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Metabolic Flux and Convex Polytope

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代謝フラックスと凸多面体

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In this paper, we apply the algebraic geometrical tools to the analysis of metabolic pathway. We analyze the metabolic flux as a convex polyhedral cone and extract the combinatorial information as convex polytope of metabolic flux. We give the new interpretation to the combinatorial quantities, such as Hilbert series and Ehrhart polynomial, from the viewpoint of metabolic flux analysis.

本研究では、代数幾何学的ツールの代謝パスウェイ解析への適用を行う。凸多面錐として代謝フラックスを解析し、代謝フラックスの凸多面体としての組合せ論的情報を抽出する。我々は、ヒルベルト級数やエールハルト多項式のような組合せ論的量に対して、代謝パスウェイ解析の視点からの新しい解釈を与える。

1 Introduction

Recent years have witnessed the progress of a new interdisciplinary field between mathematics and biology. Pure mathematics such as algebraic geometry has been applied intensively to actual problems of biology. In the field of computational biology, the approach of algebraic statistics has been developed [1]. In this line of research, phylogenetic algebraic geometry [2] and algebraic biology is also under development [3].

Metabolic pathway analysis is studied not only as a method of the analysis for metabolomics but also as one of the major fields of systems biology. The recent development of metabolic analysis is based on the flux balance analysis (FBA), in which the metabolic flux is interpreted as a convex polyhedral cone. The FBA has shown a remarkable progress after the introduction of elementary modes [4] and extreme pathways [5].

As a relevant but independent study, Clarke had already noticed that the null space can be represented by the generators of a convex polyhedral cone in the study of chemical reaction networks [6, 7, 8]. Gatermann and her colleagues focused on this nature and studied the chemical reaction networks with the methods of algebraic geometry [9, 10, 11, 12]. For instance, they enumerated the number of solutions of steady state [9, 10, 11], and analyzed Hopf bifurcation [12]. Shiu et. al. used algebraic geometrical method to analyze the global attractor point [14, 15, 16, 17], stability [18] and multistationality [19] of chemical reaction networks. Following these studies, the approach by algebraic geometry to chemical reaction networks has been further cultivated with the introduction of toric dynamical systems [13, 14, 15, 16, 17, 18, 19].

In contrast to chemical reaction networks, algebraic geometrical study of metabolic flux has been undone, while the metabolic pathway analysis has such a suitable property for the algebraic geometrical approach that the metabolic flux can be regarded as a convex polyhedral cone.

In this paper, we apply algebraic geometry of convex polytope to the metabolic pathway analysis. Our approach gives new interpretations to the results investigated in the algebraic geometry of convex polytopes from the viewpoint of the metabolic pathway analysis.

We first study the property of flux as a convex polyhedral cone by introducing the deformed toric ideal constraints. In the previous studies of the metabolic pathway analysis, without considering deformed

toric ideal constraints, the convex polyhedral cone of flux is represented as a linear combination of extreme pathways with arbitrary coefficients. With the deformed toric ideal constraints, however, the flux is no longer the convex polyhedral cone but a convex polyhedron with algebraically constrained coefficients. We show that the constraints cause significant reduction of the flux space in some cases, as we demonstrate with an example in Section 3. We also show that the deformed toric ideal constraints realize the mixing of extreme pathways. This is caused by the non-linear relation of the coefficients of generators, which originates in the deformed toric ideal constraint. To see how the mixing occurs, we discuss the perturbation of parameters. We will see that the mixed extreme pathway corresponds to the almost independent reactions and we can approximate the generators as those without the extreme pathway which corresponds to the almost independent reactions.

Next, we discuss convex polytopes in the metabolic pathway analysis using the mathematical tools of Hilbert series and Ehrhart polynomial.

The Hilbert series of a convex polytope is defined by the number of i-dimensional faces. When it is applied to the convex polytope for a metabolic pathway, it provides a combinatorially unique quantity of flux. We also show that an i-face is regarded as a face through which the exchange fluxes flow in or out.

The Ehrhart polynomial counts the lattice points inside a polytope, and the coefficient of the leading term corresponds to the volume of the polytope. It is known that the volume of flux is an indicator of genotype capability, because the genotypically capable points are realized as the points inside flux [5]. In a previous study, the approximated volume is calculated only with large flux [20]. In contrast, our method calculates the exact volume even when the flux is small, and the computation is much easier than the above work.

2 Metabolic Pathway Analysis and Deformed Toric Ideal Constraint

The metabolic pathway analysis starts with the stoichiometric equation,

$$\dot{x} = SJ, \tag{1}$$

where $\dot{x}$, S, and J denote the derivative of the concentration of metabolites with respect to time, the stoichiometric matrix, and the flux, respectively.

