

Technical University of Denmark



## MARSI: metabolite analogues for rational strain improvement

**Cardoso, Joao; Zeidan, Ahmad A; Jensen, Kristian; Sonnenschein, Nikolaus; Neves, Ana Rute; Herrgård, Markus J**

*Published in:*  
Bioinformatics

*Link to article, DOI:*  
[10.1093/bioinformatics/bty108](https://doi.org/10.1093/bioinformatics/bty108)

*Publication date:*  
2018

*Document Version*  
Peer reviewed version

[Link back to DTU Orbit](#)

*Citation (APA):*  
Cardoso, J. G. R., Zeidan, A. A., Jensen, K., Sonnenschein, N., Neves, A. R., & Herrgård, M. J. (2018). MARSI: metabolite analogues for rational strain improvement. *Bioinformatics*, [bty108]. DOI: 10.1093/bioinformatics/bty108

## DTU Library

Technical Information Center of Denmark

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## Systems biology

# MARSI: metabolite analogues for rational strain improvement

João G. R. Cardoso<sup>1</sup>, Ahmad A. Zeidan<sup>2,\*</sup>, Kristian Jensen<sup>1</sup>, Nikolaus Sonnenschein<sup>1</sup>, Ana Rute Neves<sup>2</sup> and Markus J. Herrgård<sup>1,\*</sup>

<sup>1</sup>The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark <sup>2</sup>Discovery, Chr. Hansen A/S, DK-2970, Hørsholm, Denmark.

\* To whom correspondence should be addressed

Received on XXXXX; revised on XXXXX; accepted on XXXXX

### Abstract

**Summary:** Metabolite analogues (MAs) mimic the structure of native metabolites, can competitively inhibit their utilization in enzymatic reactions, and are commonly used as selection tools for isolating desirable mutants of industrial microorganisms. Genome-scale metabolic models representing all biochemical reactions in an organism can be used to predict effects of MAs on cellular phenotypes. Here, we present the Metabolite Analogues for Rational Strain Improvement (MARSI) framework. MARSI provides a rational approach to strain improvement by searching for metabolites as targets instead of genes or reactions. The designs found by MARSI can be implemented by supplying MAs in the culture media, enabling metabolic rewiring without the use of recombinant DNA technologies that cannot always be used due to regulations. To facilitate experimental implementation, MARSI provides tools to identify candidate MAs to a target metabolite from a database of known drugs and analogues.

**Availability and Implementation:** The code is freely available at <https://github.com/biosustain/marsi> under the Apache License V2. MARSI is implemented in Python.

**Contact:** DKAHZE@chr-hansen.com, herrgard@biosustain.dtu.dk

**Supplementary information:** Supplementary data are available at *Bioinformatics* online.

## 1 Introduction

Genome-scale metabolic models (GEMs) describe the biochemical reactions in an organism and their relation to the proteome and genome (McCloskey *et al.*, 2013). These models comprehensively represent natural metabolism and they are useful for predicting the effect of metabolite analogues (MAs) as therapeutics (Agren *et al.*, 2014; Kim *et al.*, 2014).

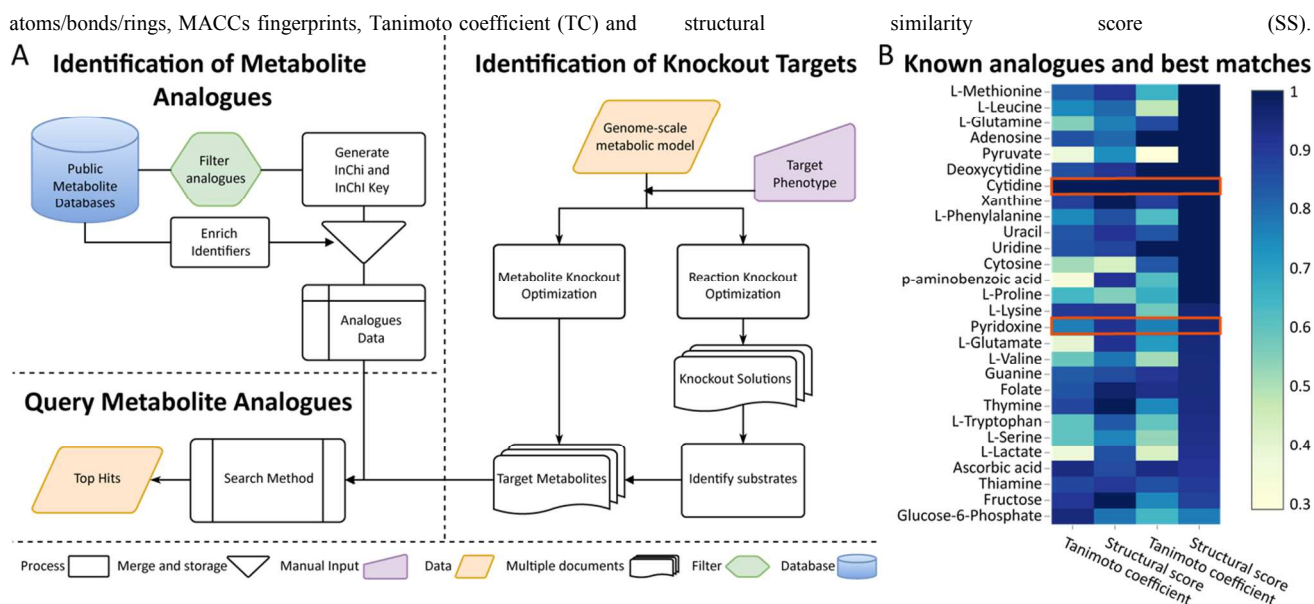
Non-rational strategies such as mutagenesis and selection or laboratory evolution can be used to develop industrial strains when the use of recombinant DNA technology is not allowed due to regulations (Derkx *et al.*, 2014; Hansen *et al.*, 2017). MAs, inhibiting the enzymatic conversion of the target metabolite, act as metabolite knockouts and can be used as the selective pressure in non-rational strategies to shape the metabolism of microorganisms (Sørensen *et al.*, 2016).

