Optimisation of Productivity and Thermodynamic Performance of Metabolic Pathways

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Abstract

In this contribution, a novel optimisation strategy has been presented that combines the metabolic flux analysis and pathway identification with the thermodynamic analysis of cellular metabolic systems. First, an optimal metabolic flux distribution among elementary pathways is identified by LP optimisation subject to constraints on flux balance analysis, pathway analysis and negative Gibbs free energy change for pathways, for achieving the maximum yield of products. The Gibbs free energy change for pathways is calculated from the new transformed Gibbs free energy of formation of external metabolites and cofactors that are in stoichiometric balance in metabolic pathways. The consideration of thermodynamic constraints on pathways ensures the selection of feasible pathways. Thereafter, the Gibbs free energy change of pathways is minimised to predict the optimal reaction conditions that facilitate such pathways. Thus the optimisation approach derives the optimal pathway distribution and conditions for the best performance of cellular systems. The effectiveness of the methodology is demonstrated by a case study on the optimisation of pentose phosphate pathway (PPP) and the glycolysis cycle of the insilico Escherichia coli.

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1. Introduction

Metabolic pathways are complex networks of enzyme catalyzed reaction steps presented in terms of internal metabolites, external metabolites and cofactors (a glossary on the terminologies has been provided in Appendix A). Such pathways, also known as elementary pathways are central to cellular biochemical activities, which channel substrate metabolites into production of energy currency, building blocks for biosynthesis, energy reserves, eliminating waste products, and for recycling reducing equivalents[1]. In this light, to quantify intracellular reaction steps and pathways and infer the objectives of cellular metabolic systems, rational modeling strategies need to be developed for altering or optimising cell properties. Optimisation of cellular properties can achieve physiological endpoints corresponding to the production of a desired external metabolite. Hence, optimal metabolic pathways responsible for the productivity of a desired metabolite can be predicted. A rational modeling approach, such as, based on thermodynamic analysis for cellular properties may also elucidate the spontaneity and existence of driving force for the occurrence of metabolic pathways responsible for a desired product. Thus, the selection of optimal metabolic pathways for the productivity of a desired cellular product based on thermodynamic analysis is driven by the relative degree of spontaneity or feasibility among competing pathways. Furthermore, representation of thermodynamic properties for metabolic pathways in terms of cellular reaction conditions attains the optimal conditions corresponding to the productivity of a desired metabolite. In this work, we have proposed an optimisation based methodology for the productivity and reaction conditions of metabolic pathways using thermodynamic analysis.

In recent years, several theoretical approaches have been developed to assign metabolic priorities through engineered cells. An optimisation-based framework, called ObjFind, has been established to infer and test hypothesized metabolic pathways and objectives depending upon experimental results[2]. A representative

modeling framework for metabolic analysis is the flux balance analysis (FBA), which can be used to infer the objectives of cellular metabolism[3,4,5]. FBA is a constrained optimisation approach based on linear programming (LP), and provides a desired physiological endpoint, e.g., the maximum growth rate, and its corresponding flux distribution under some culture conditions. In FBA, individual reaction steps and their corresponding metabolic flux balances, rather than metabolic pathways, are considered. The application of FBA has been effectively dealt with metabolic flux analysis problems for various kinds of networks[6,7,8]. Mahadevan et al.[9] have extended FBA for analyzing the dynamic reprogramming of a metabolic network. This dynamic FBA can be used to understand the dynamic behaviour of metabolic networks, and additionally, provide strategies for the design of a network with a desired objective for metabolic engineering. However, some critical issues about FBA remain unresolved[10], such as the uniqueness of flux distribution and its practical application. Firstly, the implementation of LP in FBA frequently leads to multiple (or alternate) optima depending on initial starting point. Thus for a same set of enzymes, different metabolic fluxes (that may belong to different pathways) are selected depending on an initial guess on metabolic fluxes. In practice, a set of enzymes is selectively responsible for a pathway or a set of pathways and an alteration of which disrupts such pathways to occur[11]. In addition, it is difficult to apply FBA directly to predict the metabolic genotype-phenotype relation (e.g. gene to cellular expression) from a reaction-based perspective[12]. Hence, FBA, without consideration of pathways, does not capture the genetic or enzymatic manipulation of cellular activities. Pathway analysis, on the other hand, is aimed at genetic / enzymatic manipulation of cells, which enables any steady-state metabolic network to be expressed as a collection of elementary pathways. Each elementary pathway is stoichiometrically feasible for a minimum set of enzymes[13], and the activity of which can be controlled by inhibition or activation of its responsible enzymes. All these elementary pathways should be regarded as the true functional units of metabolic systems consisting of a series of irreversible reaction steps. Thus, a cell can be regulated by selective control of activities of elementary pathways to enhance the yield and rate of a metabolic production. Hence, we combine pathway analysis with FBA to integrate metabolic engineering to genetic technology for the

synthesis of novel products or redirecting metabolite fluxes towards a desired product. This also eliminates the existence of infeasible flux distribution caused by FBA uncertainty.

Optimal metabolic fluxes based on mass balance thus obtained by combining pathway and flux balance analyses does not ensure feasibility of pathways. Hence, thermodynamic analysis can be instrumental to the selection of feasible pathways and identifying optimal cellular environment for metabolic systems. Thermodynamic insights into metabolic reaction networks or pathways are useful in estimating the key parameters in biotechnological cultures and thus to address reaction viability of bioprocesses[14]. In thermodynamic terms, the difference in Gibbs free energy sets the driving force for any system undergoing changes. For any phenomenon occurring spontaneously, its Gibbs free energy change ought to be negative. This is the basis for assessing the thermodynamic feasibility of a pathway in metabolic systems. Moreover, a pathway for which the free energy change is large and negative has an equilibrium that favors the side of products. Recently, Gibbs free energy changes for individual reaction steps in a metabolic pathway have been presented by Nolan et al.[1] in order to consider negative constraint on Gibbs free energy change for individual metabolic pathways in pathway analysis. In their study, the Gibbs free energies of formation of metabolic species are estimated using group contribution theory[15]. They have applied directionality criterion for net mss flux in the form of negative Gibbs free energy change to a pathway, as opposed to a reaction step. In many cases, cellular biochemistry, through multi-functional enzyme action, substrate channeling, or other mechanisms, couples an energetically unfavorable metabolic reaction to an energetically favorable one. In these cases, the negative Gibbs free energy change criterion applied to individual reactions would incorrectly predict opposing flux directions for consecutive reactions. Nolan et al.[1] presented an example of glycolysis which includes at least three reaction steps with unfavorable Gibbs free energy changes in the established direction from glucose to lactate[16].

In this paper, we have introduced the new transformed Gibbs free energies of formation[17] in terms of *pH*

and ionic strength, which are more suitable for biochemical reaction systems, for external metabolites and cofactors that are stoichiometrcally balanced in individual elementary pathways. Hence, in our work, Gibbs free energy changes have been directly presented for elementary pathways in a metabolic system as opposed to the work by Nolan et al.[1], who considered thermodynamic descriptors for reaction steps to predict that for pathways. Moreover, Gibbs free energy changes of pathways have been minimised to attain the optimal *pH* and ionic strength conditions to facilitate such pathways.

The paper is structured as follows. The methodology[18] for the elucidation and optimisation of metabolic systems has been detailed in the first part. In the second part, a case study on the metabolism network of pentose phosphate pathways (PPP) and glycolysis cycle of in silico Escherichia coli has been established to demonstrate the effectiveness of this methodology.

2. Methodology

2.1 Thermodynamic analysis of metabolic reaction network

Thermodynamic feasibility and optimisation of metabolic pathways based on Gibbs free energy changes is presented to formulate the optimisation problem for metabolic productivity and optimal reaction conditions. Generally speaking, there are two kinds of reaction equations, chemical equations which balance elements and charge, and biochemical equations written in terms of biochemical reactants at a specified *pH*[17]. In biochemical equations, hydrogen numbers are assumed fixed at a constant *pH*. The conventional thermodynamic properties thus can not represent biochemical systems precisely. Therefore, it is necessary to define the new transformed thermodynamic properties, like Gibbs free energy of formation, for biochemical reactants[17]. Metabolic pathways can be expressed by biochemical equations. Hence, the new transformed Gibbs free energies of formation should be computed for the metabolites in a system. The

calculation of the standard formation Gibbs free energy of biochemical reactants as a function of *pH* and an ionic strength *I* is illustrated in Fig. 1.

2.1.1 Gibbs free energy change for biochemical reaction

The Gibbs free energy G for a reaction system at a specific temperature (*T*) and pressure (*P*) is described in terms of species.

$$G = \sum_{i=1}^{N} n_i \mu_i \qquad (1)$$

In Eq. (1), μ_i and n_i are chemical potential and amount of specie *i* respectively and *N* is the number of species in the system.

Eq. (1) provides the basis for deriving the data on the Standard formation Gibbs free energy $\Delta G_f^{\,o}$ of individual species. The superscript ^{o} and the word standard mean that the species are in aqueous solutions at 1 M (1 M = 1 mol/l), while the interactions between ions and water are same as that in infinite dilution. Most of the $\Delta G_f^{\,o}$ values are taken from the NBS Tables of Chemical Thermodynamic Properties[19]. The $\Delta G_f^{\,o}$ values for the *ATP* series are from[20].

The thermodynamic properties of a solution are affected by the interactions between the species in it. Therefore, the effect of ionic strength in a solution plays an important role in determining its thermodynamic state. Since the biochemical reactions mostly work at ionic strength which ranges from 0.1 to 0.3 ($I = 0.1 - 0.3M$), the effects of the solution ionic strength have to be considered for the correction of the standard formation Gibbs free energy values. The standard formation Gibbs free energy of specie *i* at an ionic strength *I* can be calculated from the one at zero ionic strength using Eq. (2)[21].

$$\Delta G_{f,i}{}^{o} = \Delta G_{f,i}^{o}(I=0) - 2.91842 z_i{}^2 I^{1/2}/(1+BI^{1/2}) \quad (2)$$

The formation Gibbs free energy is expressed in *kJ/mol*, z_i is the charge in specie *i*, and $B = 1.6L^{1/2}mol^{1/2}$.

