# 1 Summary description of project context and objectives.

SYSCILIA, "A systems biology approach to dissect cilia function and its disruption in human genetic disease", is a large scale integrating project funded by the European Community's Seventh Framework Programme under the Health Cooperation Programme (grant agreement no: 241955). It brings together 18 partners from seven different countries. Our highly motivated, multidisciplinary consortium combines the unique expertise and experimental model systems of groups with an extensive track record in the molecular analysis of the major ciliopathies with leading experts in systems biology, bioinformatics and proteomics. Partners in the consortium have complementary expertise, apply state of the art techniques and integrate them within a well established systems biology approach. This combination is excellently equipped to unravel, integrate and employ the great number of variables involved in cilium function and its dysfunction in inherited ciliopathies.

SYSCILIA aims to apply systems approaches to understand the basic biological processes underlying the role of cilia in human disease and to develop models capable of predicting the effects of discrete perturbations or mutations in those protein networks that underpin cilia function. Cilia are ideal organelles for systems biology as they can be regarded as semi-closed systems being both largely spatially and biologically separated from other cellular structures and processes.

The project is organized in 4 scientific components and one management & dissemination component encompassing eleven interdependent workpackages. All scientific WPs "feed" the central resource (WP2) with information, and in turn employ the data from the resource to specify, modify and validate the experimental datasets. The development of a central, shared resource designed to integrate, analyze and disseminate the information from different levels of complexity will result in a unique, unprecedented tool with both a descriptive as well as predictive value for cilia biology. By its integrated approach this project reaches not only beyond the state of the art in the field but also provides general proof of principle strategies for other diseases or organ systems.

The systems approach will unveil the full regulatory repertoire of this intriguing organelle and facilitate therapeutic approaches to utilize this knowledge. In brief, our proposed studies will set a new world stage in unraveling the role of ciliary proteins in cell biology in health and disease. As a result, our project will provide important novel insights and deliverables for gene identification, protein function prediction, therapy, and diagnostics. We already see that our approach informs other systems-based disorders and as such impacts the scientific community in a broad context.

# 2 Description of the work performed since the beginning of the project and the main results achieved so far.

SYSCILIA is rapidly moving towards the **identification of molecular mechanisms which determine ciliary function**, with novel candidate protein networks, pathways, and **ciliopathy-associted genes being identified on an ongoing basis**.

### *The most prominent results per workpackage thus far:*

In WP 1 ("MAPPING THE CILIOME"), a nearly complete ciliary proteome dataset was built, using yeast two-hybrid and affinity proteomics experiments. This ciliary proteome provides a solid basis for subsequent analyses in WP2, WP3, and WP4. The network currently consists of more than 4,000 proteins and more than 30,000 interactions.

In WP 2, the "CENTRAL RESOURCE FOR DATA INTEGRATION" was built and maintained, a robust seamless storing, accessing system for the SYSCILIA project data. Also, a **Gold Standard for known ciliary genes (currently 303) has been established** (http://www.syscilia.org/goldstandard.shtml).

In WP 3 ("CONSTRUCTION, COMPARISON AND APPLICATION OF CILIARY INTERACTOMES"), a pipeline was created for ciliary membrane protein collection and classification, and regulatory ciliary protein motifs have been identified. **Detailed evolutionary analysis allowed us to trace the origin of IFT** (BBSome, IFT-A, IFT-B) and the order in which they have been added, providing new functional clues of their submodules .

In WP 4 ("INTEGRATIVE MODELLING AND PREDICTIONS OF CILIARY SYSTEM BEHAVIOUR") novel techniques for the filtering and the analysis of experimental data have been developed. A **new statistical method allowed the analysis of genome wide RNAi data** and the identification of high confidence targets for perturbations of ciliopathy-associated modules.

In WP 5 ("ASSAY SYSTEMS TO STUDY FUNCTIONAL CILIARY MODULES"), ciliary transport was monitored using FRAP-based methods in *C. elegans* and in mice, and ciliogenesis/ciliary polarity was monitored using *Xenopus* epidermis models. The characterization of different ciliopathy mouse models resulted in substantial progress towards the decipherment of the roles of different ciliary/ciliopathy-associated proteins in ciliary signaling (e.g. Wnt and Shh).

