Re-designed whole-cell biosensors for environmental monitoring (Re-Sense)

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ABSTRACT

Innovative computational algorithms were employed to boost the performance of a bacterial (*E. coli*) biosensor, harbouring a plasmid-borne fusion of a sensing element (*yqjF* gene promoter) to bioluminescence reporting genes (*luxCDABEG*, *Aliivibrio fischeri*), genetically engineered to detect explosives' vapours above buried landmines. The sequences of both the sensing and reporting elements have been modified, yielding 370 new variants of the sensing promoter and 5 variants of the *lux* cassette. New bioreporters engineered with these modifications exhibited over a 10-fold increase in signal intensity in the presence of 2,4-dinitrotoluene and a two-fold reduction in its detection threshold.

Keywords: Biosensors; bioinformatics; landmines; explosives; synthetic biology

1. INTRODUCTION

The detection of buried landmines is a humanitarian issue of global proportions that is in acute need of a practical solution. Current mine detection technologies require the presence of personnel in the immediate area of the mines, along with the obvious risks involved. We have previously presented a solution based on bacterial sensor strains, genetically engineered to emit an optical signal in the presence of explosives' vapours, thus allowing the **remote mapping of minefields**. The performance of the previously described sensors, however, was insufficient for practical field applications. In Re-Sense, we have set out to upgrade the detection capabilities of these sensors to actual field deployment requirements.

As far as we are aware, this is the first scientific report of the use of advanced computational modelling of gene expression and algorithms for gene expression design, combined with state of the art synthetic biology approaches, for the rational design of whole-cell sensors. While in the present study buried landmine detection served as a model system, the approach can be adapted to diverse environmental, industrial or medical sensing applications.

We have redesigned both the sensing element (*yqjF* gene promoter) and the bioluminescence reporting element (*luxCDABEG* genes, *Aliivibrio fischeri*) of an *E. coli* sensor strain [11] genetically engineered to detect 2,4-dinitrotoluene (DNT), the volatile "signature chemical" of 2,4,6-trinitotoluene (TNT). The new constructs exhibited a 10-fold increase in signal intensity and a two-fold reduction in DNT detection threshold.

2. STATE OF THE ART

To date, no viable physical, chemical or biological technology exists for the remote detection of buried landmines or other environmental insults. We have previously reported [4] the only scientific description of the standoff mapping of actual antipersonnel landmines locations in a simulated minefield, but in order for this solution to be applicable, a significant improvement in sensor performance was required.

In Re-sense we have set out to achieve the desired performance enhancement by employing several algorithms for gene expression modelling and engineering that we have developed in recent years [see, for example, refs 1, 2, 5, 6, 9]. Among others, based on these algorithms we are able to modulate different steps and aspects of gene expression such as transcription initiation, translation initiation, translation elongation, co-translational folding, and more. They are based among others both on biophysical modelling of intracellular processes (e.g. ribosomal movement, binding of transcription factors, folding of the genetic material) and on unsupervised models [see, for example, refs 1-3, 7, 12].

Employing these models, we have optimized the untranslated regions (UTRs) and coding sequences of the *lux* genes for improving the performances of the sensor system.

