XTMS in action: retrosynthetic design in the extended metabolic space of heterologous pathways for high-value compounds

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Abstract. Despite the increase in recent years in the portfolio of added-value chemicals that can be microbially produced, the design process still remains a complex system, costly and rather slow. To overcome such limitations, the development of Computer-Aided-Design (CAD) tools is necessary to design production pathway that systematically screen metabolic databases to select best genes to import into chassis organisms. Here, we showcase the XTMS CAD tool for pathway design, which exploits the ability for pathway ranking in our RetroPath retrosynthetic algorithm within an extended metabolic space that considers putative routes through enzyme promiscuity. The validity of the ranking function for the production of malonyl-CoA is presented.

Keywords: synthetic biology; metabolic engineering; promiscuity; computer-aided-design

1 Introduction

With recent advances in synthetic biology and metabolic engineering, synthetic production of high-value compounds in industrial hosts such as *Escherichia coli* or *Saccharomyces cerevisiae* is becoming more and more promising. Nevertheless, finding the best pathways achieving high-yield production is still challenging. Missing annotations cause ineluctably the loss of potentially interesting pathways. Moreover, it is well-known that enzymatic reactions often display the ability of accepting several similar substrates (even un-natural ones), although this promiscuous capacity has been underexploited so far.

To overcome such limitations, we hereby present the eXTended Metabolic Space server (XTMS) [2] in operation, a novel CAD pathway tool that integrates our expertise on retrosynthesis of high-value compounds (Retropath algorithm [1]) with an *in silico* metabolic space representation extended from endogenous compounds of the host organism. XTMS is designed to be user-friendly and pragmatic as it provides the user with critical information in order to assess generated pathways' quality, notably in the form of a ranking function. The validity of this ranking function has been recently highlighted by the construction of several pathways producing malonyl-CoA.

2 Material and methods

We used reactome and metabolome data from MetaCyc and EcoCyc to build our initial set of compounds and reactions covering the most part of current metabolic knowledge. Those elements were encoded by an in-house molecular representation, the molecular signature, which enable reverse engineering, meaning one can retrieve a compound's structure from its molecular signature. For a given encoded reaction rule, we iterate though all encoded compounds attempting to use the reaction rule on different substrates. When a compound is compatible with a reaction rule, the predicted product of the reaction rule might be a novel encoded compound. This process is repeated for all the reaction until no more new compound can be generated. Some of the generated compounds have not yet been reported as compounds whose synthesis is potentially accessible with natural enzymes.

Retropath is a CAD software for embedded metabolic circuits. Retropath's retrosynthesis algorithm is at the core of XTMS, using the extended metabolic space as a base to enumerate pathways. Pathway ranking is carried out using a ranking function which compiles information such as maximum pathway yield, enzyme efficiency, estimated compound toxicity or the number of enzymatic steps.

3 Results and discussion

XTMS is a user-friendly pathway CAD tool now available to the community (`http://xtms.issb.genopole.fr/`). Its strengths are to work on an extended metabolic space and to provide critical information to the user, as for example the predicted toxicity of generated pathways. In order to evaluate XTMS's ranking score, we predicted and implemented several malonyl-CoA pathways in *E. coli*. A circuit consisting on a malonyl-CoA biosensor was then used to access pathways' yield [3]. As we expected, we succeeded to predict the best pathways. Moreover, the order of the more productive pathways paralleled the one in our pathway ranking. Even if the evaluation of the ranking function needs further validation, those results are encouraging. We hope that XTMS will help metabolic engineers to design efficient circuits able to produce high-value compounds and that their comments will help us to improve XTMS.

References

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