Biochemical reactions are enzyme-catalysed reactions at a specified *pH*. Many of the reactants are weak acids so that H^+ is also a reacting specie. When the concentration of a reacting specie is specified at an equilibrium, the thermodynamic properties of the solution depend on its specific concentration, as well as on the temperature and pressure. Thus, a new standard formation Gibbs free energy for species in a biochemical reaction is introduced as a function of hydrogen ion concentration (Eq. (3)).

$$\begin{aligned}\Delta G'_{f,i}{}^{o} &= \Delta G_{f,i}{}^{o} - N_i(H)\{\Delta G_f{}^{o}(H^+) + RT\ln([H^+]/c^o)\} \\ &= \Delta G_{f,i}{}^{o} - N_i(H)\{\Delta G_f{}^{o}(H^+) - 2.303RT \times pH\} \quad (3)\end{aligned}$$

In Eq. (3), $N_i(H)$ is the number of hydrogen atoms in species *i*, and $pH = -\log([H^+]/c^o)$

In biochemical reactions, the reactants consist of species in equilibrium. Once we have calculated the standard formation Gibbs free energy $\Delta G'_{f,i}{}^{o}$ at a *pH* for all the species of a reactant, the next step is to combine the $\Delta G'_{f,i}{}^{o}$ values of these species as an integrated property for the reactant. The standard formation Gibbs free energy $\Delta G'_f$ of reactants at a specific *pH* is given by Eq. (4). Since all the external metabolites and cofactors in metabolism networks are expressed as biochemical reactants, thus, their corresponding formation Gibbs free energy $\Delta G'_{f,e\in EM}$ should also calculated by Eq. (4).

$$\Delta G'_{f,e\in EM} = \Delta G'_f(react) = -RT\ln\{\sum_{i=1}^{N_{iso}} \exp(-\Delta G'^{o}_{f,i}/RT)\} \quad (4)$$

In Eq. (4) N_{iso} is the number of species of the reactant, and $\Delta G'_{f,i}{}^{o}$ is the standard formation Gibbs free energy of species of a reactant, $\Delta G'_f(react)$.

From Eqs. (2), (3) and (4), the standard formation Gibbs free energies of the external metabolites and cofactors, $\Delta G'_{f,e\in EM}$, in a metabolic pathway can be evaluated at a given *pH* and ionic strength. In the following section, an illustration on the calculation of the standard formation Gibbs free energy of metabolites is presented.

2.1.2 An example for calculation

An example is presented herein to elucidate the procedure of formation Gibbs free energy calculation of biochemical reactants. Reactant ATP in biochemical reactions refers to an equilibrium mixture of ATP^{4-}, $HATP^{3-}$ and H_2ATP^{2-} at a specified *pH*. The standard formation Gibbs free energy for ATP^{4-}, $HATP^{3-}$ and H_2ATP^{2-} at 25℃, 1 bar, and zero ionic strength ($I=0$), as well as their ionic charges and hydrogen atom numbers are shown in Table 1.

Firstly, the formation Gibbs free energy of species ATP^{4-}, $HATP^{3-}$ and H_2ATP^{2-} at $I=0.28$ are calculated using Eq. (2).

$$\Delta G_{f,ATP^{4-}}{}^{o}(I=0.28)=\Delta G^{o}_{f,ATP^{4-}}(I=0)-2.91842 z_{ATP^{4-}}{}^{2} I^{1/2}/(1+BI^{1/2})$$

$$=-2573.49-2.91842\times 4^{2}\times 0.28^{1/2}/(1+1.6\times 0.28^{1/2})=-2586.87\ kJ/mol$$

$$\Delta G_{f,HATP^{3-}}{}^{o}(I=0.28)=\Delta G^{o}_{f,HATP^{3-}}(I=0)-2.91842 z_{HATP^{3-}}{}^{2} I^{1/2}/(1+BI^{1/2})$$

$$=-2616.87-2.91842\times 3^{2}\times 0.28^{1/2}/(1+1.6\times 0.28^{1/2})=-2624.40\ kJ/mol$$

$$\Delta G_{f,H_2ATP^{2-}}{}^{o}(I=0.28)=\Delta G^{o}_{f,H_2ATP^{2-}}(I=0)-2.91842 z_{H_2ATP^{2-}}{}^{2} I^{1/2}/(1+BI^{1/2})$$

$$=-2643.58-2.91842\times 2^{2}\times 0.28^{1/2}/(1+1.6\times 0.28^{1/2})=-2646.93\ kJ/mol$$

$$\Delta G_{f,H^{+}}{}^{o}(I=0.28)=\Delta G^{o}_{f,H^{+}}(I=0)-2.91842 z_{i}{}^{2} I^{1/2}/(1+BI^{1/2})$$

$$=0-2.91842\times 2^{2}\times 0.28^{1/2}/(1+1.6\times 0.28^{1/2})=-0.84\ kJ/mol$$

Next, the formation Gibbs free energy of species at a *pH=6.8* are calculated by Eq. (3).

$$\Delta G'^{o}_{f,ATP^{4-}} = \Delta G_{f,ATP^{4-}}{}^{o} - N_{ATP^{4-}}(H^{+})\{\Delta G_{f}{}^{o}(H^{+}) - 2.303RT \times pH\}$$

$$= -2586.87 - 12 \times \{-0.84 - 2.303 \times 0.008314 \times 298 \times 6.8\} = -2111.24 \ kJ/mol$$

$$\Delta G'^{o}_{HATP^{3-}} = \Delta G_{f,HATP^{3-}}{}^{o} - N_{HATP^{3-}}(H^{+})\{\Delta G_{f}{}^{o}(H^{+}) - 2.303RT \times pH\}$$

$$= -2624.40 - 13 \times \{-0.84 - 2.303 \times 0.008314 \times 298 \times 6.8\} = -2109.13 \ kJ/mol$$

$$\Delta G'^{o}_{f,H_2ATP^{2-}} = \Delta G_{f,H_2ATP^{2-}}{}^{o} - N_{H_2ATP^{2-}}(H^{+})\{\Delta G^{o}{}_{f}(H^{+}) - 2.303RT \times pH\}$$

$$= -2646.93 - 14 \times \{-0.84 - 2.303 \times 0.008314 \times 298 \times 6.8\} = -2092.02 \ kJ/mol$$

Reactant ATP is a mixture of the three species: ATP^{4-}, $HATP^{3-}$ and H_2ATP^{2-}. Thus, the number of species for ATP is 3 ($N_{iso} = 3$). The formation Gibbs free energy of reactant ATP is calculated using Eq. (4).

$$\Delta G'_{f}(ATP) = -RT \ln\{\exp(-\Delta G'^{o}_{f,ATP^{4-}}/RT) + \exp(-\Delta G'_{f,HATP^{3-}}{}^{o}/RT) + \exp(-\Delta G'_{f,H_2ATP^{2-}}{}^{o}/RT)\}$$

$$= -2376.47 \ kJ/mol$$

2.2 Flux balance analysis and pathway analysis

Flux balance analysis (FBA) and pathway analysis are used for the systematic enumeration of elementary pathways and metabolic flux distributions among them.

FBA is used to describe metabolic system models that include a complete list of reactions and metabolites (external as well as internal) and cofactors involved in each reaction step, in a quantitative manner. For FBA of metabolic systems, the information required is the stoichiometry of metabolic reaction steps, mass balance around internal metabolites under pseudo-steady state and the uptake of external metabolite sources [22]. The process of flux balance analysis is illustrated in Fig. 2.

In FBA, a stoichiometry of metabolic reactions is presented by a $X \times N$ stoichiometric matrix $\overline{S}_{(i,j)}$, in terms of X number of internal metabolites $i \in IM$, in a total of N reactions $j \in R$. Since it is reasonable to place the internal metabolites of a system into a steady state, a set of linear homogeneous equations based on mass balance can be derived. In matrix notation, the expression is presented in Eq. (5). $\overline{V}$ is a $N \times 1$ flux vector and represents the flux distribution of reactions in a metabolic system.

$$\overline{S} \times \overline{V} = 0 \tag{5}$$

Pathway analysis is aimed at genetic / enzymatic manipulation of cells, which enables any steady-state metabolic network to be expressed as a collection of elementary pathways. Each elementary pathway is stoichiometrically feasible for a minimum set of enzymes, and its overall activity can be controlled by inhibition or activation of its responsible enzymes. Therefore, pathway analysis is combined with FBA to eliminate the existence of infeasible flux distribution caused by FBA uncertainty, and also integrate metabolic engineering to genetic technology for achieving the synthesis of novel products or redirecting metabolite fluxes towards a desired product. Based on the combination of FBA and pathway analysis, the optimal mass flux distribution among these elementary pathways for the maximum productivity of a desired metabolite can be predicted. Moreover, each elementary pathway is a collection of individual reaction steps. The overall reaction of an elementary pathway includes only external metabolites and cofactors. Thus, two stoichiometric matrices, $\overline{A}$ and $\overline{U}$, can be derived from pathway analysis. $\overline{A}_{(j,p)}$ represents a $N \times M$ stoichiometric matrix, in terms of N number of reactions $j \in R$, in a total of M elementary pathways $p \in P$. $\overline{U}_{(p,e)}$ is a $M \times Y$ stoichiometric matrix in terms of Y external metabolites and cofactors $e \in EM$ in a total of M elementary pathways $p \in P$. $\overline{B}$ is a $M \times 1$ flux vector referring to the flux distribution in elementary pathways p ($p \in P$) detected in the system, the values of which can not be negative. The relationship between reaction flux distribution $\overline{V}$ and pathway flux distribution $\overline{B}$ can be expressed as:

$$\overline{V} = \overline{A} \times \overline{B} \quad (6)$$

If $\overline{VE}$ is a $Y \times 1$ flux vector in terms of Y number of external metabolites e ($e \in EM$) in a system, the overall equations for elementary pathways give rise to the stoichiometry of overall reactions (Eq. (7)).