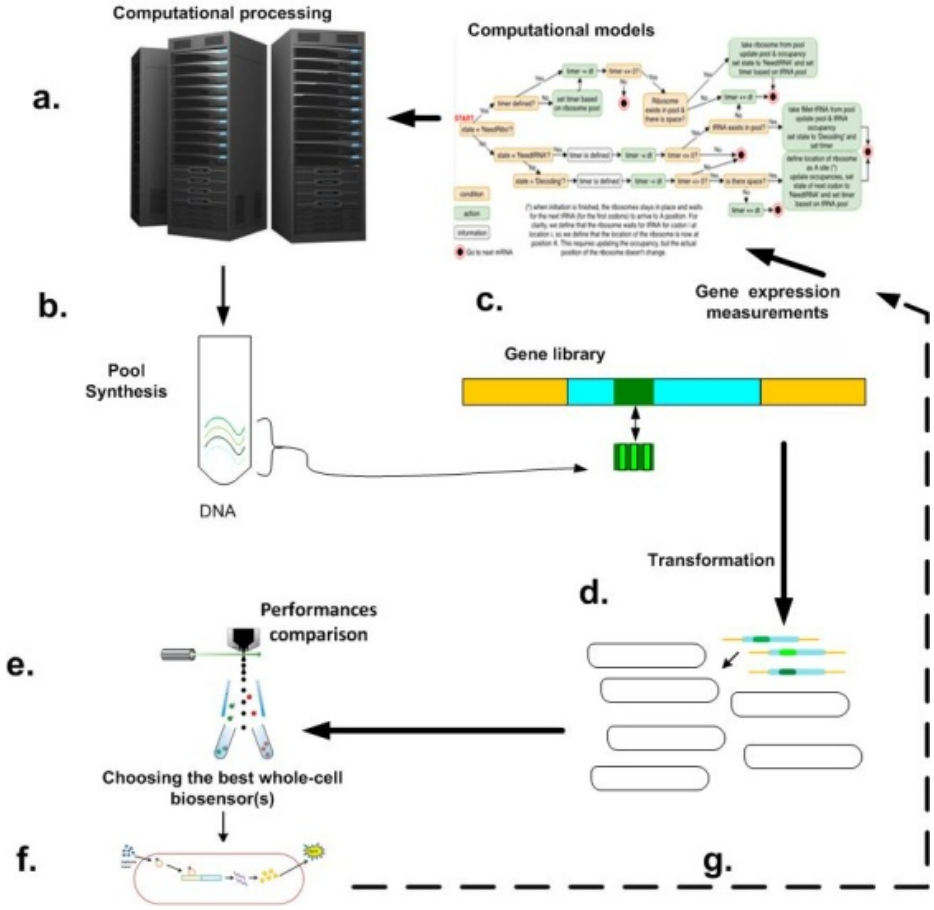

Fig. 1. The Re-Sense pipeline: computational models of gene expression are used for designing a large-scale library of constructs (a); these were generated using state of the art synthetic biology approaches (b-d) and were compared to the best previous bioreporters (e-f). Our pipeline can perform a few iterations of design-generate-screen for further improving the performances of the system (g).

1. BREAKTHROUGH CHARACTER OF THE PROJECT

For the first time, state of the art computational models and algorithms, based on in-depth studies of relevant gene induction, transcription and translation processes, have been employed for boosting biosensor performance.

Re-Sense has succeeded in dramatically enhancing the remote detection capabilities of buried explosive devices, thereby providing a potential answer to a humanitarian problem of global proportion that presently has no technical solution. This breakthrough is expected to be further utilized in other applications of cell-based environmental monitoring [10]. The pipeline employed is presented in Fig. 1.

2. PROJECT RESULTS

Based on our novel computational-synthetic biology pipeline, we have designed and generated ca. 400 new variants of the sensing promoter (out of which, 370 were successfully synthesized and cloned) and 5 variants of the *lux* gene cassette. The performance of these variants was compared to the current state of the art bioreporter [8].

Many of the new variants outperformed the current bioreporter. While improvement in DNT detection threshold was relatively minor (ca. two-fold, not shown), enhancement of signal intensity was dramatic, thus greatly facilitating future outdoor imaging of the bioluminescent signal. As shown in Fig. 2, over 50% of the modified strains responded to the presence of DNT (5 mg/L) by emitted luminescence intensities that were significantly higher than that of the unmodified control. The increase in signal intensity is further demonstrated in Fig. 3, in which luminescence development in a single representative (#394) of the enhanced strains is compared to that of the unmodified control. These results clearly demonstrate the advantages of our approach, which – following additional iterations - will further improve the performances of the bioreporter.

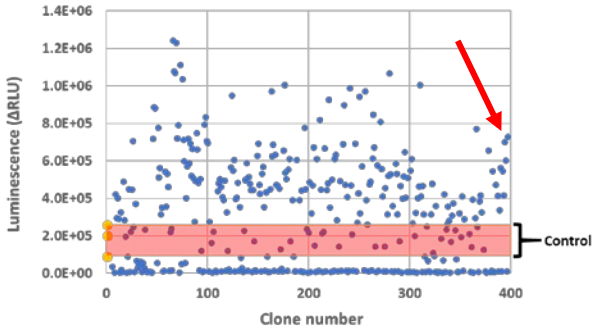

Fig. 2. Luminescent responses of all sensor variants to a single DNT concentration (5 mg/L). Values are presented as the difference between luminescence intensity in the presence of DNT and that in its absence, in the plate reader's arbitrary relative luminescence units (ΔRLU). Signal intensity range of the unmodified control is represented by the shaded area. The red arrow denotes variant #394 (see Fig. 3).

