Anexo I: Resultados de la optimización

La optimización fue llevada a cabo para obtener las mejores soluciones de tres objetivos concretos: (J1) la producción de naringenina, (J2) el error de naringenina tras la producción y (J3) el tiempo de respuesta del enzima. Asimismo, se propusieron 5 variables de decisión que serían fáciles de modificar en el laboratorio. Las gráficas Frente Pareto de las 26 soluciones se encuentran en las Figuras 1 y 2. De ellas, 4 fueron seleccionadas como las mejores (sección 4.3.2).

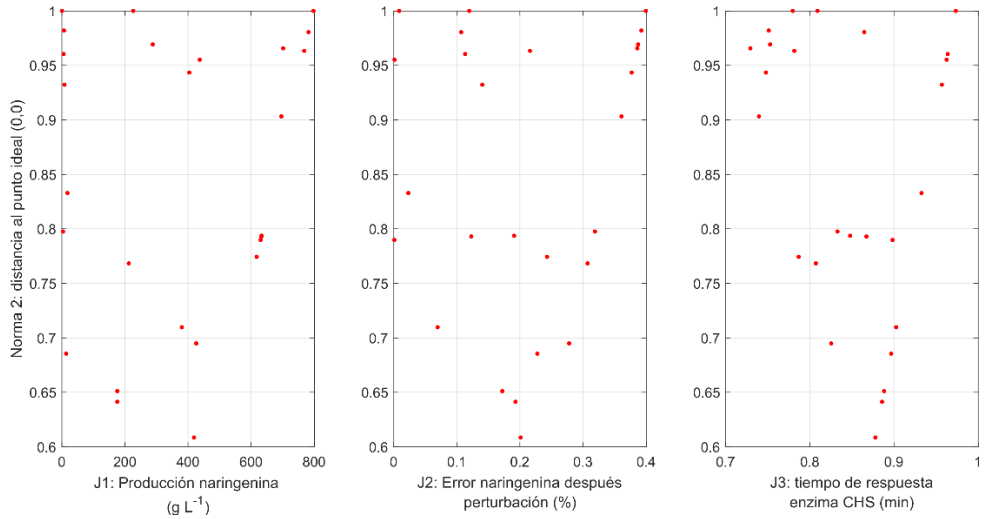

Figura 1. Frente Pareto obtenido de la optimización de parámetros. *Cada punto representa una de las soluciones de las 26 obtenidas para cada objetivo, siendo mejor aquella que aumente la producción de naringenina y se aleje menos del punto ideal (0,0).*

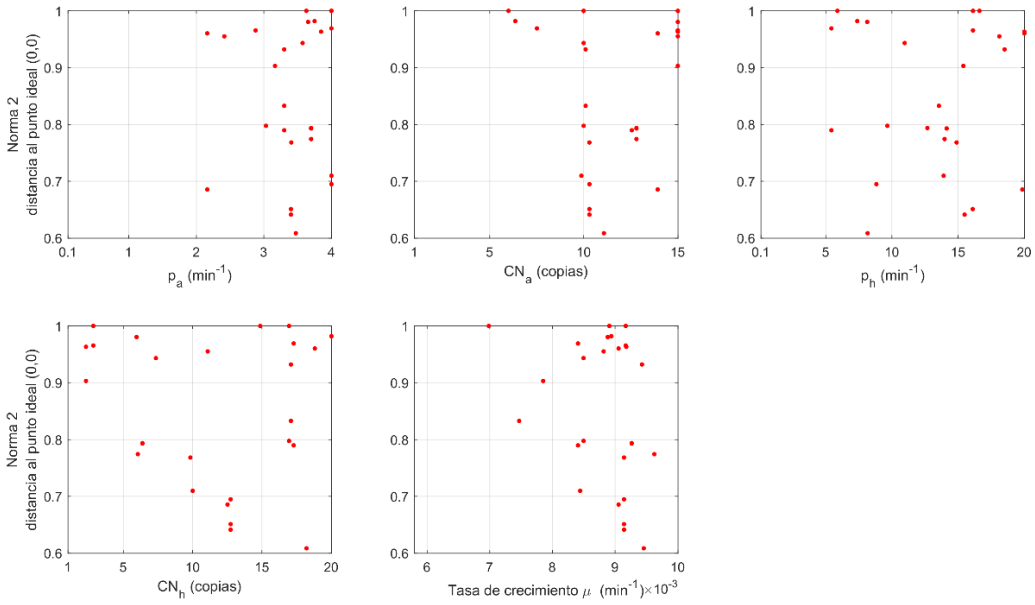

Figura 2. Soluciones de Frente Pareto obtenidas para las variables de decisión. *Cada variable de decisión (pa, CNa, pchs, CNchs, μ) presenta un valor para cada solución de los objetivos.*

Anexo II: Artículos de Revista y de Congreso derivados de este trabajo

Dynamic pathway regulation: extended biosensor and controller tuning with multiobjective optimization

Yadira Boada[1], **Alejandro Vignoni**[1], **Ana Fraile**[2], **Jesús Picó**[1], **Pablo Carbonell**[1]
[1]Synthetic Biology and Biosystems Control Lab, Instituto de Automática e Informática Industrial, Universitat Politécnica de València, [2]Escuela Téc. Sup. de Ingeniería Agronómica y del Medio Natural, Universitat Politécnica de València
{yaboa,alvig2,anfrlo,jpico,pjcarbon}@upv.es

1 BACKGROUND

Natural cells preserve robust growth and endure environmental changes by dynamically adapting cell metabolism by means of complex regulatory networks [6]. However, these complex regulation strategies are the result of years of evolution and they are not compatible with production levels demanded by the industry. Major improvements in yield, titer, and productivity can be achieved by balancing pathway gene expression. There are mainly two different ways of doing this balancing: static control, and dynamic pathway regulation.

