**Diagnostic et résolutions pour systèmes complexes dérégulés :**
**De la sécurité aérospatiale à la médecine personnalisée**

La sécurité dans le domaine aérospatial a toujours été une contrainte forte, prévalant sur les aspects techniques ou économiques. L'augmentation constante de la complexité et du nombre de systèmes constituant un aéronef permet de répondre à ce besoin, mais en contrepartie l'analyse d'une défaillance de l'un des systèmes est plus difficile, surtout pour les membres de l'équipage. Ce problème est résolu, de manière paradoxale, par l'ajout de systèmes, tels que les Flight Warning System (FWS), qui analysent les pannes, remontent les causes racines, prédisent l'évolution du système global et proposent des procédures à l'équipage.

Un humain, ou plus simplement un organe, sont des systèmes biologiques extrêmement complexes avec un haut niveau de régulation. Une « panne » de ces systèmes correspond à une dérégulation induite par une mutation dans un gène ou une agression extérieure. La dérégulation de systèmes critiques, comme le métabolisme affecte le système biologique global et mène à une pathologie.

Un médecin se trouve face à la même problématique qu'un FWS. Il analyse les symptômes pour diagnostiquer la cause racine et propose ensuite un traitement adapté. La médecine personnalisée aborde cette question avec une considération supplémentaire sur la diversité biologique de chaque patient, et pour cela elle s'appuie sur des systèmes de diagnostic qui analysent les données biologiques personnelles.

La biologie des systèmes est un domaine récent qui a pour objectif d'utiliser les approches et les outils de l'ingénierie pour l'étude des systèmes biologiques complexes. Elle est particulièrement adaptée aux problématiques de la médecine personnalisée. Le parallèle établi entre le FWS et un système de diagnostic biologique permet d'évaluer les connaissances communes nécessaires à leur étude : la sureté de fonctionnement, l'automatique (identification, estimation, optimisation), l'ingénierie des systèmes, les mathématiques, l'informatique. Le parallèle réalisé entre le FWS et les symptomes d'une pathologie ne concerne pas l'étude des facteurs humains.

Cette thèse aborde par une démarche de type biologie des systèmes l'étude d'une rétinopathie, l'« Atrophie Optique Dominante Autosomale de type 1 » (ADOA-1), afin de proposer à terme des pronostics et un traitement personnalisé.. L'ADOA-1 est une des causes principales d'atrophie optique héréditaire est due à des mutations du gène nucléaire OPA1 codant une protéine mitochondriale. L'ADOA-1 est une maladie génétique rare qui se caractérise par une atrophie du nerf optique qui est formée par le prolongement (les axones) des cellules nerveuses ganglionnaires de la rétine. Cette affection conduit dans de nombreux cas à une cécité légale (acuité visuelle inférieure à 1/20), sans recours thérapeutique pour l'instant ni possibilité de pronostic de la sévérité.

L'objectif de cette thèse est de modéliser les mécanismes moléculaires impliqués dans l'AODA-1 pour à terme simuler l'évolution de la pathologie à l'aide des caractéristiques personnelles du patient qui peuvent moduler la sévérité de la maladie. A plus long terme, des traitements personnalisés pourront être proposés grâce au modèle.

**Diagnostic and resolving For dereguLated complex sYstems:**
**From aErospace safety to peRsonalised medicine- FLYER**

Safety has always been a matter of concern in aerospace, beyond technical or economic aspect. On the one hand the growing number of systems and their complexity increase the safety, but in the other hand they make the analysis of failures very difficult, especially for the crew. One solution is to create a new system, another one, like Flight Warning System (FWS), which analyses failures, identifies root causes, predicts overall aircraft system evolution and displays appropriated emergency procedures.

A human, or more basically an organ, are biological systems extremely complex with a high level of regulations. A "failure" in these systems corresponds to a deregulation induced by a mutation, intoxication or external aggression. Deregulation of a critical function, such as metabolism or oxidative respiration, affects the global biological system and leads to a disease.