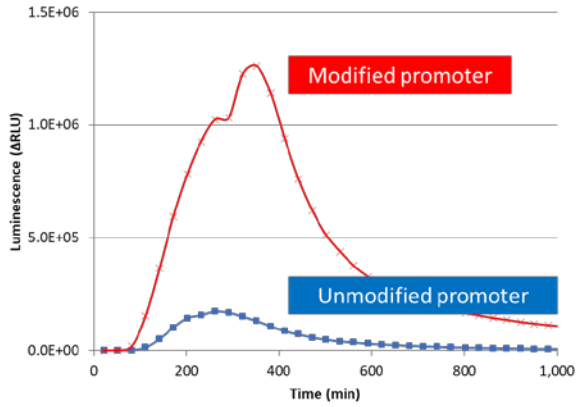

Fig. 3. Response to DNT (1.8 mg/L) of the "wild type" sensor strain (blue) and modified strain # 394 (red). Luminescence values are presented as the difference between light intensity in the presence of DNT and that in its absence, in the plate reader's arbitrary relative luminescence units (ΔRLU).

3. FUTURE PROJECT VISION

3.1. Technology Scaling

Three complementary components need to be considered in the design of a biosensor-based environmental monitoring network: hardware, software and bioware. While Re-Sense has concentrated its effort on the latter aspect, in order for the developed technology to be scaled up, it is required that progress be made in all three directions. To reach at least TRL6, the following steps need to be taken:

- Design, construct and test a hardware module incorporating the sensor cells
- Continue molecular enhancement of sensor strains performance, aimed at specific applications and targets
- Optimize sensor strain integration into the hardware platform
- Develop the necessary software for signal acquisition and analysis

3.2. Project Synergies and Outreach

The principle strengths of the Re-Sense partners are in synthetic biology, computational biology and environmental sciences. As is indicated in section 5.1, the main fields of expertise that need to be enter the collaboration are engineering/electronics/physics, for designing and constructing the hardware platform that will incorporate the sensor cells. Out of the current ATTRACT Phase 1projects, SENSEI seems to be an ideal partner for Phase 2. Other potential partners will be sought over the next few months, inside and outside of the ATTRACT community.

Envisioned dissemination activities will include a public website and proactive involvement in community outreach activities, with a particular focus on high schools.

3.3. Technology application and demonstration cases

Whole-cell biosensors may be applied towards diverse environmental monitoring applications. In Phase 1, we have proven the biocomputation enhancement concept using explosives' detection as a model application; in Phase 2 we will concentrate on water quality monitoring, demonstrating two different implementations: (a) a stationary online flow-through device for real time monitoring of water quality; (b) a portable hand-held unit for spot checks. We envision a partnership that will include research groups across Europe, end-users in the water quality industry, and commercial entities for the production and marketing the envisaged systems.

3.4. Technology commercialization

As indicated above, the consortium that will be put together in Phase 2 for the design, construction and testing of water quality monitoring biosensors will include at least one entity dedicated to the commercialization of the technology.

3.5. Envisioned risks

The main risk we see is in the public acceptance of the use of genetically engineered microorganisms. To mitigate this risk, we will introduce molecular modifications that will prevent the bacterial sensors from (a) surviving outside the environment of the monitoring device and (b) transfer antibiotic resistance genes to other organisms. In addition, in our outreach and dissemination activities we will emphasize the non-

existent risks involved in the use of our sensor microorganisms, as well as our intention to fully abide with all relevant laws and regulations.

3.6. Liaison with Student Teams and Socio-Economic Study

Both the Tel Aviv University and the Hebrew University Faculty of Science and Business Schools lead several entrepreneurship programs, in which graduate students teams join forces with a specific research group, providing insights and ideas as well as contributing to the actual research efforts and to the conceptualization of the final product. Re-Sense Phase 2 will be integrated into one of these programs, and hopefully to at least one additional program in one of the consortium's partner institutions. A PI in each of the relevant universities will be appointed as a mentor to the students and a liaison to their parent program. Re-Sense Phase 2 will also be integrated into at least one of the universities' iGEM programs, to be lead by one of Re-Sense PIs. Furthermore, aspects of Re-Sense Phase 2 will be taught in the synthetic and systems biology courses that the PIs teach at TAU and HUJI.

Re-Sense Phase 2 will gladly contribute to all socio-economic studies initiated by the ATTRACT initiative.

4. ACKNOWLEDGEMENTS

This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222. This study was also supported in part by a fellowship from the Edmond J. Safra Center for Bioinformatics at Tel-Aviv University, by the Ofakim Research Fellowship by Miri and Efraim, and by the Minerva Center for Bio-Hybrid Complex Systems.

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