

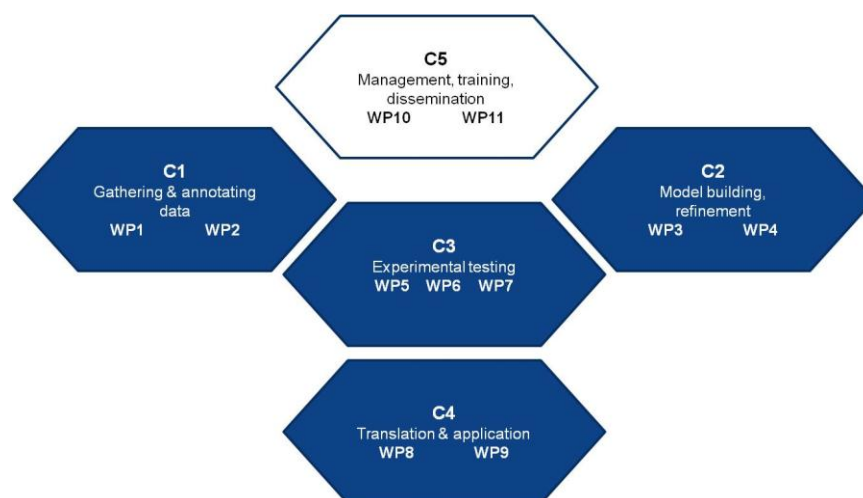
1 Summary description of project context and objectives.

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 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. 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.

As all proposed workpackages are strongly integrated in this resource, their potential to generate biological and medical knowledge of cilia function in a multidisciplinary fashion is similarly unprecedented. 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 suggest that our approach will inform other systems-based disorders and as such impact the scientific community in a broad context.



SYSCILIA: Components and Workpackages.

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

During the first year of SYSCILIA work in all areas was focused on establishment of experimental assays, development and improvement of required tools and recruitment of personnel. During the kick-off meeting at hotel Erica, Berg en Dal, The Netherlands in July 2010, the project was initiated and since then has been running smoothly and according to plan.

In *Workpackage 1*, specific progress has been made in setting up a robust protocol for cilia isolation, one of the most tedious tasks to be performed within the first 12 months. As proposed, ciliary interactome description based on native protein complex analysis as well as based on yeast two hybrid binary interaction screens has progressed, delivering a first significant set of binary protein-protein interaction data, both hypothesis-driven and hypothesis-free, which was annotated in the central resource. To assess complex stoichiometry and combine this with targeted perturbations of interactions, quantitative immunoprecipitation combined with knock down (QUICK) was established. This method was then applied to discriminate specific binders in a immunoprecipitation experiment from unspecific ones: **purified complexes can be quantified against a control lacking the bait protein, thereby enabling highly confident identification of endogenous protein complexes**. The method can also be used to determine relative stoichiometries when combined with data interpretation tools such as MaxQuant.

In *Work package 2* systems for sharing and storing data have been created, substantial data from the literature has been collected, and a database of Ciliary molecules, interactions and functional data has begun to populate. **A web site for public accesses to the principle findings within SYSCILIA has been set up**. Analysis of internally generated datasets has begun and **the first draft of the database and viewer is available** to the consortium.

In *Workpackage 3* ciliary protein-protein interaction data from yeast two-hybrid and tandem affinity purification experiments is collected in the central database. Spatial interactions of ciliary proteins are analysed and it became evident that the few cilium associated targeting motifs are limited to special proteins like rhodopsin and cannot be represented as universal ciliary targeting motifs. To further characterize the assembly of ciliary interactomes investigations of the RNA sequence composition now need to be performed. It is demonstrated with the BBSome, how evolutionary analysis can be applied to identify functional modules that are crucial for the cilium. The identified ciliary submodules will be used in WP4 and mapped to phenotypes and diseases. Sequence associated properties of the key ciliopathy protein Meckelin were analysed while a destruction motif was identified in SANS, enabling further experimental validations by partners. **Structural models have been made and are being sorted**.

In *Workpackage 4* the work has been focused on familiarization with the biological system and the available modelling techniques. The modelling approaches were presented in the WP3/WP4 meeting in Heidelberg (March 14-15, 2011) and in the SYSCILIA annual meeting in Mallorca (April 18-20, 2011). Discussions with the other partners allowed the evaluation of the different frameworks and the identification of the ones that could be used for SYSCILIA. **Mutagenesis and protein-protein interaction data will be integrated for the implementation of a biological network model for the prediction of phenotypes**. The aim is to exploit the model to determine the phenotypic impact of gene mutations and explain how single gene mutations produce complex disease phenotypes, improving this way our understanding about ciliopathies.