A physician faces the same challenge than the FWS. He analyzes the symptoms to diagnose the root cause and therefore proposes a treatment. Particularly, the field of personalized medicine addresses this challenge with a consideration for human diversity (age, genotype, etc...) and it relies on diagnostic systems, which can be seen as FWS.

System biology is a recent approach based on engineering sciences and tools to study complex biological systems. It is especially relevant for topics concerning personal medicine. The links made between FWS and diagnostic systems enable to list common knowledge necessary for their study: safety analysis, automatic (identification, estimation, optimization), system engineering, mathematics, computing. The parallel between FWS and the symptoms of a pathology does not concern the study of human factors.

This thesis uses a system biology approach for the study of an optic neuropathy "Autosomal Dominant Optic Atrophy type 1" (DOA-1), in order to predict pathology evolution and to give appropriated treatment for a specific patient. DOA-1, is characterized by moderate to severe loss of visual acuity with insidious onset in early childhood associated with optic nerve atrophy. This complex pathology remains without effective treatment or predictive diagnostic to date.

The objective is to model molecular mechanisms involved in DOA to predict pathology evolution considering individual patient's factors such as modulator genes. In the future, personalised treatments will be proposed based on model analyses.

**Diagnostic and resolving For dereguLated complex sYstems:**
**From aErospace safety to peRsonal medicine- FLYER**

The project "FLYER" is integrated in the area of personalised medicine, which is the fifth goal of "Innovation 2030" which inventories the main questions for the future in France, underlined a most important collective and individual efficiency of medicine with adapted treatments. It is clearly written that "french school of mathematics" will be a real support for the valorisation of biological data. In this context, **MOVE ON objective is to predict DOA pathology evolution and to give appropriated treatment based on in-silico analysis with physiological parameters for a specific patient. Those analyses will be realised with a mathematical model of molecular mechanisms involved in DOA customized with individual patient's factors. A Systems Biology approach will be used to achieve this work.**

- A link with the aerospace domain

The Flight Warning System (FWS) reports to the crew the failures of all aircraft systems. Depending on the failures, it suggests a list of procedures. The increasing number of systems, their complexity and their interconnexions make the analysis of failures very difficult for the crew under pressure. Thus, FWS has to be more and more "smart" to identify root causes, to predict overall aircraft system evolution and to display appropriated emergency procedures.

A human, or more basically an organ, are biological systems extremely complex with a high level of regulations. A "failure" in these systems corresponds to a deregulation induced by a mutation, intoxication or external aggression. Deregulation of a critical function, such as metabolism or oxidative respiration, affects the global biological system and leads to a disease.

A physician faces the same challenge than the FWS. He analyzes the symptoms to diagnose the root cause and therefore proposes a treatment. Particularly, the field of personalized medicine addresses this challenge with a consideration for human diversity (age, genotype, etc...) and it relies on diagnostic systems, which can be seen as FWS.

Consequently, the findings made during the development of biological diagnostic system (in terms of concepts, robustness analyses or prediction of evolution of complex systems) will certainly benefit to the safety in the aerospace domain.

- Objective

Mitochondria are double membrane organelles, acting as cellular powerhouse via oxidative phosphorylation. Mitochondrial diseases, can be caused by mutations in their genes.. Optic neuropathy is a frequent disease manifestation as Autosomal Dominant Optic Atrophy type 1 (DOA). Although the responsible genes OPA1 has been identified the physio-pathological mechanisms involved are still unknown and there is no therapy to date.

Thanks to the originality and transversality of our collaborative project, which lies in the conjunction of a well thought-of working hypothesis, complementary outstanding expertise as well as versatile experimental models, we hope to unravel the pathophysiological mechanisms leading to DOA and build a mathematical model to predict DOA pathogenesis. We have already shown that OPA1 inactivation impacts mitochondria oxidative metabolism. Since, oxidative metabolism alterations are priming events of apoptosis in many neurodegenerative diseases, we want to demonstrate that OPA1-induced mitochondrial defects pre-sensitize neurons to further insults like oxidative stress leading to late onset apoptotic death, that thus could be prevented. Beyond unravelling the mechanisms at the origin of DOA, our project aims to establish a proof of principle of a new therapeutic avenue, based on the neuro-protective effect of antioxidant compounds based on a personalised prediction thanks to a mathematical model and personal data, for DOA.