$$\overline{VE} = \overline{U} \times \overline{B} \quad (7)$$

Substituting Eq. (6) into Eq. (5), Eq. (8) is resulted.

$$\overline{S} \times (\overline{A} \times \overline{B}) = 0 \quad (8)$$

2.3 Optimisation Approach and Inclusion of Thermodynamic Analysis

An optimisation approach is developed, which combines the metabolic flux analysis and pathway identification as illustrated by Eqs. (5)-(8) in section 2.2 and the thermodynamic analysis discussed in Eqs. (2)-(4) in section 2.1 (Fig. 3). The optimisation approach is presented as two modules. In module 1, the objective is the productivity of an external metabolite (desired product) and is maximised by linear programming (LP). In module 2, thermodynamic optimisation (NLP) in terms of minimisation of the total Gibbs free energy change of a metabolic system is carried out to predict the optimal cellular conditions. The formulations for module 2 are non-linear due to the calculation of formation Gibbs free energy of external metabolites and cofactors. The detailed algorithm of the optimisation strategy is presented in Fig. B1 in Appendix B, and illustrated in the following sections.

2.3.1 Productivity Maximisation

In addition to the existing approaches[3,5,13], we have combined the flux balance analysis together with pathway analysis (section 2.2; Eqs. (5)-(8)) to achieve the optimal flux distribution among pathway modes,

rather than for individual reactions, which satisfies the desired physiological endpoint, eg., the maximum productivity of external metabolites. Moreover, an inequality thermodynamic constraint defining the negative Gibbs free energy change for individual metabolic pathways is used to ensure the feasibility of flux balance analysis (Eq. (9)). The new thermodynamic properties designed for biochemical reactants[17] in terms of the standard formation Gibbs free energy change ($\Delta G'_{f,e\in EM}$) are predicted for external metabolites and cofactors in individual metabolic pathways (Eqs. (2), (3)-(4)). Based on these values, the standard pathway Gibbs free energy changes ($\Delta G'^{o}_{p\in P}$) are determined using stoichiometric balance among external metabolites and cofactors in individual elementary pathways. The constraint for the negative Gibbs free energy change of pathways is expressed in Eq. (9).

$$\overline{GP} = \overline{U} \times \overline{GE} \leq 0 \qquad (9)$$

$\overline{GP}$ is a $M \times 1$ vector of the standard Gibbs free energy changes for elementary pathways ($\Delta G'^{o}_{p\in P}$), $\overline{GE}$ is a $Y \times 1$ vector of the standard formation Gibbs free energy of external metabolites and cofactors ($\Delta G'_{f,e\in EM}$) at specified *pH* and ionic strength, and *M* is the number of elementary pathways included in a system.

The strategy for a productivity maximisation is depicted in Fig. 4. The productivity of a desired external metabolite is defined as the objective function to be maximised for a given set of reacting rates of substrates. The decision variable is the pathway flux distribution $\overline{B}$. The LP problem formulation can be expressed as follows:

Maximise $\overline{OBJECT}$ $\{e \in OBJ\}$ (Productivity Maximisation)

Subject to Eqs. (6), (8), (9) and $\overline{B} \geq 0$

$$\overline{r_{reacting}} = \{r_{reacting_1}, r_{reacting_2}, \ldots, r_{reacting_e}, \ldots / e \in UPT\} \qquad (10)$$

$\overline{r_{reacting}}$ in Eq. (10) presents the reacting rates of external metabolites ($e \in UPT$) acting as precursors or substrates (sources) in a system and is user specified or a controlling variable. $\overline{OBJECT}$ is the property vector of the productivity of a desired external metabolite e, which is defined as the objective function to be maximised for a given set of substrates.

2.3.2 Gibbs free energy minimisation

From productivity maximisation, the optimal flux distribution of pathways ($\overline{B}^{opt}$) and the maximum generation of the desired product are achieved. The next step is to determine the minimum total Gibbs free energy change of metabolic pathways. Therefore, the optimal flux distribution achieved by the productivity maximisation is used as an input to the thermodynamic evaluation (Eq. (11)). The NLP problem formulation can be expressed as follows:

Minimise G_{tot} (Gibbs free energy change minimisation)

Subject to: Eqs. (2)-(4), (9), (10)

$$\overline{B} = \overline{B}^{opt} \quad (11)$$

$$G_{tot} = \overline{GP}^{T} \times \overline{B} \quad (12)$$

$$\overline{B} \geq 0$$

Negative Gibbs free energy change is also used as a constraint in optimisation. G_{tot} is the total Gibbs free energy change of the system (Eq. (12)), which is defined as the objective function to be minimised. Since $\overline{GP}^{T}$ is presented as a function of cellular *pH* and ionic strength (Eqs. (2), (3) and (4)), the minimisation of Gibbs free energy change results into optimal cellular conditions such as *pH* and ionic strength *I*. Since

cellular metabolism is a very tightly controlled process, the above two input variables can be optimised within very narrow ranges of limits specified, e.g. $pH = 6.8\text{-}7$ and $I = 0.28\text{-}0.3$.

In the context of the overall metabolism engineering design procedure, the proposed methodology (Eqs. (1)-(12) and Figs. 3-5) can be integrated with the experimental program and industrial processes. Its purpose is to systematically screen and set up the incentives for the promising pathways towards a performance target. A priority selection among metabolic elementary pathways can be achieved from flux and energy constrained optimisation analysis of a metabolism system. The differences in thermodynamic driving forces for the selected pathways provide a basis for experiment measurements. The optimal cellular conditions of *pH* and ionic strength attained by thermodynamic optimisation are valuable for practical experiments or industrial processes. In addition to biological insights, this approach can be adopted in the construction of engineered metabolism pathways by enzyme controlling or some other gene altering technologies. Based on our model, a functional network of elementary metabolic pathways and their predicted flux distribution can be established to govern the cellular metabolic system towards achieving an objective.

3. Case Study

A case study on the synthesis of pentose phosphate pathways (PPP) and glycolysis of *in silico* model of *Escherichia coli* metabolism has been used to illustrate the proposed optimisation approach.

3.1 The representation of the metabolic network

The metabolism network under consideration is embedded with the glycolytic pathway and the pentose phosphate pathway in the *in silico* model of *E. coli* metabolism. This network incorporated 26 metabolites

(4 external metabolites, 15 internal metabolites, 7 cofactors) and 19 metabolic reactions (Tables 2-3). An overview of the reaction scheme for the model is indicated in Fig. 6. Among the external metabolites, glucose-6-phosphate is considered as the only carbon source consumed through the system while producing metabolic products. Other external metabolites include carbon dioxide, Ribose *5*-phosphate, and pyruvate. Pyruvate has been assumed as the objective sink for the productivity maximisation.

Formulation of the pathway analysis in a network has been described previously[23]. Thirteen elementary path modes have been derived from computation as shown in Table 4. Fig. 7 describes the pathway mode 3 as an example to illustrate the reactions involved in this pathway.

Based on the results of flux balance analysis and pathway analysis, a stoichiometry of metabolic reactions is presented by a 15×19 stoichiometric matrix $\overline{S}_{(i,j)}$ (Table 5), and a 19×13 stoichiometric matrix $\overline{A}$ is derived from the stoichiometry of reactions in each pathway (Table 6). A 13×11 stoichiometric matrix $\overline{U}$ presenting the stoichiometry of external metabolites and cofactors in each pathway is shown in Table 7.

3.2 Thermodynamic properties of the external metabolites and cofactors

All the biochemical species of the external metabolites and cofactors involved in the metabolism network (Fig. 6) as well as their corresponding thermodynamic properties of the standard formation Gibbs free energy at 25℃, 1 *bar* and *I*=0, are illustrated in Table 4. Firstly, the standard formation Gibbs free energy of these species, at an initial *pH* and ionic strength ($pH = 6.8, I = 0.28$) is calculated using Eq. (2) and (3) respectively, based on their hydrogen atom numbers $N_i(H^+)$ and the charge z_i (Table 8). Next, the standard formation Gibbs free energy for all the external metabolites and, like ATP, ADP and inorganic phosphate, are calculated using Eq. (4), shown in Table 9.