We discuss the steady state condition,

$$SJ = 0. \tag{2}$$

Study of the steady state solutions of chemical reaction networks was initiated by Clarke, and is called "stoichiometric network analysis (SNA)." Using the vector space of the steady state solutions and analyzing the null space (or the kernel space) of stoichiometric matrix S is called "Flux Balance Analysis (FBA)." [21, 22]

In the rest of this section, we treat the metabolic flux as the monomial vector of of metabolite concentrations, and discuss an illustrative example. We analyze the relation between the elements of a monomial vector, and treat the metabolic flux as the vector space spanned by the generators of the null space. These generators are called "extreme pathways."

Example 1.1:
Feedback Inhibition of pathway, Palsson (2011) [23]

In a biosynthetic pathway, the first reaction is often inhibited by the end product of the pathway. We discuss the example in [23], since it is one of the simplest realistic pathways in which there is an inhibitory feedback and the monomial vector form of flux is known. Fig.1 illustrate the example.

The differential equations that describe this feedback loop are

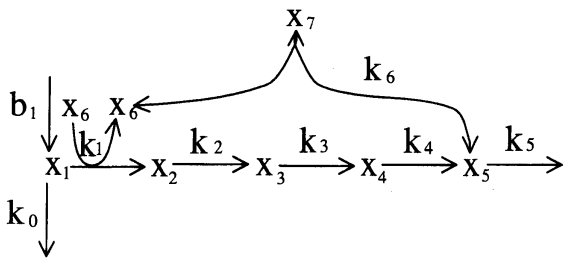

図 1: Feedback Inhibition of pathway

$$\dot{x_1} = b_1 - k_0 x_1 - k_1 x_6 x_1, \tag{3}$$
$$\dot{x_2} = k_1 x_6 x_1 - k_2 x_2, \tag{4}$$
$$\dot{x_3} = k_2 x_2 - k_3 x_3, \tag{5}$$
$$\dot{x_4} = k_3 x_3 - k_4 x_4, \tag{6}$$
$$\dot{x_5} = k_4 x_4 - k_5 x_5 - (k_6 x_5 x_6 - k_{-6} x_7), \tag{7}$$
$$\dot{x_6} = -k_6 x_5 x_6 + k_{-6} x_7, \tag{8}$$
$$\dot{x_7} = k_6 x_5 x_6 - k_{-6} x_7, \tag{9}$$

which are obtained by the mass action kinetics. We consider this system with FBA. For this example, the stoichiometric matrix is

$$S = \begin{pmatrix} -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \end{pmatrix}, \tag{10}$$

and the flux vector is

$$J = (k_1 x_6 x_1, k_6 x_5 x_6, k_0 x_1, k_2 x_2, k_3 x_3, k_4 x_4, k_5 x_5, k_{-6} x_7, b_1)^T. \tag{11}$$

The generators of null space computed from the stoichiometric matrix are

$$E_1 = (0,1,1,0,0,0,0,1,1)^T, \tag{12}$$
$$E_2 = (1,0,-1,1,1,1,1,0,0)^T, \tag{13}$$
$$E_3 = (0,1,0,0,0,0,0,1,0)^T. \tag{14}$$

These generators are extreme pathways. By taking a linear combination of the extreme pathways, the metabolic flux at a steady state is given by

$$J = j_1E_1 + j_2E_2 + j_3E_3$$
$$= \begin{pmatrix} j_2 \\ j_1 + j_3 \\ j_1 - j_2 \\ j_2 \\ j_2 \\ j_2 \\ j_2 \\ j_1 + j_3 \\ j_1 \end{pmatrix}. \quad (15)$$

The difference from our analysis from the ordinary theory is that we introduce internal structure of the metabolic flux which is realized as a monomial vector form given by the mass action kinetics. The monomial vector form typically affects to the genotype capability as the deformed toric ideal constraints [24]. We thus consider the deformed toric ideal of the above pathway, which was not discussed in the previous work [23]. The ideal $I_{Y_L}^{def} = \{f \in \mathbb{Q}[z] | f(x) \equiv 0\} \subseteq \mathbb{Q}[z]$ is called a deformed toric ideal, where Y_L is the configuration whose entries are the exponents of the monomials in the flux vector. The generators of the deformed toric ideal are the binomial relation between the elements of flux with the monomial representation. The substruction of the second term from the first term vanishes with the adjusted coefficients. This has the property of toric ideal. Because of the adjustment with the coefficients, this might be called 'deformed' toric ideal. Introducing Laurent monomials, from the monomial vector representation of J in Eq.(11), the deformed toric ideal is given by

$$I_J = \langle J_1k_6k_0/J_3 - J_2k_1k_5/J_7 \rangle. \quad (16)$$

From the corresponding representation of flux in Eq.(15), the deformed toric ideal represented by j_l is given by

$$I_j = \langle j_2k_6k_0/(j_1 - j_2) - (j_1 + j_3)k_1k_5/j_2 \rangle. \quad (17)$$