- Autosomal Dominant Optic Atrophy (DOA): a subject for personalised medicine

DOA, is characterized by moderate to severe loss of visual acuity with insidious onset in early childhood associated with optic nerve atrophy. The disease affects primary the retinal ganglion cells (RGC) and their axons forming the optic nerve,. There is a considerable inter- and intra-familial variation in visual acuity.. This complex pathology remains without effective treatment to date. The majority of patients (about 75%) with DOA harbors mutation in the *OPA1* gene.. Interestingly, recent studies evidenced a severe multi-systemic disorder associated with some *OPA1* mutations, named 'DOA plus' syndrome. Patients present additional neurological complications as ataxia, sensorineural deafness or myopathy in adult life. These observations highlight the widespread deleterious consequences of OPA1 mutations, not only for RGCs, but also for other neuronal populations and skeletal muscle.

The *OPA1* gene encodes a mitochondrial protein anchored to mitochondrial inner membrane. To what extent inactivation of OPA1 functions contribute to DOA and DOA+ pathogenesis still has to be elucidated. We addressed the question of the general impact of OPA1 inactivation on oxidative metabolism by down-regulating OPA1 in rat cortical neurons in primary culture and in HeLa cells as a control, in which OPA1 was silenced by RNA interference, in $OPA1^{enu/+}$ mice as well as in fibroblasts from DOA or DOA+ patients.

We found that cellular respiration is diminished and leads to a **pro-oxidative state**,.. 4 and 10 months old DOA mice cortices were analysed and clearly demonstrated that **they are in oxidative stress.** The *in vivo* data reinforce *in vitro* experiments previously described. Finally, we checked the oxidative metabolism machinery in DOA patients' and healthy volunteers' fibroblasts. **We clearly found, that some patients showed altered expression of genes implicated in the oxidative metabolism. These results are the basic elements of a European Patent (EP 14305448) ANTIOCHE (actually in the phase of international extension) centred on the pronostic and therapy of DOA and glaucoma which affects 80 billions of patients worldwide.** On the one hand, these data reveal modifier genes of the severity of DOA and one explanation for the inter- and intra-familial variations and on the other hand the results direct pharmacological approaches to prevent and treat DOA pathogenesis. Thus, we can propose that mutations or decreased quantity of OPA1 induce an imbalance in the cellular redox state, weakening cells to exogenous pro-oxidative stresses. **This phenomenon would be the major key of the molecular mechanisms involved in DOA pathogenesis and glaucoma.**

- Modelling biological phenomenons

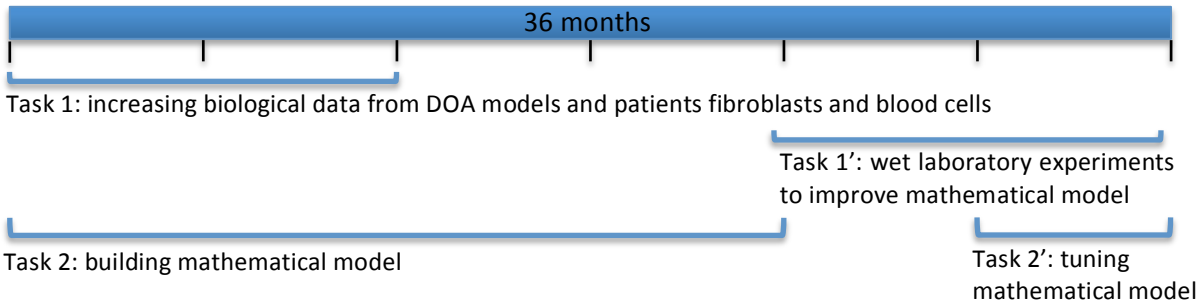
From recent years it can be observed that biology has become a new application domain for classical engineering topics like mathematical modelling, dynamical systems theory, or even process control. On the other hand, the growing results in biological science force the researchers to consider new design principles. Thus, enabling synergies at the interface of biology and engineering will allow to access to new challenges in both areas. It is now admitted that the general principles of feedback and control systems are central when trying to answer key questions in biological systems science.