3.3 Optimisation Approach

3.3.1 Productivity maximisation

For the productivity maximisation, the objective is to maximise the product flux of pyruvate using the flux balance analysis (Eqs. (5)-(10)). The reacting rate of glucose-6-phosphate has been specified to be 115*mmol/gDCWh*[24]. Eqs. (6), (9)-(10) are solved using the LP optimisation solver in General Algebraic Modeling System (GAMS)/BDMLP on a Pentium® 4CPU, 3 GHz and 512 MB of RAM. The computational time is 1.7 seconds. It yields the theoretical maximum productivity rate of pyruvate of 115 *mmol/gDCWh*. This is obviously maximised compared to the unoptimised pyruvate rate of 44.3 *mmol/gDCWh*, from experiments[24]. The corresponding pathways obtained that are responsible for the maximum productivity of pyruvate, are modes 2, 4, 7 and 9 in Fig. 8. To demonstrate the importance of Gibbs free energy change as a sufficient constraint to optimisation, two sets of optimal pathway distributions derived with and without Gibbs free energy constraint is illustrated in Fig. 9. Their corresponding Gibbs free energy changes are summarized in Table 10. The optimal pathway modes derived from optimisation without Gibbs free energy constraint are modes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, but some of them have positive Gibbs free energy changes which oppose their thermodynamic feasibility. However, with Gibbs free energy constraint, the corresponding optimal pathways derived are modes 2, 4, 7, and 9, all of which have been strictly constrained to ensure the feasibility and direction of the pathways. The Gibbs free energy change for this process at the initial conditions is -54696.774 $kJ \cdot mol^{-1}$. Additionally, the results in terms of reaction flux distribution and productivity solely from FBA[3,4,5] are presented in Table 11 in order to establish the distinction of the combined FBA and pathway analysis, introduced in this work. Although the maximum productivity derived from FBA alone is also 115 *mmol/gDCWh*, which is the same as that obtained from the combined FBA and pathway analysis, the feasibility of this reaction flux distribution is uncertain. Therefore, pathway analysis is still needed further to distribute these reaction

fluxes into different pathways so as to capture the genetic or enzymatic manipulation of cellular activities. Based on the reaction flux distribution from FBA alone, a set of pathway distributions with the corresponding Gibbs free energy changes is derived as shown in Table 12. The pathways selected from FBA alone are 3 and 6, between which the pathway 3 is an infeasible pathway resulting into positive Gibbs free energy change of 17539.340 *kJ/mol*. Thus, we may conclude that only the methodology combining FBA and pathway analysis incorporating thermodynamic constraints can ensure the representation of the metabolic genotype-phenotype relations and related cellular control activities.

3.3.2 Gibbs free energy minimisation

To predict the optimal reaction conditions, the optimal flux distribution achieved by the productivity maximisation in section 3.3.1 is used as an input to thermodynamic optimisation. Using Eqs. (2), (3)-(4), the standard formation Gibbs free energy of the external metabolites and cofactors of the system (Fig. 6) as functions of *pH*, ionic strength *I* is computed. The expression of the standard pathway Gibbs free energy changes for pathway modes are derived from the stoichiometry of the overall reaction equations of these pathways. The objective function is the minimisation of the total Gibbs free energy for all the pathways included (Eq. (13)). The problem is non-linear due to Eqs. (2), (3)-(4). This NLP optimisation problem is solved via the NLP optimisation solver in General Algebraic Modeling System (GAMS)/CONOPT on a Pentium® 4CPU, 3 GHz and 512 MB of RAM. The computational time is 2.3 seconds. The result of the Gibbs free energy minimisation is presented in Table 13. The optimal cellular level reaction conditions obtained are $pH = 7$ and $I = 0.3$. The corresponding Gibbs free energy change for this process is -67608.11 $kJ \cdot mol^{-1}$, which is significantly minimised compared to the initial value (-54696.774 $kJ \cdot mol^{-1}$). Fig. 10 shows that the Gibbs free energy changes for pathway 2, 4 and 9, as well as the total Gibbs free energy change is obviously minimised after minimisation, although the Gibbs fee energy change of mode 7 is slightly increased.

4. Conclusion

A novel optimisation methodology has been presented for the productivity and thermodynamic performance of metabolic systems. The theoretical connection between flux balance analysis and pathway analysis is well established. Their combined application has been integrated with the thermodynamic constraints on Gibbs free energy based driving force in order to predict the maximum productivity of desired products and the optimal metabolic flux distribution. Moreover, thermodynamic optimisation in terms of the Gibbs free minimisation has been successfully developed for metabolic systems, from which, the best cellular conditions are predicted. The heuristic idea of introducing thermodynamic analysis into metabolic engineering presents a new way to rationalize metabolic pathway analysis, hence, providing a better control mechanism for industrial bioprocesses. The work presented in this paper is an essential step forward in establishing cellular pathway level control for productivity maximisation.

Appendix A

Glossary

Metabolism:
The processes occur within living cells or organisms that are necessary for the maintenance of life. In metabolism some substances are broken down to yield energy for vital processes while other substances, necessary for life, are synthesized.

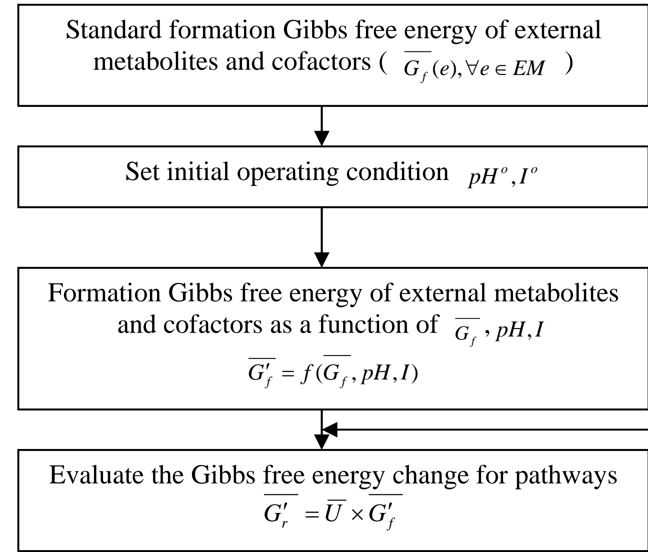
Metabolites:
Substances consumed or produced by metabolism, can be external or internal to a cellular system.

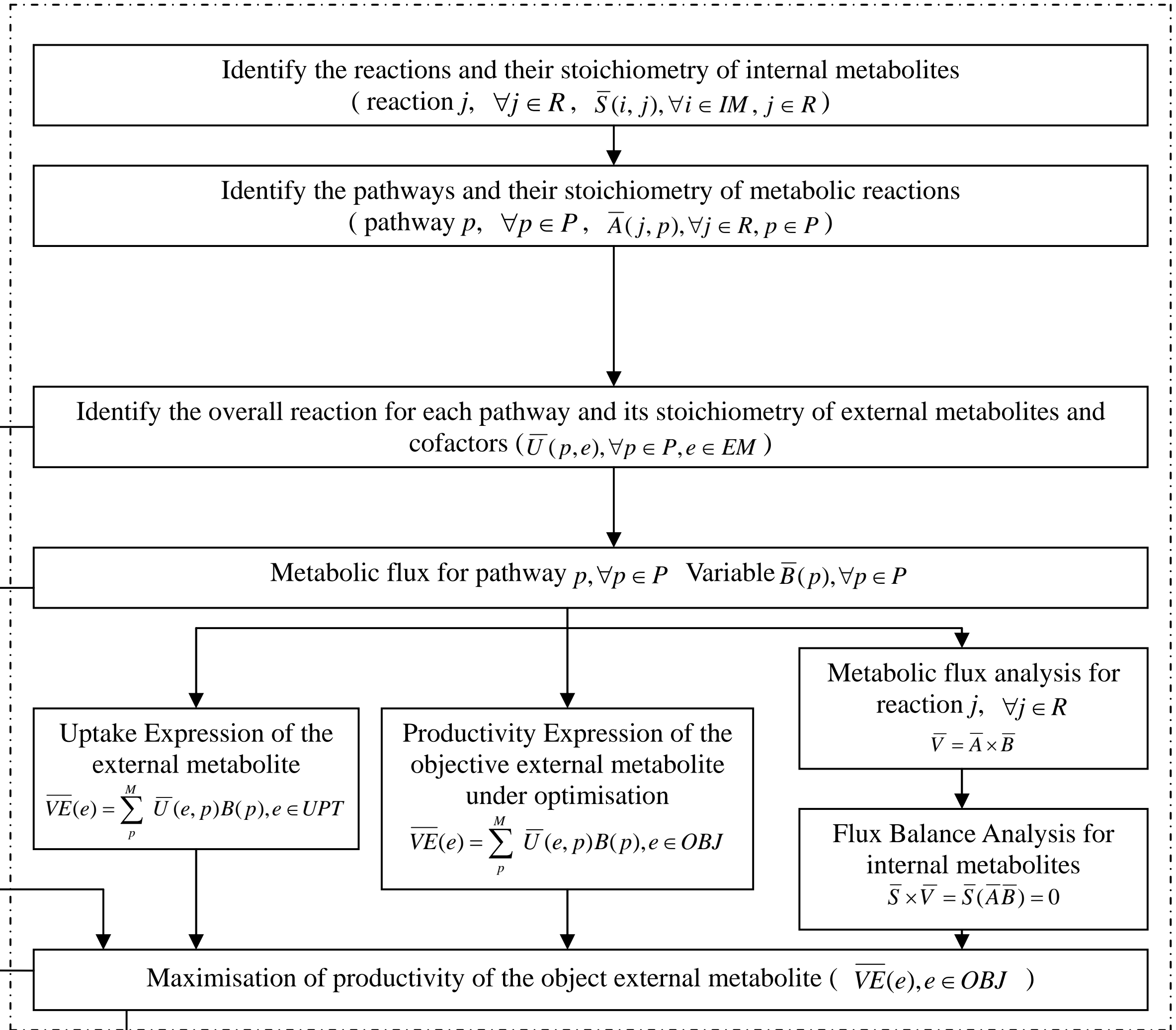
External metabolites:
Metabolites buffered by connection to reservoirs. They can be considered to be sources and sinks (nutrients and waste products, stored or excreted products, or precursors for further transformations), exchanged be tween a cellular system and its environment.

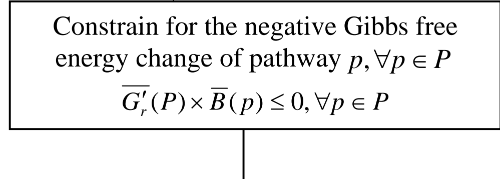
Internal metabolites:
Metabolites only participate in reactions of the model, the formation of which are exactly balanced by consumption within a cellular system.

