

Development of new codon optimization tools and development of a synthetic gene expression system in the green alga *Chlamydomonas reinhardtii*

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The Idea

Synthetic biology aims to apply principles of engineering to the construction of complex biological systems building up from readily available and well-characterized individual components, also referred as biological parts. Elements that control gene expression such as promoters and transcriptional factors were the first biological parts used in the construction of human-designed genetic circuits. As they are key components of genetic circuits, promoters have been progressively improved in order to drive inducible and tunable gene expression, while at the same time to be insulated from global cell physiology. These parts have been optimized mostly to work in bacteria, which is the favorite chassis in synthetic biology because of its simplicity to grow and amenable genetic manipulation.

The lack of compatibility of parts between organisms is one of the main challenges for the expansion of synthetic biology. It is frequent that parts that work in one organism do not reproduce their behavior in another organism. Although several reasons can lie behind this lack of compatibility, one of the first things to consider is the adequacy of the sequence to be expressed to the codon usage of the host organism. Although most organisms share the same genetic code based on three nucleotide codons that encode for a particular amino acid, synonymous codons that encode for a same amino acid are used in an organism-specific frequency.

Several features make *Chlamydomonas reinhardtii* an attractive target of synthetic biology: (1) it is a suitable microorganism for biofuel and valuable compound production; (2) cell physiology and metabolism are relatively well studied; (3) it is a haploid organism, and therefore gene manipulation is relatively simple and transformation protocols are well established; (4) it can be used as a platform to develop tools of synthetic biology for land plants, including crops. Several promoter sequences have been used to drive expression of sequences in this alga. However, there is no promoter sequence that allows tunable gene expression, and those which are inducible require conditions that create major changes in cell physiology and metabolism.

Taken all this into consideration, our idea for this OpenPlant fund is to develop publicly available tools of synthetic biology for *Chlamydomonas reinhardtii*. In particular, we would like:

- 1) to improve tools of codon optimization and gene synthesis for the expression of transgenes in the alga

2) to test an optimized synthetic gene expression system in the alga for tunable gene expression

We would like to create a tool for gene sequence optimization for *Chlamydomonas* that refines codon selection by taking into consideration gene expression levels.

Additionally, our tool will allow a more money-efficient gene synthesis, as it will allow the user to control the level of optimization of the sequence. We will use these tools to create an optimized version of the TetON transcriptional factor, which is going to be the base of our synthetic gene expression system. This system has been successfully adapted to mammalian cells but has not been tried to our knowledge in algal systems. We will test different promoter architectures to control the expression of a reporter gene in a TetON-dependent fashion.

Independently of the levels of success of this project, our efforts will contribute to the development of synthetic biology in algae.

Who We Are

Francisco Navarro: Molecular Biologist with a keen interest in developing new gene expression tools in *Chlamydomonas*. Francisco has worked mostly in yeast studying cell metabolism and cell cycle, and has also experience in developing new promoter sequences for the biotechnologically interesting species *Hansenula polymorpha*. He is now an OpenPlant postdoc in the laboratory of Prof. David Baulcombe at the department of Plant Sciences, University of Cambridge, and works in the application of RNA silencing in synthetic genetic circuits in the green alga *Chlamydomonas reinhardtii*.
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Marielle Vigouroux: Computational biologist at the John Innes Centre.
Marielle has worked on several projects related to *Blumeria graminis*, mainly involving the development of analysis pipelines, phylogenetic analysis and most recently CRISPR/Cas9. She also provides bioinformatics support to the various proteomics teams on the Norwich Research Park.
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Implementation

We plan to start by redesigning the available tools for codon optimization for *Chlamydomonas*, which currently do not take into consideration gene expression levels. We plan to construct tools that allow the user to define the optimization parameters and that will estimate expression level of constructs. We will use these tools for the construction of TetON factors which will be the base of the synthetic gene expression system. Vectors containing this expression system will be constructed using Golden Gate Cloning. We will follow the syntax proposed for exchange of DNA parts in plant synthetic biology. The development of this expression system will include the “domestication” of selection markers, constitutive promoters and reporter genes, facilitating the use of these parts for other applications.

The funding requested will cover expenses in gene and oligo synthesis, reagents of molecular biology, strains and media.

MV will be in charge of the bioinformatics analysis for codon optimization and online tools design. FN will be in charge of the construction of components and test their functionality in the alga.

Benefits and outcomes

Our proposal fits well with one of the possible outcomes of this fund (“Synthesis and sharing of useful DNA parts or vectors”) and will also lead “to tangible, publicly documented and open outcomes”, as bioinformatic tools and biological parts will be available from public repositories. Additionally, our proposal satisfies also the condition of interdisciplinary collaboration between Norwich and Cambridge.

We predict three possible outcomes and benefits:

1. “Good”

- New bioinformatic tools for codon optimization and gene synthesis are created. They are open to the community through an appropriate webpage host.
- Synthetic gene expression system based on the TetOn repressor provides tunable gene expression in *Chlamydomonas reinhardtii*. Parts will be made available from the *Chlamydomonas* Resource Centre (CRC) and the Registry of Standard Biological parts (iGEM).
- Additional bioparts (domesticated for Golden Gate Cloning) are also made available from the previous repositories.

2. “Average”

- Synthetic gene expression drives transcription of reporter gene but it does not respond in a dose-dependent manner to inducer. No useful for tunable gene expression.
- New bioinformatic tools for codon optimization and gene synthesis are created. They are open to the community through an appropriate webpage host.
- Additional bioparts (domesticated for Golden Gate Cloning) are also made available from the previous repositories.

3. “Bad”

- TetOn promoter does not drive transcription in *Chlamydomonas*.
- New bioinformatic tools for codon optimization and gene synthesis are created. They are open to the community through an appropriate webpage host.
- Additional bioparts (domesticated for Golden Gate Cloning) are also made publicly available.

Budget

Gene and oligo synthesis: 2500

Other Molecular Biology reagents: 1000

Bacterial and algal strains: 250

Bacterial and algal media: 250