Here, we present software that implements workflows to identify metabolite knockouts instead of gene or reaction knockouts. We also provide a pipeline to identify structural analogues for those targets.

## 2 Materials and Methods

The first workflow consists of systematically replacing reaction knockouts (identified by other strain design methods) by metabolite knockouts, until we can find metabolite targets that result in a similar flux distribution. The second workflow consists of searching for metabolite targets using heuristic optimization, without the need to specify reaction knockouts *a priori*. A metabolite knockout consists of blocking all reactions consuming a given metabolite, excluding transporters.

After identifying the metabolite targets, we search for MAs similar to them. We compiled a database of potential MAs from publicly available sources (see Supplementary Information). We use OpenBabel (O'Boyle *et al.*, 2011) and RDKit (<http://www.rdkit.org>) to calculate the features used to compare candidate MAs to the target metabolite: number of



**Fig. 1. Metabolite target identification workflow and examples of MA targets.** (A) The workflow for identifying for metabolite knockouts and candidate MAs. (B) Comparison between the known MAs (columns 1 and 2) and the best MARSIs hits (columns 3 and 4) used to calibrate the search parameters. We show the Tanimoto coefficient and the structural score. We highlighted rows where the best MARSIs hit and the known MA are the same.

### 3 Results

We implemented a software package containing algorithms to generate strain design strategies using MAs. Our software could generate metabolite targets for a published knockout-based design (Harder et al., 2016). We also provide the tools to identify candidate MAs that could be used for implementation of the designs.

#### 3.1 Identification of replacement targets

We used an experimentally validated strain design for itaconic acid production in *Escherichia coli* (Harder et al., 2016) and the *E. coli* GEM iJO1366 (Orth et al., 2014) to demonstrate the use of MARSIs. MARSIs identified acetyl-phosphate as a metabolite knockout target that can replace the PTAr reaction knockout and sustain the same flux for itaconic acid production (Table 1). Using a SS cutoff of 0.5 (see Supplementary Information), we found 182 MAs for acetyl-phosphate (Table S1 shows the top 10 hits). More examples of replacement targets in other *E. coli* strain designs can be found in Supplementary Information.

**Table 1.** Knockout replacements for the strain design. We use Biomass Product Coupled Yield (Patil et al., 2005) as fitness measure.

| Non-replaced knockouts                   | Replaced reaction | Metabolite | Original fitness | New fitness |
|--|-------------------|------------|------------------|-------------|
| PTA2, ICL, ALDD2x, PYK, SUCOAS, GGGABADr | PTAr              | Acetyl-P   | 0.001            | 0.001       |

#### 3.2 Query calibration with known metabolite analogues

In order to validate the ability of MARSIs to find known analogues for a target metabolite, we selected 42 known metabolite-MA pairs from the literature (Table S3). We compared the structural features between the MAs and their target metabolites (Figure S1). We used a distance of 4 for the number of atoms, 3 for the number of bonds and 2 for the number of rings as our query cutoff. The TC cutoff changes dynamically with the size of the metabolites (see Supplementary Information). In Figure 1B, we show the SS and TC for different targets and their known analogues

as well as the best hit analogue in the database. For most targets MARSIs found candidate MAs that showed higher structural similarity to the target metabolite than the known analogue.

### Acknowledgements

We would like to thank Miguel Campodonico for input on cheminformatics tools.

### Funding

This work has been supported by the Novo Nordisk Foundation. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 686070.

*Conflict of Interest:* None declared.

### References

Agren, R. et al. (2014) Identification of anticancer drugs for hepatocellular carcinoma through personalized genome-scale metabolic modeling. *Mol. Syst. Biol.*, **10**, 721–721.

Derkx, P.M. et al. (2014) The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. In *Microbial Cell Factories*, p. S5.

Hansen, A.S.L. et al. (2017) Systems biology solutions for biochemical production challenges. *Curr. Opin. Biotechnol.*, **45**, 85–91.

Harder, B.-J. et al. (2016) Model-based metabolic engineering enables high yield itaconic acid production by *Escherichia coli*. *Metab. Eng.*, **38**, 29–37.

Kim, H.U. et al. (2014) Integrative genome-scale metabolic analysis of *Vibrio vulnificus* for drug targeting and discovery. *Mol. Syst. Biol.*, **7**, 460–460.

McCloskey, D. et al. (2013) Basic and applied uses of genome-scale metabolic network reconstructions of *Escherichia coli*. *Mol. Syst. Biol.*, **9**, 661.

O'Boyle, N.M. et al. (2011) Open Babel: An Open chemical toolbox. *J. Cheminform.*, **3**, 1–14.

Orth, J.D. et al. (2014) A comprehensive genome-scale reconstruction of *Escherichia coli* metabolism—2011. *Mol. Syst. Biol.*, **7**, 535–535.

Patil, K.R. et al. (2005) Evolutionary programming as a platform for in silico metabolic engineering. *BMC Bioinformatics*, **6**, 308.

RDKit: Open-source cheminformatics (2016).

Sørensen, K.I. et al. (2016) Enhancing the sweetness of yoghurt through metabolic remodeling of carbohydrate metabolism in *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Appl. Environ. Microbiol.*, **82**, AEM.00462-16.