The equality $j_2k_6k_0/(j_1 - j_2) - (j_1 + j_3)k_1k_5/j_2 = 0$ is the only deformed toric ideal constraint. The constraint is not only the relation between the elements of flux, but also gives the restriction of parameter space of flux [24]. This significantly reduces the possible parameter space, which is closer to the true set of steady states than the standard FBA. Additionally, while the algebraic constraints may introduce a complex structure, we can derive useful information on the mixing of the extreme pathways as we demonstrate in the next section.

3 Metabolic Flux as Convex Polyhedron with Algebraically Constrained Coefficients

We have seen that the genotypically capable metabolic flux J is expressed by a linear combination of the extreme pathways. Each element of internal fluxes metabolic flux, however, has to take a positive value. Metabolic flux J can be thus interpreted as an element in the convex polyhedral cone;

$$J = j_1E_1 + j_2E_2 + j_3E_3, \quad (18)$$

where j_ℓ is non-negative real numbers. With this representation, we can analyze the metabolic flux from the viewpoint of a convex polyhedral cone. The geometrical properties of the metabolic flux can be related with the combinatorial properties of the extreme pathways.

For the convex polyhedral cone, we also consider the deformed toric ideal constraint. For the current example, the constraint in (17) is represented by

$$j_3 = \frac{j_2^2 k_6 k_0 - j_1^2 k_1 k_5 + j_1 j_2 k_1 k_5}{(j_1 - j_2) k_1 k_5}, \tag{19}$$

in which j_3 is the function of j_1 and j_2. With this j_3, the metabolic flux J is given by

$$J = j_1 E_1 + j_2 E_2 + \frac{j_2^2 k_6 k_0 - j_1^2 k_1 k_5 + j_1 j_2 k_1 k_5}{(j_1 - j_2) k_1 k_5} E_3. \tag{20}$$

The combination coefficients are represented as the function of j_1 and j_2. This is the important effect of the deformed toric ideal constraint, because this changes the picture of flux from the linear combination of extreme pathways to the nonlinear, algebraic combination of extreme pathways.

From (20) we observe nonlinear mixings of extreme pathways: the metabolic flux J is no longer a convex polyhedral cone, but a subset in the convex polyhedron defined by the nonlinearly constrained coefficients of the generators.

While it is difficult to see how the mixing occurs directly, we can nonetheless extract useful information on this mixing by local expansion. Consider perturbation along j_1 and j_2;

$$\begin{aligned} j_1 &\mapsto j_1 + \Delta j_1, \\ j_2 &\mapsto j_2 + \Delta j_2. \end{aligned}$$

These perturbations can be interpreted as deformation of the polyhedron which causes the mixing of generators. With these perturbations, j_3 is approximated by

$$\begin{aligned} j_3 \simeq\ & \frac{k_6 k_0}{k_1 k_5} \frac{j_2^2}{j_1 - j_2} - \frac{j_1^2}{j_1 - j_2} + \frac{j_1 j_2}{j_1 - j_2} \\ & \frac{1}{(j_1 - j_2)^2} \left\{ -\frac{k_6 k_0}{k_1 k_5} j_2^2 + j_1^2 + (j_1 - 2j_2)(j_1 - j_2) - j_1 j_2 \right\} \Delta j_1 \\ & + \frac{1}{(j_1 - j_2)^2} \left\{ \frac{k_6 k_0}{k_1 k_5} j_2^2 + 2j_2(j_1 - j_2) - j_1^2 + j_1(j_1 - j_2) + j_1 j_2 \right\} \Delta j_2 \\ \equiv\ & A_0 + A_1 \Delta j_1 + A_2 \Delta j_2, \end{aligned}$$

which is the first order approximation of j_3 along j_1 and j_2. The metabolic flux J is then

$$\begin{aligned} J \simeq\ & (j_1 + \Delta j_1) E_1 + (j_2 + \Delta j_2) E_2 + A_0 E_3 + A_1 E_3 \Delta j_1 + A_2 E_3 \Delta j_2 \\ \equiv\ & E_0' + E_1' \Delta j_1 + E_2' \Delta j_2, \end{aligned} \tag{21}$$

