SynSignal - Synthetic Cellular Signaling Circuits

EXECUTIVE SUMMARY

First Periodic Report

Context and Objectives

Cellular signaling systems are crucially important for a broad range of critical health and disease areas and high value industrial applications. Signaling systems are the target for more than half of the medicines marketed by the pharmaceutical industry, and form the main R&D area for the nutrition, flavour and fragrance industries. SynSignal is a multidisciplinary high-tech consortium working in synthetic biology's area of greatest untapped potential, delivering a synthetic biology toolbox and finished products custom designed for major present and future industrial applications of cellular signaling.

Synthetic Cellular Signaling Circuits of interest for SynSignal. Natural cellular signaling cascades are comprised of multiple functional components, with each individual component of the system typically being a protein, or a multiprotein complex. Traditional biochemical and molecular biology methods focused on studying the individual component parts of signaling cascades in isolation, while more modern approaches including systems biology, genomics and proteomics have provided insight into the function of signaling cascades as a whole. Synthetic biology provides a powerful and new perspective - bringing additional methodologies and thought processes to bear- particularly classical engineering disciplines. From a synthetic biology perspective, synthetic cellular signaling circuits are perceived as being analogous to electronic circuits. Each component of the circuit, encoded by a DNA sequence of defined structure and function, is physically interchangeable with compatible modular building blocks of similar or dissimilar function, which makes the system on the whole designable and thus accessible for engineering. The cellular context of the signaling circuit, the "chassis", is considered as being similarly modular and designable. This way of thinking is beginning to provide powerful new tools and methods to understand, and more importantly *to control and modulate* the complex signaling systems of cells.

Industry and Cellular Signaling. Development times for new products across a broad range of industries that focus on cellular signaling are excessively long, with associated high costs. In addition, the lack of effective screening platforms for a number of high value industrial applications involving signaling has a severely adverse effect on innovation and discovery, impeding the delivery of new products. These deficiencies create an economic bottleneck that makes the search for promising technologies to overcome this bottleneck a high priority. Therefore, design of synthetic cellular signaling circuits is one of the key areas of development in synthetic biology, with a number of high value industrial applications,

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spread across several key European industry sectors. SynSignal will address this urgent and imposing bottleneck, by providing new and sophisticated synthetic biology tools to overcome the challenges facing signaling-based product development in the pharmaceutical, flavour, fragrance and nutritional industries.

Essential Objectives of SynSignal are:

1. Implement a development program consisting of an iterative cycle of i) Design & Engineering, ii) DNA Assembly and Protein Production, and iii) Testing to accumulate a toolbox of synthetic parts (bio-bricks), cell lines (chassis), and complete signaling circuits.

2. To develop tools for a signaling toolbox with broad combinatorial potential applicable for different types of signaling cascades.

3. To develop whole synthetic signaling pathways that are applicable as screening platforms for creating new medicines in key disease areas, particularly Cancer and Diabetes.

4. To develop synthetic signaling cascades which are transferrable into human cells for the treatment of specific diseases.

5. To develop human signaling pathways which are applicable as screening platforms for creating new flavour, fragrance and nutritional ingredients.

6. To generate technologies and intellectual property that is widely disseminated to the broader European SME and large industry community.

7. To identify societal perceptions and concerns about synthetic biology in Europe in order to ensure long-term impact and sustainability of research efforts in the field.

Main Results

WP2 (Computer Aided Design): (i) General mathematical models have been developed to describe GPCR activation and an oscillating MAPK/ MP1 synthetic system. (ii) A generic method has been developed to characterize mobilities of single GPCRs in plasma membranes at different stages of cellular signalling. (iii) Assays have been developed measuring interactions between proteins using BRET. (iv) Work in progress models GPCR signaling in taste and olfaction.

WP3 (Taste) established a cell line expressing a chimeric Gα subunit coupling to taste GPCRs. Transient receptor potential channels TRPC1 and TRPC4 were expressed and functional tests carried out and optimized. DNAs for baculovirus production of sweet and umami taste receptors, signaling subunits and optical readout systems were produced and receptors functionally expressed.

Methods have been established to measure TRPC mediated signaling in insect cells via BRET.

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WP4 (Olfaction) developed different modular optical readout systems: (i) a G-protein dissociation assay, (ii) a cAMP reporter gene assay, and (iii) a cAMP BRET assay. A focus was the assembly of DNA elements to generate viral based multiprotein expression vectors to deliver entire multicomponent synthetic olfactory signaling cascades into cultured insect cells. Further, molecular dynamics simulations revealed detailed conformational changes of GPCRs during activation. Additional in silico screening for an olfactory receptor discovered new activating compounds. To miniaturize cellular signaling assays we developed a purification/characterization protocol for cell-derived vesicles, and microfluidic single-cell analysis with affinity beads. In addition, we tested the function of human olfactory receptors present in pancreatic cells.