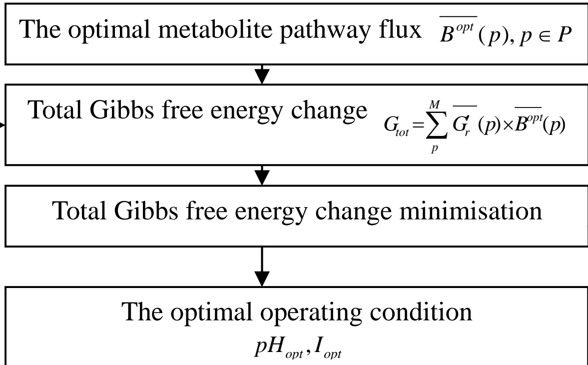
Cofactors:
Substances, such as metallic ions or coenzymes, must be associated with an enzyme for the enzyme to function.

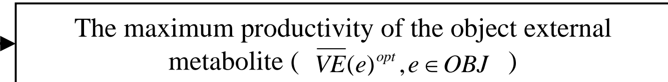
Appendix B

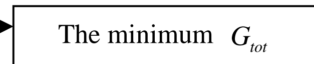












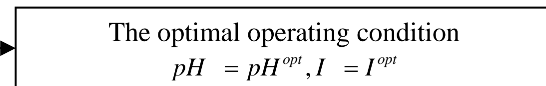


Fig. B1 Optimisation Algorithm

Figures

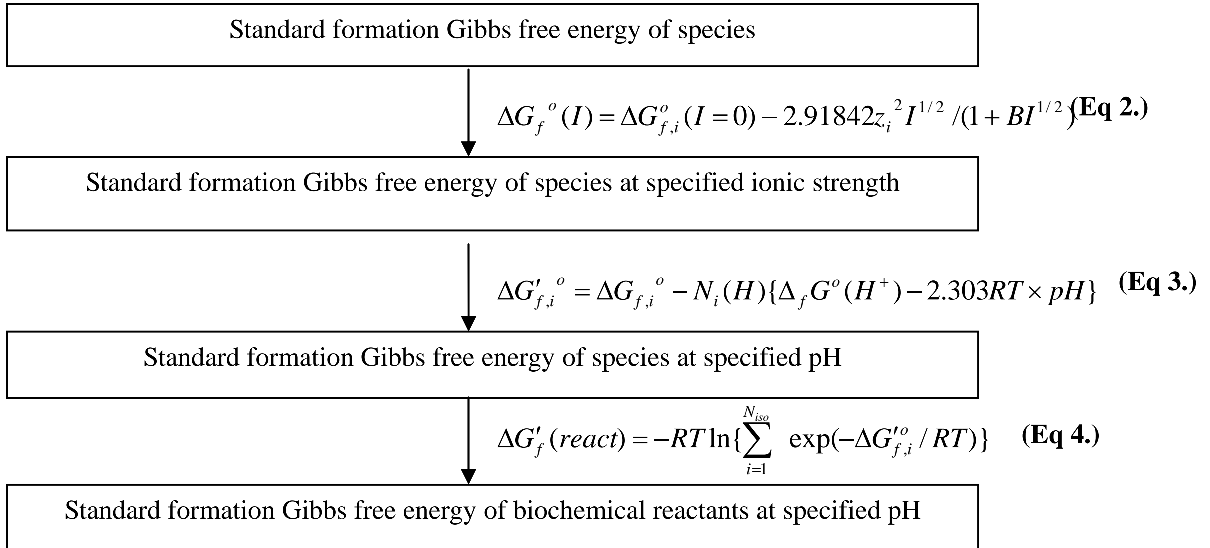


Fig. 1   Calculation of the standard formation Gibbs free energy for biochemical reactants

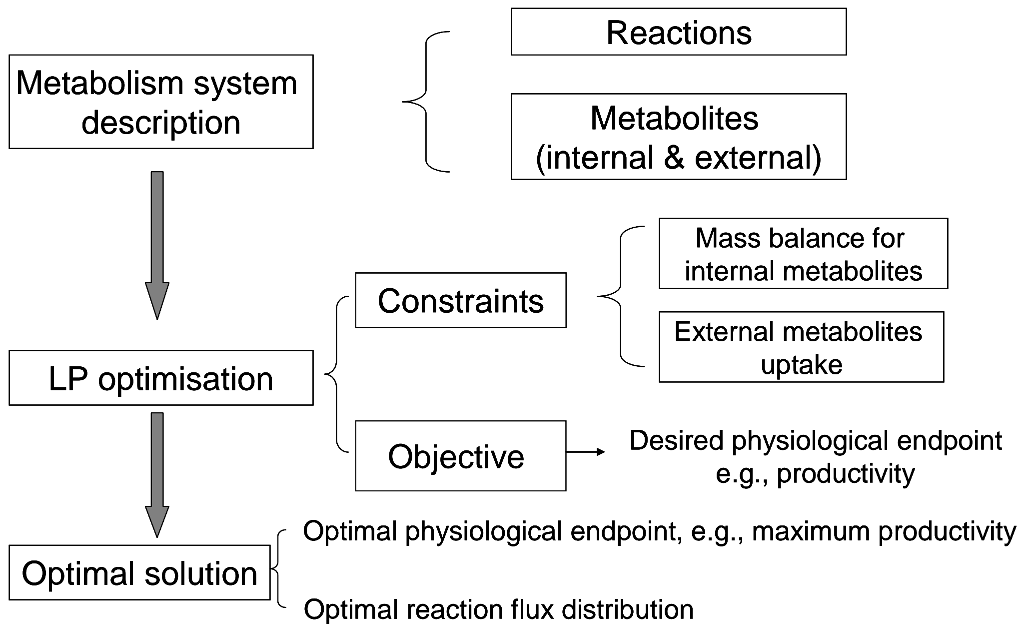


Fig. 2   Flux balance analysis

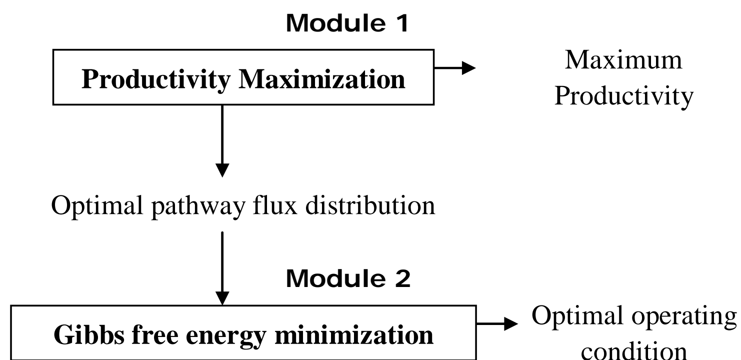


Fig. 3 Modules for Optimisation

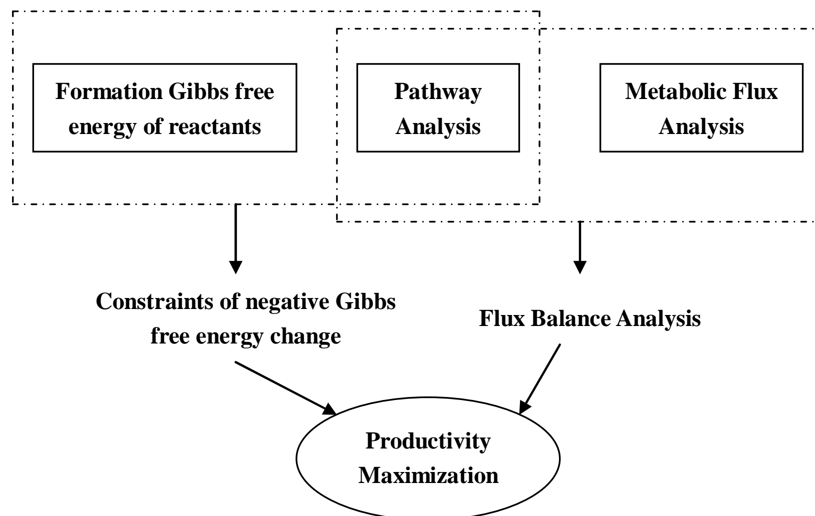


Fig. 4 Module 1: Productivity maximization

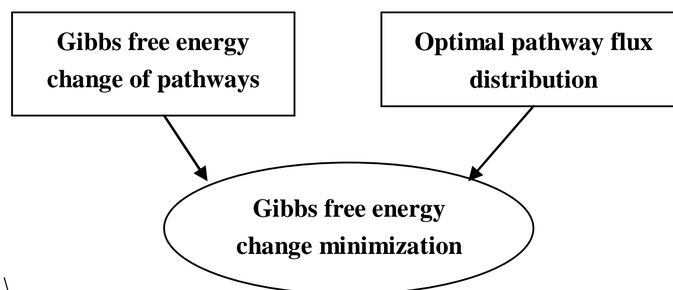


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Fig. 5 Module 2: Gibbs free energy minimization