Work in WP 6 ("ASSAYS TO DISTORT CILIOPATHY-ASSOCIATED MODULES"), has resulted in major progress including the identification of a novel NPHP module containing a new member ANKS6, the linking of a cilia-related gene *Ircc50* to zebrafish and human seminoma formation, the finding that murine OFD1 is required for brain patterning, the discovery of spermatogenesis functions for murine centrin, and the realisation that complex deregulation of Wnt/Shh signalling underpins the phenotypic variability of *Tmem67* mice.

The "**SYSTEMATIC RNAi SCREENS** TO DISTORT AND IDENTIFY CILIOPATHY-ASSOCIATED MODULES" of WP 7 **have been completed** and the data analyzed. The results have begun to be integrated with the central resource and are included in the SYSCILIA workflow, and a comparison with human genetic variation and mutation data is on-going.

Partners in WP 8 ("ASSESSMENT OF THE INVOLVEMENT OF THE PREDICTED CILIARY MOLECULAR MACHINES IN THE PATHOGENESIS OF CILIOPATHIES" have generated high-coverage (99% capture at 10x coverage) **sequencing data for 766 ciliary target genes in 500 patients**. The consortium provided clear evidence that systems biology, linked with expert modelling, opens new horizons via the identification of novel protein networks as novel therapeutic targets.

In WP 9 (" TRANSLATIONAL SYSTEMS BIOLOGY: CILIOTHERAPEUTICS") **we completed in-depth analyses of pharmaceutical inhibitors** of different signaling pathways in *in vitro, ex vivo* and *in vivo* ciliopathy models, **drugs repurposing screens** in zebrafish, and **translational read-through drugs**, with promising results.

The enthusiastic contribution and involvement of all partners has allowed our SYSCILIA consortium to generate **78 peer-reviewed publications** in three years, of which 13% in top-journals such as Cell, Science and the Nature journals with an impact factor >30, and almost 50% in journals with an impact factor >8.

# 3 Description of the expected final results and their potential impacts and use.

SYSCILIA is the first and largest comprehensive project on Systems Biology of ciliary disease ever conducted. One big problem in the field of rare disease is genetic heterogeneity or classical pharmacological approaches fall short when searching for improved diagnostics and novel therapy. SYSCILIA uses systems tools developed or applied by the project to acquire knowledge on the overarching principles of systems failure in these diseases. The cilia model systems and associated discoveries will ultimately be employed to accurately diagnose and therapeutically target the growing number of human diseases associated with ciliary dysfunction. The combined output of our work will clearly inform multiple aspects of ciliary biology and genetics. For example, we will not only generate and validate both the total protein complement of the ciliary proteome, but also describe the majority of physical relationships of that proteome and how those relationships determine the behaviour of the cilium. Moreover, we will identify novel signalling components of the ciliary proteome (many of which will be likely relevant to disease pathogenesis), which, together with the current knowledge base of the roles of the organelle in vertebrates, will continue to inform our functional work. Thirdly, the combinatorial information from SYSCILIA will be projected to address human genetic disease, since we have both well-validated targets for next-generation medical

resequencing, which analysis currently is near-complete, and the means to test the effect of mutations found. Importantly, this work will not only lead to the systematic identification of new disease genes (which can be accomplished, albeit less comprehensively, by independent investigators), but will describe the total mutational load in ciliary dysfunction in humans and imbibe predictive power to the genotype.

Finally, through our consortium, we are generating a plethora of new ciliopathy models and developing new therapeutic paradigms that are based not on the dysfunction of a particular gene/protein, but on the quantitative assessment of an entire functional module. It is our strong expectation that this approach will represent the most efficient means towards drug discovery and that it will overcome the difficulties typically associated with a proteinocentric/single pathway approach to therapeutics. The excellent results from the project are confirming these high expectations, as the positive outcome of our ciliotherapeutic efforts, well ahead of schedule, already urge us to think of ways to implement these results in human trials. It is only through such collaborative structures that we will be able to screen the entire gamut of ciliopathy phenotypes and transition from a genocentric view of genetic disease to a systems-based expression of gene/allele causality and modification.

**"SYSCILIA is providing proof of principle that an integrated approach to a model organelle (io. cilium) substantiates the utility of a systems biology approach to the analysis of complex biological systems."**

**More information: www.syscilia.org**