Recruitment of cargo proteins is mediated by specific transport processes that are crucial for ciliogenesis and cilia maintenance. *Workpackage 5* focused on establishing methods to measure ciliary transport in no less than three different assay systems. Ciliary transport was successfully monitored, using a FRAP-based method in MDCK cells. This method confirmed the known

importance of distinct Rab molecules, but in addition **revealed previously unknown functions based on the quantitative analysis of our data**. Fluorescent dye-based methods were also established in the two other model systems, providing a comprehensive tool-box for further studies.

Motile cilia (e.g. in the brain or lungs) need to be polarized to establish a directional fluid or particle flow. To characterize the polarization process, the motile cilia of the *Xenopus* epidermis is studied. This model system to follow the orientation of cilia is successfully established and it is found that measuring the speed of fluorescent particles across the *Xenopus* epidermis adequately reflects the degree of polarization, while ciliary angles appeared less precisely regulated, at least in this model system. Using this system, **the consortium is now well positioned to determine how disease genes affect ciliary polarization**. Regarding ciliary signaling processes, significant progress has been made in deciphering the precise events that control mTOR activity.

Workpackage 6 was principally involved in addressing the establishment of cellular and animal models of cilia-related genes, and especially known ciliopathy components, which will subsequently be used to perform the various cilium structure/function assays outlined in WP5. Some work has been conducted towards the genetic inter-relatedness of ciliary/disease genes. This work has been proceeding very well, with many cellular (e.g., NIH3T3, IMCD3, RPE-1, HEK293T) and animal (e.g., mouse, zebrafish, worms) models now established for ciliopathy genes such as *Mks1/2/3*, *Odf1*, *Nphp1/2/4/5/6/8*, *Tctn1/2* and *Bbs2/8*, as well as various IFT gene components. Cilium structure/function assays are now underway, and this has resulted in significant new knowledge. This includes **mTOR regulation by mammalian ciliary signaling, roles for *Nphp* genes in apical organization, regulation of transition zone structure/function by combinations of nematode MKS and NPHP components, Wnt signaling regulation by *Mks1/3*, a role *Odf1* in digit number and Shh signaling**, and further **demonstration of genetic associations between ciliopathy genes and planar cell polarity**. Work has yet to commence on characterizing new ciliary/disease genes that will be predicted by the discovery workpackages pipeline (WP1-3; WP7). Once predictions start to come through in Year 2, the powerful functional characterization capabilities of WP6 will be invoked. By the end of this project, WP6 will have been a major driver in deriving major new knowledge for known and novel ciliary and ciliopathy disease components, and the networks that underlie their functions.

In *Workpackage 7* **the infrastructure and equipment for the whole-genome reverse genetics siRNA screen for ciliogenesis and cilia maintenance have now been established**. The reagents and a protocol for reverse transfection and immunofluorescence imaging has been optimized. These are suitable for high-throughput liquid handling and high content imaging. The efficacy of negative and positive screening controls have been determined from manual assessment using confocal microscopy, and have now been thoroughly validated by high content imaging. Automated image acquisition and analysis for controls were completed well ahead of schedule, and an image analysis algorithm has now been written and validated.

Workpackage 8 has made good progress towards collating the expected cohort, capturing the necessary phenotypic diversity and **constructing the database that houses both clinical and, in time, genetic and functional data**.

Workpackage 9 has set its aims high; intending to generate all the preclinical data necessary from SYSCILIA WP1-8 and prepare it for clinical administration in ciliopathy patients. **The ultimate goal is to have the Molecular Medicine dictated by the Systems Biology**; however in the beginning of this project we are testing proof of principle using knowledge-derived candidate approaches. For example, these first 12 months **three SYSCILIA groups have made progress in testing a novel human-approved drug called PTC124 in cell-based systems as well as mouse and human retinas**. PTC124 holds great potential in treating the 10-20% of patients harboring nonsense mutations in ciliopathy genes. Our work with PTC124 and related drugs has generated two papers already published with two more being drafted, and feasibility studies are being considered for a small-scale clinical trial in retinal-renal ciliopathies (where disease progression can be delayed). In addition, **we**



are performing drug re-purposing screens with FDA-approved drugs on ciliopathy models (zebrafish and cell-based assays). Some candidates from that screen have already been validated and will be tested more extensively in additional animals and cell assays. Working closely with the systems builders, our choices of targeted pathways are being guided towards novel therapeutic approaches, with the hope of improving both the quality and quantity of life ciliopathy patients' experience.

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

The models 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 will have both well-validated targets for next-generation medical resequencing 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 will be able to generate a plethora of new ciliopathy models and develop new therapeutic paradigms that will be 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.

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.

More information: www.syscilia.org