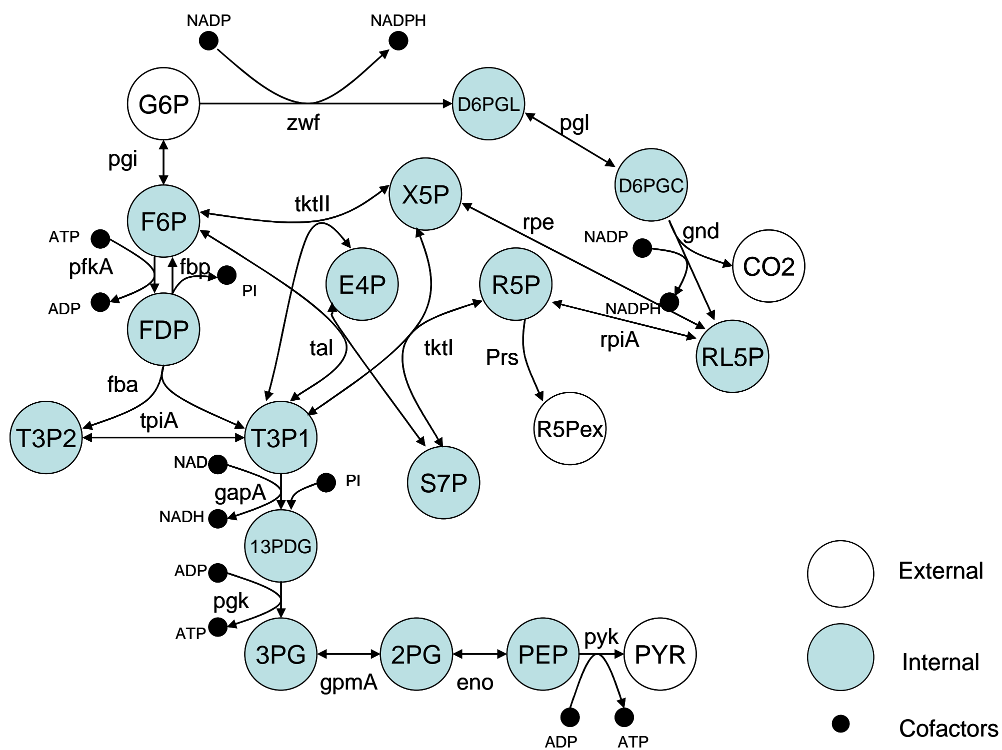

Fig. 6 Overview of the metabolic network of Glycolysis and PPP in *E.coli* model

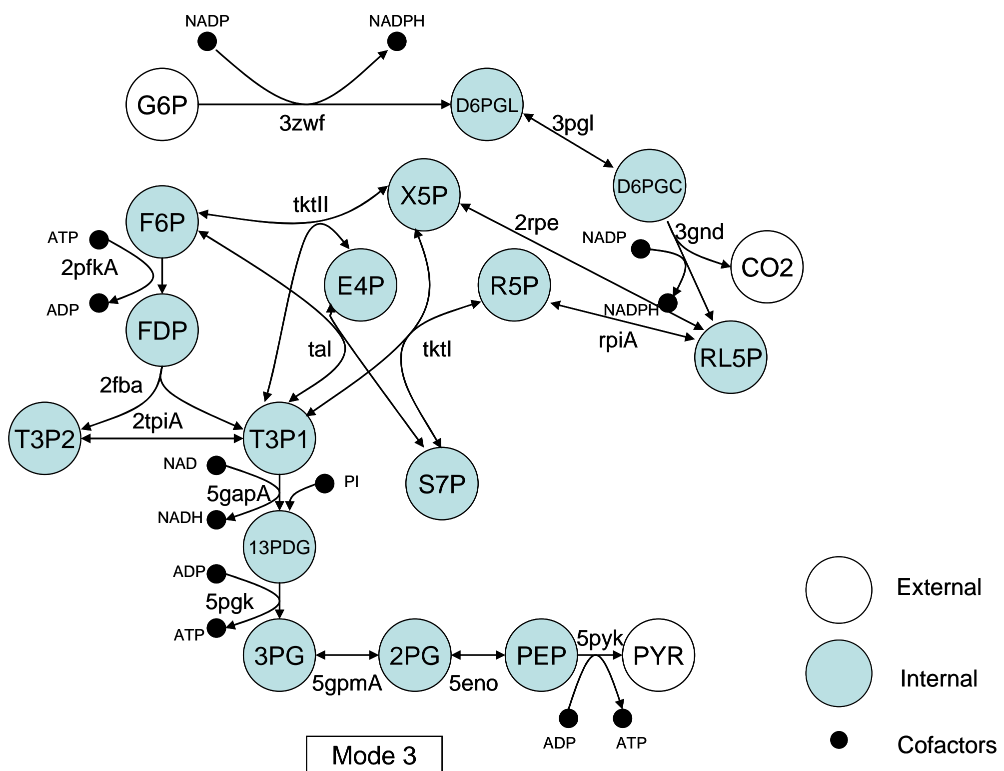

Fig. 7 Graphical representation of the pathway mode 3 pertaining to the reaction scheme

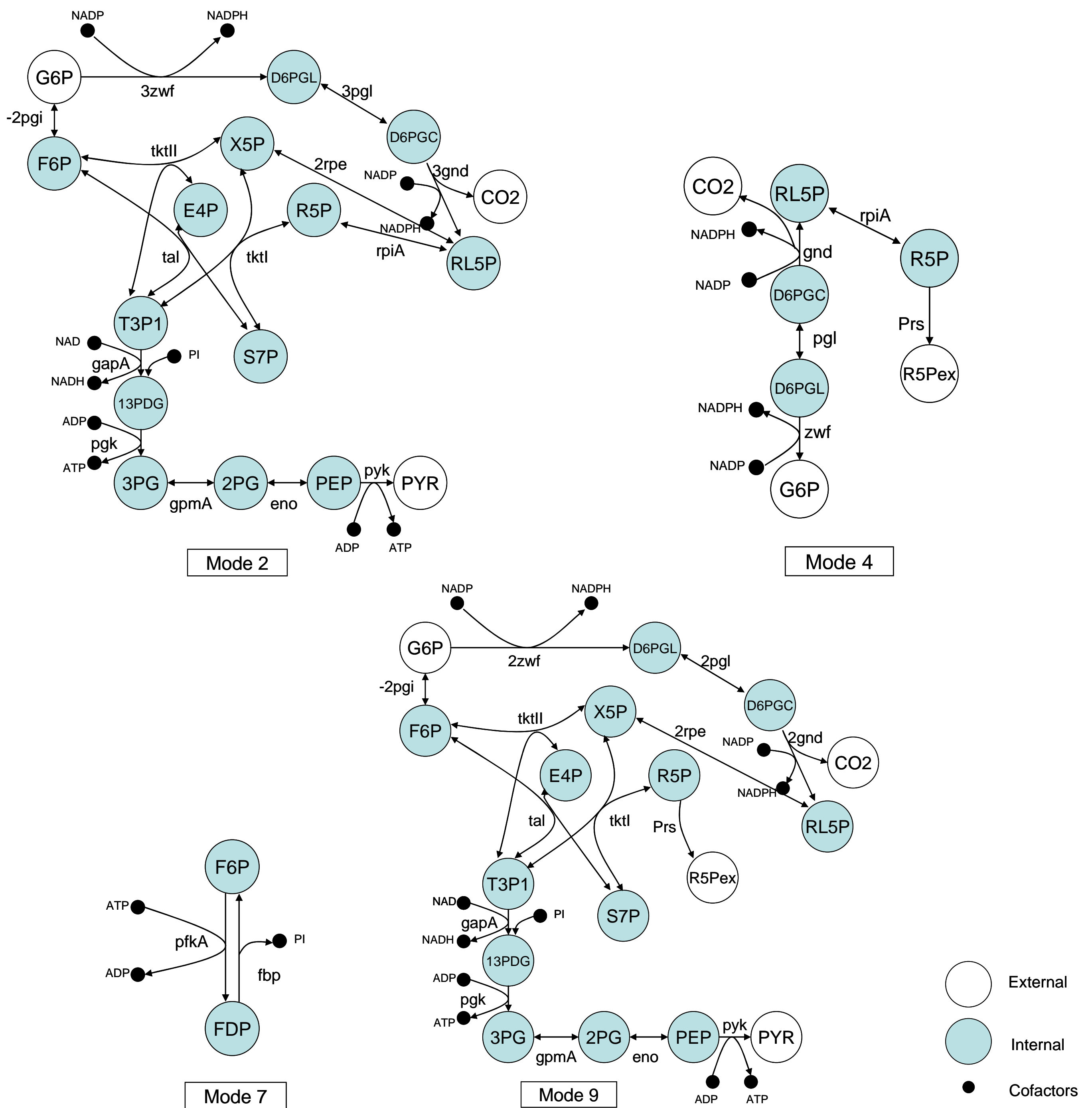

Fig. 8 Graphical representation of the pathway mode 2, 4,7 and 9

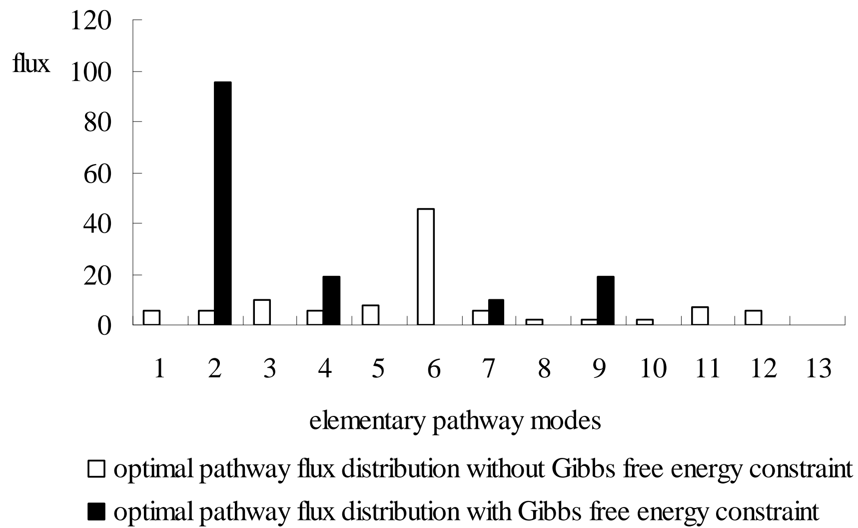

Fig. 9 Comparison of the optimal pathway flux distribution with and without Gibbs free energy constraint