where the first order approximation of the mixed generators are given by

$$E_0' = j_1 E_1 + j_2 E_2 + A_0 E_3, \tag{22}$$

$$E_1' = E_1 + A_1 E_3, \tag{23}$$

$$E_2' = E_2 + A_2 E_3. \tag{24}$$

The degree of freedom of j_3 is lost and the degree of freedom along Δj_1 and Δj_2 is left. Including the remianing degree of freedom, the generators are mixed with E_3 by the coefficients as the function of j_1 and j_2. From eqs. (23) and (24), we notice that the perturbation along Δj_1 and Δj_2 are causing the mixture of E_3 to E_1 and E_2. The mixing coefficients of the original E_i are the functions of j_i.

Notice that E_3 has the 2nd and 8th elements, which correspond to the fluxes of $k_6 x_5 x_6$ and $k_{-6} x_7$, respectively. Since the reaction from x_5 and x_6 to x_7 is reversible (see figure 1), this reaction forms the cycle and is almost independent from the other reactions. Therefore, adding E_3 has little effect to the original E_1 and E_2. So, when Δj_1 and Δj_2 are small, we can approximate the generators as $E_0' = j_1 E_1 + j_2 E_2, E_1' = E_1, E_2' = E_2$.

4 Stanley-Reisner Ring of Metabolic Flux

In this and the next sections, we introduce an upper bound to the coefficients j_ℓ, and discuss convex polytopes rather than convex polyhedra. This bounding comes from both mathematical and realistic reasons: it is natural to assume that the flux is bounded by some chemical or physical constraints, and an upper bound may be given by the linear programming of constrained system of flux balance analysis. By considering polytopes, we can discuss the combinatorial properties more easily.

We consider the convex polytope whose vertices are E_1, E_2, E_3 and origin. This convex polytope is generated by a finite set of vertices and the vertices of this polytope are restricted to integer points. Hilbert series is invariant under scale transformation, because Hilbert function depends only on the combinatorial quantity, i.e. the number of faces and the number of how to choose the monomials. The f-vector of the current convex polytope is $(4, 6, 4, 1)$. This can be confirmed also by the mathematical software Macaulay2. We calculate the Hilbert series of this convex polytope.

$$F(k(\Delta), \lambda) = \frac{1 + \lambda + \lambda^2 + \lambda^3 + \lambda^4}{(1 - \lambda)^4}. \tag{25}$$

Hilbert series can be interpreted as the combinatorially unique quantity of metabolic flux.

5 Ehrhart Polynomial of Metabolic Flux

Going back to the original form of flux, $J = j_1 E_1 + j_2 E_2 + j_3 E_3$, j_i are real numbers. Here, j_i are assumed to be bounded. We approximate the real number j_i by rational number. Then, multiply LCM of the denominators of j_i to $\mathcal{P}$, we obtain the number of integer points inside this polytope as $i(\mathcal{P}, n)$. The multiplication of constant integer factor to rational $\mathcal{P}$ is used in the derivation of Ehrhart polynomial.

Note that the coefficient of leading term of Ehrhart polynomial is the volume of polytope $\mathcal{P}$ [25]. The points inside polytope are the genotypically realizable points of metabolic flux, the volume gives the genotype capability of flux. Then, we can calculate the volume from Ehrhart polynomial.

For example, we show the case of the convex polytope with the vertices, E_1, E_2, E_3 and origin as $\mathcal{P}$. Ehrhart polynomial is

$$\frac{1}{6}n^3 + n^2 + \frac{11}{6}n + 1. \tag{26}$$

Therefore, the volume is $1/6$. This indicates the genotype capability.

6 Conclusions

In this paper, we discussed how the metabolic flux can be interpreted from the viewpoint of the algebraic geometry of convex polytope.

At first, we reviewed the metabolic pathway analysis by FBA, with the concrete example of the feedback inhibition of metabolic pathway.

The later sections are devoted to give the interpretation from metabolic pathway analysis to the analysis of former sections. At first, we gave the formulation of metabolic flux as the convex polyhedral cone and analyzed the nonlinear mixing of generators which is caused by deformed toric ideal constraint. Next, we studied the Stanley-Reisner ring of metabolic flux and gave the interpretation of Hilbert series as a combinatorial unique quantity of flux. Then, we gave the interpretation of Ehrhart polynomial as the indicator of genotype capability of flux.

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