. Therefore, in the last year of my Ph.D. I focused on a better characterization of the molecular mechanism underlying the action of (4’-OH)-PVL on AβOs. (4’-OH)-PVL proved to be able to remodel preformed AβOs into amorphous, supposedly non-toxic aggregates *in* vitro, as showed by atomic force microscopy imagining and the loss of reactivity with an anti-toxic amyloid oligomer antibody (A11) in dot blot analysis. Moreover, (4’-OH)-PVL reduced neuroinflammation in AβO-injected mice, decreasing mouse brain section immunoreactivity with antibodies against the microglia/macrophage-specific protein Iba1 and the glia/astrocyte intermediate filament protein GFAP. PVLs thus lend themselves as novel AβO-selective, candidate AD-preventing compounds worth of further investigation. Ongoing experiments are also aimed at assessing their ability to cross the blood brain barrier, in order to evaluate their use as potential nutraceutical drugs.

Additionally, my Ph.D. project concerned the use of yeast models to identify cellular players involved amyloid toxicity. I carried out a comparative transcriptional analysis of four different ND yeast models, namely: 1) β23-based AD model; 2) Parkinson’s disease (PD) model, based on the over-expression of α-synuclein (α-syn); 3) Huntington’s disease (HD) model, based on the expression of Huntingtin exon 1 with expanded polyQ tract (Htt72Q); 4) amyotrophic lateral sclerosis (ALS) model, based on the expression of TAR-DNA binding protein (TDP43). I set out to evaluate the transcriptional response at different time points (4, 6 or 15 hours after induction), in order to identify both early and late changes associated with amyloid structure formation or aimed at their detoxification. RNA-sequencing (RNA-seq) of total yeast RNAs, followed by enrichment analysis and confirmation of the main hits by quantitative real-time PCR, revealed a fast and differentiated *S. cerevisiae* response to the formation of different amyloid structures. Protein folding and unfolded protein response, as well as response to oxidative stress, resulted affected in different yeast models and the expression of heat-shock proteins, whose role in neurodegeneration has not been fully elucidated yet, resulted modulated both positively and negatively in different samples. Other pathways associated with neurodegeneration, e.g. nutritional pathways involved in the uptake of essential metal ions, like iron or copper, were involved and metal ion supplementation in yeast medium evidenced the delicate equilibrium between their beneficial and detrimental action. The AD model presented the highest number of deregulated genes and mitotic cell cycle emerged as one of the most affected processes with strong gene down-regulation, in line with an aberrant cell morphology. Unexpectedly, the AD and the HD models showed a significant enrichment in terms concerning sporulation and meiosis, revealing an even more profound alteration of DNA replication and cellular division processes. Another signature of β23 over-expression was the mis-regulation of genes involved in mitochondrial function. Interestingly, mitochondrial-encoded genes resulted strongly down-regulated, while an opposite profile was shown by the PD model. Fluorescence microscopy analysis of mitochondrial morphology pointed to an increased mitophagy in the PD model and defects in mitochondrial fission and fusion processes, revealed by fragmentation of the mitochondrial network, in the AD model. Future experiments will move forward with characterizing the involvement of different cellular components in amyloid toxicity and assess a possible transcriptional effect of the (4’-OH)-PVL metabolite.