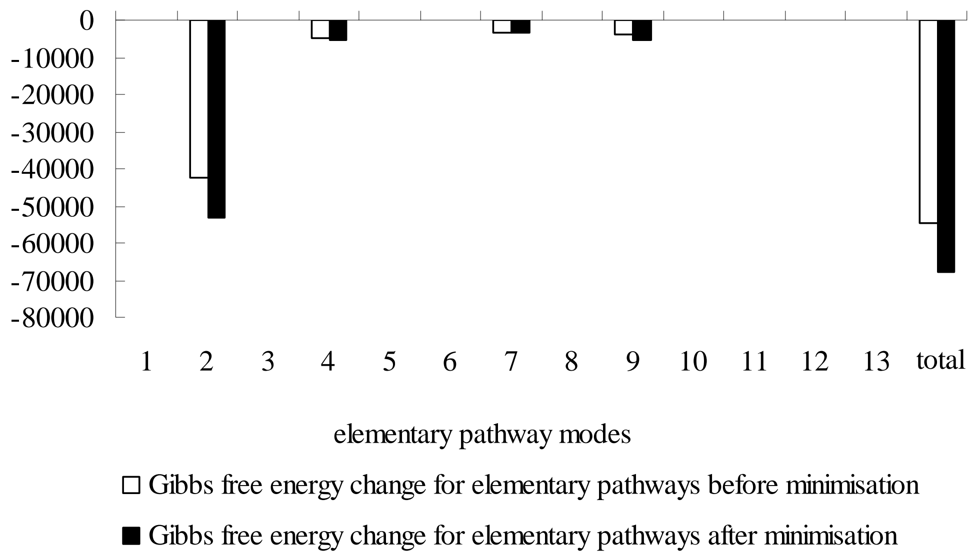

Fig. 10 Comparison of the Gibbs free energy change for elementary pathways before and after minimisation

Tables

Table 1 Properties for ATP^{4-}, $HATP^{3-}$, H_2ATP^{2-} at 25℃, 1 bar, and I = 0

Species	$\Delta G_f^{\,o} / kJ \cdot mol^{-1}$	Charges z_i	Hydrogen atom numbers $N_i(H)$
ATP^{4-}	-2573.49	4	12
$HATP^{3-}$	-2616.87	3	13
H_2ATP^{2-}	-2643.58	2	14

Table 2 Metabolic reactions of glycolysis and PPP in *E.coli* model

Enzyme	Gene	Rxn no.	Reaction
Glycolysis(10)			
Phosphoglucose isomerase	pgi	1	$G6P \leftrightarrow F6P$
Phosphafructokinase	pfkA	2	$F6P + ATP \rightarrow ADP + FDP$
Fructose-1,6-bisphosphatase	fbp	3	$FDP \rightarrow F6P + PI$
Fructose-1,6-bisphophate aldolase	fba	4	$FDP \leftrightarrow T3P1 + T3P2$
Triosphosphate isomerase	tpiA	5	$T3P1 \leftrightarrow T3P2$
Glyceraldehyde-3-phosphate dehydrogenase	gapA	6	$T3P1 + PI + NAD \rightarrow NADH + 13PDG$
Phosphoglycerate kinase	pgk	7	$13PDG + ADP \rightarrow ATP + 3PG$
Phosphoglycerate mutes	gpmA	8	$3PG \leftrightarrow 2PG$
Enolase	eno	9	$2PG \leftrightarrow PEP$
Pyruvate kinase	pyk	10	$PEP + ADP \rightarrow ATP + PYR$
Pentose phosphate pathway (PPP)(9)			
Glucose-6-phosphate dehydrogenase	zwf	11	$G6P + NADP \rightarrow NADPH + D6PGL$
6-Phophogluconolactonse	pgl	12	$D6PGL \leftrightarrow D6PGC$
6-Phosphogluconate dehydrogenase	gnd	13	$D6PGC + NADP \rightarrow NADPH + CO_2 + RL5P$
Ribose-5-phosphate isomerase	rpiA	14	$RL5P \leftrightarrow R5P$
Ribulose phosphate 3-epimerase	rpe	15	$RL5P \leftrightarrow X5P$
Transketolase 1	tktI	16	$X5P + R5P \leftrightarrow T3P1 + S7P$
Transaldolase	tal	17	$T3P1 + S7P \leftrightarrow E4P + F6P$
Transketolase 2	TktII	18	$X5P + E4P \leftrightarrow F6P + T3P1$
5-Phosphoribosyl-1-pyrophosphate synthetase	Prs	19	$R5P \rightarrow R5Pex$

Table 3 Metabolites of glycolysis and PPP in *E.coli* model

Abbreviation	Compound
External metabolites(4)	
G6P	Glucose *6*-phosphate
PYR	Pyruvate
CO_2	Carbon dioxide
R5Pex	Ribose *5*-phosphate (external)
Internal metabolites(15)	
F6P	Fructose *6*-phosphate
FDP	Fructose *1,6*-diphosphate
T3P1	Glyceraldehyde-*3*-phosphate
T3P2	Dihydroxyacetone phosphate
13*PDG*	*1,3-P-d* glycerate
3*PG*	*3-P-d* glycerate
2*PG*	*2-P-d* glycerate
PEP	Phosphoenolpyruvate
D6PGL	*d*-*6*-Phosphogluconate
D6PGC	*d*-*6*-Phosphoglucono-δ-lactone
RL5P	*d*-Ribulose *5*-phosphate
R5P	Ribose *5*-phosphate
X5P	Xylulose-*5*-phosphate
S7P	*d*-Sedoheptulose-*7*-*P*
E4P	Erythrose *4*-phosphate
Cofactors(7)	
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
NAD *NADH*	Nicotinamide adenine dinucleotide
NADP *NADPH*	Nicotinamide adenine dinucleotide phosphate
PI	Phosphate

Table 4 Elementary path modes of the combined glycolysis and PPP system

Mode	Overall equation	Steps
1	$G6P + 3ADP + 2Pi + 2NAD \rightarrow$ $3ATP + 2NADH + 2Pyr$	pgi, pfkA, fba, tpiA, 2gapA, 2pgk, 2gpmA, 2eno, 2pyk
2	$G6P + 2ADP + Pi + NAD + 6NADP \rightarrow$ $2ATP + NADH + 6NADHP + 3CO_2 + Pyr$	-2pgi, gapA, 3zwf, 3pgl, 3gnd, rpiA, 2rpe, tktI, tal, tktII, pgk, gpmA, eno, pyk,
3	$3G6P + 8ADP + 5Pi + 5NAD + 6NADP \rightarrow$ $8ATP + 5NADH + 6NADHP + 3CO_2 + 5Pyr$	2pfkA, 2fba, 2tpiA, 5gapA, 3zwf, 3pgl, 3gnd, rpiA, 2rpe, tktI, tal, tktII, 5pgk, 5gpmA, 5eno, pyk
4	$G6P + 2NADP \rightarrow 2NADPH + CO_2 + R5Pex$	zwf, pgl, gnd, rpiA, Prs
5	$5G6P + ATP \rightarrow ADP + 6R5Pex$	5pgi, pfkA, fba, tpiA, 4rpiA, -4rpe, -2tktI, -2tal, -2tktII, 6Prs
6	$G6P + 12NADP \rightarrow 12NADPH + Pi + 6CO_2$	-5pgi, -fba, -tpiA, 6zwf, 6pgl, 6gnd, 2rpiA, 4rpe, 2tktI, 2tal, 2tktII, fbp
7	$ATP \rightarrow ADP + Pi$	pfk, fbp
8	$2ADP + Pi + NAD + 3R5Pex \rightarrow$ $2ATP + NADH + 2G6P + Pyr$	-2pgi, gapA, -2rpiA, 2rpe, tktI, tal, tktII, pgk, gpmA, eno, pyk, -3Prs
9	$2ADP + Pi + NAD + 4NADP + R5Pex \rightarrow$ $2CO_2 + 2ATP + NADH + 4NADHP + Pyr$	-2pgi, gapA, 2zwf, 2pgl, 2gnd, 2rpe, tktI, tal, tktII, pgk, gpmA, eno, pyk, -Prs
10	$8ADP + 5Pi + 5NAD + 3R5Pex \rightarrow$ $8ATP + 5NADH + 5Pyr$	2pfkA, 2fba, 2tpiA, 5gapA, -2rpiA, 2rpe, tktI, tal, tktII, 5pgk, 5gpmA, 5eno, 5pyk, -3Prs
11	$8ADP + 5Pi + 5NAD + 4NADP + 2G6P + R5Pex \rightarrow$ $2CO_2 + 8ATP + 5NADH + 4NADHP + 5Pyr$	2pfkA, 2fba, 2tpiA, 5gapA, 2zwf, 2pgl, 2gnd, 2rpe, tktI, tal, tktII, 5pgk, 5gpmA, 5eno, 5pyk, -Prs
12	$6R5Pex \rightarrow 5G6P + Pi$	-5pgi, fbp, -fba, -tpiA, -4rpiA, 4rpe, 2tktI, 2tal, 2tktII,-6 Prs
13	$8NADP + 2R5Pex \rightarrow 4CO_2 + 8NADPH + G6P + Pi$	-5pgi, fbp, -fba, -tpiA, 4zwf, 4pgl, 4gnd, 4rpe, 2tktI, 2tal, 2tktII, -Prs