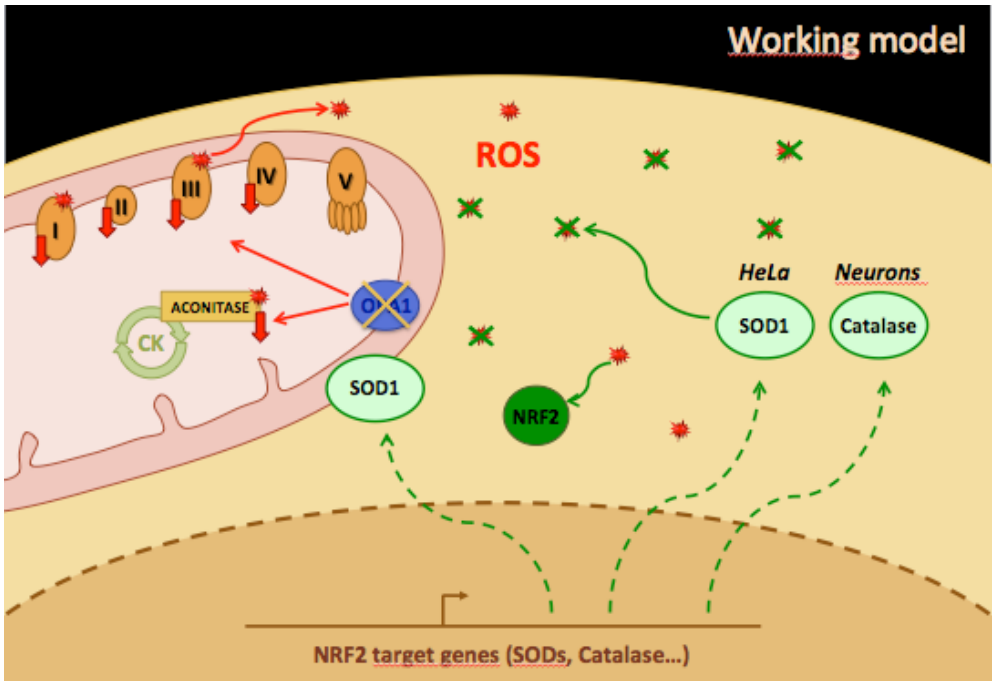
System Biology is a recent approach (since 2000), which uses large biological analysis results combined to computational sciences to address complex biological problems. This approach is based on an iterative methodology and is a holistic rather than reductionist. It uses a collaborative, cross-disciplinary approach and integrates many multi-scale types of biological information. Systems biology develops new experimental approaches to capture temporal and spatial dynamics of biological networks and allows the development of predictive and actionable models of biology or disease.

This approach has been recently used in the field of neurodegenerative pathologies. In 2013 Poliquin et al. set up a mathematical model of metabolic energetic pathways involved in Parkinson's disease. Their simulation suggested, among other results, that following a genetic disorder, in this case a mutation of Park2 gene, a cell performs naturally a rebalancing of fluxes, suggesting a robust energy regulation. The methodology used in this project is organized as follow: (i) they built a first model based on ODE (Ordinary Differential Equation) from literature, (ii) they created new experimental protocols to establish the cell's metabolome, (iii) they tuned their model to fit experimental data and simulated external or internal stresses revealing new biological networks. Simulation measurements are then compared to wet lab measurements and model parameters are then adjusted to fit measurements. Parameters estimation is realized with a function of software Systems Biology Toolbox® (Schmidt & Jirstrand, 2006) implemented in Matlab® (The Mathworks, Inc.®). With the adjusted model they performed simulation of different oxidative stress profiles (long term/low stress, short term/medium stress, short term/high stress) on cells to evaluate, among other results, ROS production (Reactive Oxydative Species).