WP5 (Cancer) engineered chimera between human CXRR4 and yeast Ste2. Cloning and functional testing in yeast and HEK cells have been performed. The TET-inducible MGEV system for protein expression was adapted to control expression of yeast proteins in HEK cells. **WP6** (Diabetes) improved the in vivo imaging platform to assess the effects of ligands and implementation of synthetic signalling pathways on beta cell function and replication. Furthermore a procedure was established to quantify gene expression in a beta cell line and in primary cells from pancreatic islets. Potential activating ligands of pancreatic olfactory receptor OR1Q1 were assessed by in-silico screening; till now one of the predicted compounds was tested which turned out to be an agonist inducing insulin release in pancreatic Min6 cells. Furthermore, ablation of pancreatic beta cells has been studied either by streptozotocin or in diphtheria toxin receptor mouse model. Both strategies lead to hyperglycemia, which for the STZ model can be cured by transplanting intact islets into the anterior chamber of the eye. Based on these results, it is now possible to study engineered islets in the absence of endogenous non-engineered islets.

WP7 (Functional antibody fragments): 31 receptors and 50 signaling proteins have been defined as targets. Stable cell lines for expression of 23 receptors have been created. Several receptors and signaling proteins have been expressed and purified. Camel antibodies for several receptors and signaling proteins have been produced. Functional characterization of the antibodies is ongoing. Further, a generic assay has been developed to probe activation of GPCRs in living cells observing the lateral diffusion of post-translationally labelled GPCRs (using antibodies or covalent chemical labelling).

WP8 (Dissemination & Exploitation) analyzed in detail the public discourse on synthetic biology in Germany, serving as a model for the other country reports. The SynSignal website was created as a management and communication platform. In addition, a project corporate identity and a dissemination toolkit were developed and made available on the internal project website. First dissemination activities include press releases, a radio interview, presentation of the SynSignal consortium at different scientific academies and organizations, scientific conference presentations and a number of scientific publications.

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Expected Results and Impact

A central objective of SynSignal is to radically improve synthetic biology technologies and to create novel tools for a wide range of applications to discover molecules for health and industrial purposes. The SynSignal collaborative teams have implemented this mission and achieved significant results in the first 18 months of the work program. The consortium created several synthetic multicomponent heterologous signaling cascades, which comprise the highest complexity synthetic signaling systems ever produced. The consortium has created, tested, and implemented several different readout strategies that provide unique benefits for molecule discovery for different industrial purposes including pharmaceutical drug development, and flavor and fragrance molecule development. SynSignal particularly focuses on i) designing individual synthetic signaling building blocks, ii) combinatorially assembling them, and iii) testing signaling of the cascade in low background cell lines. The aim is to produce disruptive next generation high-throughput compatible synthetic signaling regulatory circuits which provide uniquely powerful signal to noise profiles over state of the art systems.

Notably, the work plan of the SynSignal consortium answers the call to focus on new technologies that will greatly accelerate discovery in the life sciences. The ongoing joint effort will open entirely new avenues for advancing large-scale European and global efforts that aim at catalyzing and exploiting the emerging field of synthetic biology, and industrial biotechnology. SynSignal tackles and will overcome present challenges and bottlenecks, which impede these fields in Europe and worldwide, to the benefit of industrial and academic R&D, and human health. SynSignal stands for innovation, catalyzed by drawing on the best available technologies in European SMEs and the academic sector for its ambitious endeavor. The work plan fosters a productive mindset, which fully supports fluid exchange between the academic and private life science sectors, and embraces the entrepreneurial spirit.

SynSignal Impact: The work plan In the Europe 2020 strategy for growth, competitiveness in the future requires first and foremost a “strengthening of the sources of growth in Europe's industrial base“ (Barroso, 2009). The European pharmaceutical and biotech industries face increasingly difficult challenges from countries with significantly lower labor costs. To maintain on European soil highly paid, highly skilled jobs in these industries, we must immediately develop competitive advantages that compensate for our higher labor costs. The vision of SynSignal is to provide European pharmaceutical and industrial biotechnology companies with these competitive advantages through development of innovative technology platforms which change the way we discover and produce novel products. SynSignal‘s high throughput, synthetic biology-based technology platforms will boost both the speed and efficiency at which essential signaling pathways that dictate cellular processes can be modified, modulated and interfered with, thereby reducing cost of product development, and speeding the time of delivery of new products to market.

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SynSignal technologies will make accessible, for the first time, cellular signaling and metabolic pathways that are currently impossible to address using established technologies, thereby creating opportunities to develop entirely novel classes of potent and efficient therapeutics, and new and better molecules for the fragrance, flavor and nutritional industries, opening multibillion € markets. Strengthening of these markets with innovative technologies such as those SynSignal will provide is imperative to strengthen and maintain Europe's leading position in this large and growing sector, thus maximizing the impact of research and innovation on European societies and economies. Synthetic Biology is an emerging technology with the potential to be transformational in a large number of key areas of important socioeconomic challenges in healthcare, nutrition, green technology, and manufacturing. The United States has led the way in synthetic biology as judged by publication output and investment, outcompeting Europe in this viral and emerging field. This can be countered only with highest level technology development by the best European research teams and SMEs. We formed the SynSignal team and created the development plan of this proposal exactly with the objective to close this competitive gap.

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