Table 5 Stoichiometric matrix $\overline{S}_{(i,j)}$ in terms of internal metabolites $i \in IM$ in reactions $j \in R$

reactions	*internal metabolites*														
	F6P	*FDP*	*T3P1*	*T3P2*	*13PDG*	*3PG*	*2PG*	*PEP*	*D6PGL*	*D6PGC*	*RL5P*	*R5P*	*X5P*	*S7P*	*E4P*
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	-1	1	1	0	0	0	0	0	0	0	0	0	0	0
5	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0
6	0	0	-1	0	1	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	-1	1	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	-1	1	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	-1	1	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	-1	1	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	-1	1	0	0	0
15	0	0	0	0	0	0	0	0	0	0	-1	0	1	0	0
16	0	0	1	0	0	0	0	0	0	0	0	-1	-1	1	0
17	1	0	-1	0	0	0	0	0	0	0	0	0	0	-1	1
18	1	0	1	0	0	0	0	0	0	0	0	0	-1	0	-1
19	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0

Table 6 Stoichiometric matrix $\overline{A}_{(j,p)}$ in terms of reactions $j \in R$ in elementary pathways $p \in P$

Elementary pathways	*Reactions*																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1	1	0	1	1	2	2	2	2	2	0	0	0	0	0	0	0	0	0
2	-2	0	0	0	0	1	1	1	1	1	3	3	3	1	2	1	1	1	0
3	0	2	0	2	2	5	5	5	5	5	3	3	3	1	2	1	1	1	0
4	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1
5	5	1	0	1	1	0	0	0	0	0	0	0	0	4	-4	-2	-2	-2	6
6	-5	0	1	-1	-1	0	0	0	0	0	6	6	6	2	4	2	2	2	0
7	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	-2	0	0	0	0	1	1	1	1	1	0	0	0	-2	2	1	1	1	-3
9	-2	0	0	0	0	1	1	1	1	1	2	2	2	0	2	1	1	1	-1
10	0	2	0	2	2	5	5	5	5	5	0	0	0	-2	2	1	1	1	-3
11	0	2	0	2	2	5	5	5	5	5	2	2	2	0	2	1	1	1	-1
12	-5	0	1	-1	-1	0	0	0	0	0	0	0	0	-4	4	2	2	2	-6
13	-5	0	1	-1	-1	0	0	0	0	0	4	4	4	0	4	2	2	2	-1

Table 7 Stoichiometric matrix $\overline{U}_{(p,e)}$ in terms of external metabolites and cofactors $e \in EM$ in elementary pathways $p \in P$

External metabolites and cofactors	*Elementary pathways*												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ATP	3	2	8	0	-1	0	-1	2	2	8	8	0	0
ADP	-3	-2	-8	0	1	0	1	-2	-2	-8	-8	0	0
$G6P$	-1	-1	-3	-1	-5	-1	0	2	0	0	-2	5	1
PYR	2	1	5	0	0	0	0	1	1	5	5	0	0
$R5Pex$	0	0	0	1	6	0	0	-3	-1	-3	-1	-6	-2
CO_2	0	3	3	1	0	6	0	0	2	0	2	0	4
NAD	-2	-1	-5	0	0	0	0	-1	-1	-5	-5	0	0
$NADH$	2	1	5	0	0	0	0	1	1	5	5	0	0
$NADP$	0	-6	-6	-2	0	-12	0	0	-4	0	-4	0	-8
$NADPH$	0	6	6	2	0	12	0	0	4	0	4	0	8
PI	-2	-1	-5	0	0	1	1	-1	-1	-5	-5	1	1

Table 8 Standard formation Gibbs free energy, charges and hydrogen atom numbers for species

Species	$\Delta G_f^{\,o} / kJ \cdot mol^{-1}$	**Charges** z_i	**Hydrogen atom numbers** $N_i(H)$
$G6P^{2-}$	-1763.94	2	11
PYR^{-}	-472.27	1	3
$R5P^{2-}$	-1605.34	2	9
NAD^{-}	0	1	26
$NADH^{2-}$	22.65	2	26
$NADP^{3-}$	0	3	25
$NADPH^{4-}$	25.99	4	25
$HPO_4^{\,2-}$	-1095.1	2	1
$H_2PO_4^{\,-}$	-1137.3	1	2
ATP^{4-}	-2573.49	4	12
$HATP^{3-}$	-2616.87	3	13
H_2ATP^{2-}	-2643.58	2	14
ADP^{3-}	-1711.55	3	12
$HADP^{2-}$	-1752.53	2	13
H_2ADP^{-}	-1777.42	1	14

Table 9 Standard formation Gibbs free energy for external metabolites and cofactors at $pH = 6.8$, $I = 0.28$

External metabolites and cofactors	$\Delta G_f'^o / kJ \cdot mol^{-1}$
G6P	-1331.29
PYR	-354.198
R5Pex	-1251.96
NAD	1029.701
NADH	1049.842
NADP	983.3745
NADPH	1003.511
PI	-1230.57
ATP	-2376.47
ADP	-1508.15

Table 10 Comparison of optimal pathway flux distribution and their corresponding Gibbs free energy changes

Elementary Modes	without ΔG constraint		with ΔG constraint	
	Pathway flux distribution *B(p)* (*mmol/gDCWh*)	ΔG kJ / mol	Pathway flux distribution *B(p)* (*mmol/gDCWh*)	ΔG kJ / mol
1	5.62	3390.493	0	0
2	5.62	-2495.21	95.746	-42509.4
3	10	7625.8	0	0
4	5.62	-1375.01	19.254	-4710.65
5	7.976	185.676	0	0
6	45.785	-85583.7	0	0
7	5.62	-2124.45	9.627	-3639.07
8	1.808	524.388	0	0
9	1.808	-360.417	19.254	-3837.68
10	1.808	2706.131	0	0
11	7.096	7147.792	0	0
12	5.62	-2255.29	0	0
13	0	0	0	0

Table 11 Reaction flux distribution derived from FBA alone

Reaction	Reaction flux distribution *V(j)* (*mmol/gDCWh*)
1	-230
2	46
3	46
4	0
5	0
6	115
7	115
8	115
9	115
10	115
11	345
12	345
13	345
14	115
15	230
16	115
17	115
18	115
19	0

Table 12 Pathway analysis based on the result of reaction flux distribution derived from FBA alone

Pathway	Reaction flux distribution *B(p)* (*mmol/gDCWh*)	ΔG kJ / mol
1	0	0
2	0	0
3	23	17539.34
4	0	0
5	0	0
6	46	-85985.5
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0

Table 13 Standard formation Gibbs free energy at optimal condition

External metabolites and cofactors	$\Delta G'^{opt}_{f} / kJ \cdot mol^{-1}$
$G6P$	-1318.634
PYR	-350.745
$R5Pex$	-1241.617
NAD	1059.736
$NADH$	1079.833
$NADP$	1012.137
$NADPH$	1032.171
PI	-1228.872
ATP	-2361.616
ADP	-1493.225
$\Delta G^{opt}_{tot} / kJ \cdot mol^{-1}$	-5577.197

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Nomenclature

Symbol	Name	Unit
c^o	standard state concentration (1M)	mol/l
G	extensive Gibbs free energy of a system	kJ
G'	extensive transformed Gibbs free energy of a system	kJ
$\Delta G_f{}^o{}_i$	standard formation Gibbs free energy of species *i* at specified *T, P*, and *I*	kJ/mol
$\Delta G_f'^o(react)$	standard formation Gibbs free energy of reactant *i* at specified *T, P, pH* and *I*	kJ/mol
$\Delta G'_{f,e\in EM}$	standard formation Gibbs free energy of external metabolites and cofactors *e* at specified *T, P, pH* and *I*	kJ/mol
$\Delta G'^o_{p\in P}$	standard Gibbs free energy change of pathway *p* at specified *T, P, pH* and *I*	kJ/mol
I	ionic strength	mol/l
$N_i(H)$	number of H atoms in species *i*	dimensionless
pH	$-\log([H^+]/c^o)$	dimensionless
P	pressure	bar
R	gas constant ($8.31451 JK^{-1}mol^{-1}$)	$JK^{-1}mol^{-1}$
T	temperature	K
z_i	charge of ion *i*	dimensionless
μ_i^o	standard chemical potential of species *i* at specified *T, P,* and *I*	kJ/mol
$r_{reacting_e}$	reacting rate of external metabolites *e* ($e \in UPT$)	$mmol/gDCWh$

Sets

IM = {*1, 2..., X* / internal metabolites}
EM = {*1, 2..., Y* / external metabolites and cofactors}
R = {*1, 2..., N* / reactions of the metabolic system}
P = {*1, 2..., M* / pathways of the metabolic system}
OBJ = { e / objective product under optimisation, $OBJ \subset EM$ }
UPT = { e / external metabolite uptake from external metabolite measurement, $UPT \subset EM$ }

Variables & Parameters

Symbol	Description
$\overline{V}$	property vector of metabolic flux for individual reactions $j, \forall j \in R$
$\overline{B}$	property vector of metabolic flux for elementary pathways $p, \forall p \in P$
$\overline{B}^{opt}$	property vector of optimal metabolic flux for elementary pathways $p, \forall p \in P$
$\overline{S}$	stoichiometric matrix for internal metabolite i ($i \in IM$) in reaction j ($j \in R$)
$\overline{A}$	stoichiometric matrix for reaction j ($j \in R$) in elementary pathway p ($p \in P$)
$\overline{U}$	stoichiometric matrix for external metabolites e ($e \in EM$) in pathway p ($p \in P$)
$\overline{VE}$	property vector of metabolic flux for external metabolites and cofactors $e, \forall e \in EM$
$\overline{GE}$	property vector of standard formation Gibbs free energy of external metabolites and cofactors e ($e \in EM$) at specified *pH* and ionic strength
$\overline{GP}$	property vector of standard Gibbs free energy changes for elementary pathways p ($p \in P$)
G_{tot}	total Gibbs free energy change of the system
$\overline{r_{reacting}}$	property vector of the reacting rates of given steady external metabolites e ($e \in UPT$)
$\overline{OBJECT}$	property vector of the productivity of desired external metabolite e ($e \in OBJ$)