But even if these models are becoming common in biological studies, it is still difficult to use them as efficient tools to explain and predict biological phenomena. Indeed, classical models are generally built so as they match the experimental results from initial hypothesis. But the capability of explaining and/or predicting complex phenomena from observations remains limited. This limitation can be overcome if we consider an engineering-based procedure: the overall model will be composed of many basic models interconnected together. Typically these basic models should render the input-output behaviour of elementary components of the biological process, like cells for instance. This procedure is widely used in electrical, mechanical or aerospace engineering. This system-level model, which has been built taking into account the desired level of detail, is then used to explain, predict and analyse the behaviours of the biological system. Thus it becomes possible to use the model as a way of proving hypothesis and performing predictions.

**This thesis project is well anchored in the field of biology and of automatic control and builds a link with aerospace domain, the ED-AA is perfectly adapted for this specific subject.**






**Laboratory:** Center of Developmental Biology (CNRS UMR5547, Toulouse, France) Université Fédérale de Toulouse (Toulouse 3), 118 route de Narbonne 31062 Toulouse cedex 09

**PhD director 1 :** The project will be coordinated by **N. Davezac (PhD, HDR)** who recently joined the team directed by P. Belenguer at the Center of Developmental Biology (CNRS UMR5547, Toulouse, France). **N. Davezac** possesses a recognized expertise on proteomics, oxidative stress and genetic diseases (Davezac et al, 2004 Proteomics; Lipecka et al, 2006 JPET; Moriceau et al., 2009 J Immunol; Witko-Sarsat et al., 2010 J Exp Med; Colas et al, 2012 HMG; Bouayad et al., 2012 J Biol Chem) and acquired an expertise in oxidative metabolism. She patented novel findings in understanding DOA pathogenesis driving to a potential therapy and named: "Method, process and kit for prognosis of OPA1 gene or OPA1 gene product deficit induced diseases" (Millet et al submitted to PLOS Biology; European Patent 14305448 ANTIOCHE ). The team has a long time expertise in the mitochondria field. They participated to the discovery of the first gene controlling mitochondrial dynamics (OPA1) involved in a neurodegenerative process (Dominant Optic Atrophy) (Delettre et al., 2000 Nat Genet.). Since then, the team has investigated at the molecular and cellular levels the functions of this protein (Olichon et al., 2003 JBC; Olichon et al., 2007 Cell Death Differ), and the consequences of its inactivation on DOA pathological mechanisms (Olichon et al., 2007 J Cell Physiol, Landes et al., 2010 Semin Cell Dev Biol).

**PhD director 2: Joel Bordeneuve-Guibé** is working in the Department of Mathematics, Computer Science and Automatic Control of ISAE, which aims to develop methods, techniques and tools that make it possible to understand, analyze, evaluate, control and design, the functional and operational behavior and the performances of complex systems. They have a recognized expertise in Control (N. Guy et al, Journal of Dynamic Systems, Measurement and Control, 2014. Bordeneuve-Guibé et al, Journal Européen des Systèmes Automatisés, 2011 ; D. Alazard, et al, AIAA Guidance Navigation and Control Conference, 2013; J. Bordeneuve-Guibé et al, AIAA Guidance Navigation and Control Conference, 2014) In this context, the Control team mainly focuses on: (i) the modeling of complex dynamical systems, (ii) the development of control law design methods (multivariable, robust, self-scheduled, adaptive, non-linear) and their implementation within realistic applications (aircraft and space craft). More particularly, many research projects handled by the Control team are focused on the interdependence between the modeling process and the controller design: Indeed the main challenge under consideration is to find the best-required model to solve a specific control problem. Especially when robustness analysis is considered, the model complexity should be strongly related to the analysis methods. A special attention is given to the multidisciplinary aspects: indeed in the case of complex systems, it is necessary to consider the optimality of the models for every subsystem and the interactions between them. Mastering the couplings between subsystems considered as elements of a whole physical system is a key problem addressed by several projects in the Control team. Model reduction techniques, extensively used in Aerospace control engineering, are also under study.