Static pathway regulation strategies (Figure 1A) are optimized for a particular situation, and therefore they are incapable of responding to growth and environmental changes that occur during fermentation in a bioreactor [10]. Dynamic balancing addresses the robustness pitfalls of static control through the application of feedback and feedforward regulation (Figure 1B). This makes it possible to obtain higher titers as compared to static regulation [9]. Despite a growing number of success stories, engineering dynamic control remains extremely challenging [5]. Moreover, the performance specifications for synthetic gene circuits and components change significantly with variations in parameters such as temperature, host organism, growth media formulation, and position of the genes in the genome [8]. Model-based design relying on the principles of control engineering can provide a powerful formalism to engineer dynamic control circuits and address these challenges. These, together with the tools of synthetic biology, can lead to robust and efficient microbial production at industrial levels [6, 8].

2 METHODS

In this work, we propose using a multiobjective optimization (Figure 1C) approach to optimally tune a recently developed dynamic pathway regulation strategy [4]. The metabolic pathway is a phenylpropaniod pathway to produce the metabolite Naringenin (Figure 1A). The controller used to regulate the pathway is the novel antithetic controller [1] in combination with our recently proposed extended metabolic biosensor [4] (Figure 1B). These two pieces together imply a complexity that needs several objectives to be fulfilled simultaneously (i.e. low titer error, fast response to perturbations, parametric robustness, and closed-loop stability among others). In general, these objectives are in conflict and a trade-off must be reached. Multiobjective optimization has shown to be a valuable tool in these situations [3]. With the dynamic system model at hand (developed in [4]), the following steps are necessary for a successful multiobjective optimization: i) define the multiobjective problem (objectives to be optimized), ii) perform the optimization to obtain the solutions (Pareto Front and Pareto Set), and iii) select among the resulting solutions the ones that fulfill the requirements of the design. The result of this optimization is a set of guidelines for the implementation of the biosensor and the controller *in vivo*. Then, when this approach is combined with a collection of parts previously characterized, the results can be interpreted as suggestions about how to select parts like RBS, promoter, plasmid, or enzyme (gene variant) from the collection.

3 DISCUSSION

Several authors have recently explored approaches to help in the tuning of the antithetic controller [2, 7]. Nevertheless, these models and their level of detail are not enough to assist in the *in vivo* implementation of the system.

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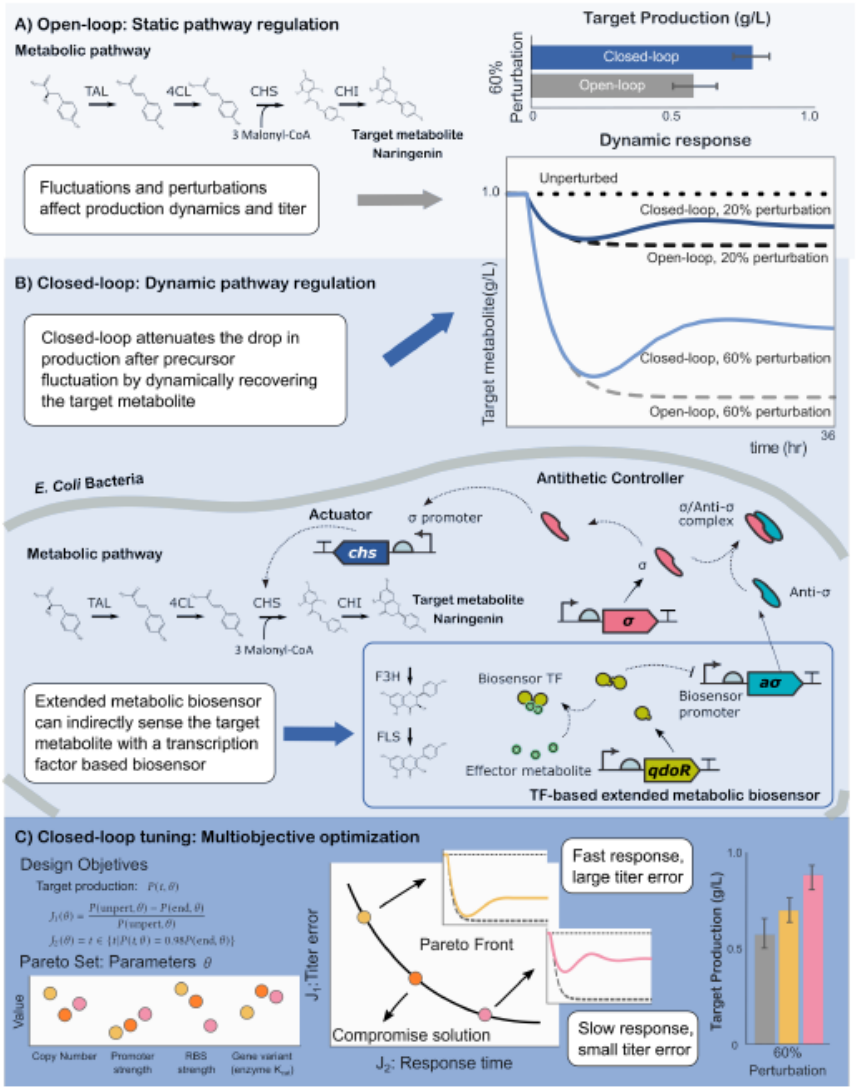

Figure 1: Dynamic pathway regulation: Biosensor and controller parameter tuning.

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