

# Use of the Matrix Form of the Fundamental Equations for Transformed Gibbs Energies of Biochemical Reaction Systems at Three Levels

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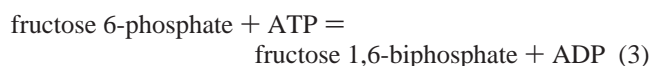
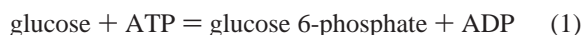
The thermodynamics of a biochemical reaction system can be treated at three levels. At level 1 the fundamental equation for the Gibbs energy  $G$  is written in terms of species, at level 2 the equilibrium pH is specified and the fundamental equation for the transformed Gibbs energy  $G'$  is written in terms of reactants (sums of species), and at level 3 the equilibrium pH and equilibrium concentrations of one or more reactants are specified and the fundamental equation for the further transformed Gibbs energy  $G''$  is written in terms of pseudoisomer groups of reactants. At each level it is convenient to use matrix notation because the conservation matrixes  $A$ ,  $A'$ , and  $A''$  are useful for identifying components and choosing alternate sets of components. These conservation matrixes can also be used to calculate the corresponding stoichiometric number matrixes  $\nu$ ,  $\nu'$ , and  $\nu''$ , which are useful for grouping terms for an independent set of reactions in the fundamental equation. The treatments of reactions at the three levels are similar, and there are mathematical relations between the thermodynamic properties at the three levels. The matrix form of the fundamental equation at each level is useful for identifying the set of properties that have to be specified to describe the extensive state of the system at equilibrium. These variables are used to specify the criterion for spontaneous change and equilibrium. The matrix form of the Gibbs–Duhem equation at each level is useful for identifying the set of intensive properties required to describe the intensive state of the system at equilibrium. The total number of degrees of freedom  $F$  is the same at all three levels.

## Introduction

Stoichiometric number matrixes are useful in discussing the thermodynamics of chemical reaction systems because reactions can be represented by vectors of stoichiometric numbers and systems of chemical reactions can be represented by matrixes.<sup>1,2</sup> These matrixes are related to the corresponding conservation matrixes. Conservation matrixes bring in the concept of components, and components are important in considering reaction systems because amounts of components are conserved. Chemical reactions are discussed in terms of species, but under some conditions it is useful to consider reaction equilibria at a specified partial pressure or concentration of a species. When this is done, the criterion for spontaneous change and equilibrium is expressed in terms of a transformed Gibbs energy  $G'$  defined by use of a Legendre transform by subtracting from the Gibbs energy  $G$  the product of the chemical potential of the species that is held constant and the amount of the corresponding component.<sup>3</sup> Reactions catalyzed by enzymes generally are considered at a specified pH. In this case the criterion for spontaneous change and equilibrium is expressed in terms of a transformed Gibbs energy  $G'$ , which is defined by use of a Legendre transform by subtracting from  $G$  the product of the specified chemical potential of the hydrogen ion and the amount of the hydrogen component.<sup>4,5</sup> At specified pH, species that differ only with respect to the number of hydrogen atoms become pseudoisomers. It is convenient to refer to a pseudoisomer group as a reactant and to calculate the standard transformed thermodynamic properties of the reactant. When  $Mg^{2+}$  is bound by reactants,  $pMg$  ( $-\log[Mg^{2+}]$ ) can be specified in addition. This process of using Legendre transforms can be taken a step further in considering the thermodynamics of biochemical reaction systems in which adenosine triphosphate

(ATP) and adenosine diphosphate (ADP) are at steady-state concentrations.<sup>6</sup> The further transformed Gibbs energy  $G''$  is defined by use of a Legendre transform by subtracting from transformed Gibbs energy  $G'$  the product of the specified transformed chemical potential of ATP and the amount of the ATP component, and also subtracting the product of the specified transformed chemical potential of ADP and the amount of the ADP component. Thus thermodynamic calculations on a biochemical reaction system can be carried out at three levels: level 1 in terms of species, level 2 at specified pH in terms of reactants (sums of species), and level 3 at specified pH and specified concentrations of ATP and ADP in terms of pseudoisomer groups of reactants. The treatment given here differs from those given previously in its emphasis on the conservation matrixes and stoichiometric number matrixes. These matrixes provide the basis for the reconceptualization of a reaction system whenever the concentration of a species or a reactant is specified. The matrix forms of the fundamental equations and the Gibbs–Duhem equations for  $G$ ,  $G'$ , and  $G''$  provide the basis for identifying the thermodynamic variables required to describe the intensive and extensive states of the system at each level.

To make the thermodynamic treatments of a system of biochemical reactions at these three levels clear, they will be applied to the first three reactions in glycolysis:



These three biochemical reactions are catalyzed by hexokinase (EC 2.7.1.1), glucose-6-phosphate isomerase (EC 5.3.1.9), and

6-phosphofructokinase (EC 2.7.1.11), respectively. The EC numbers are from *Enzyme Nomenclature*.<sup>7</sup> In the pH range 5–9 and in the absence of cations such as  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , which are bound by the negative ions, the level 1 discussion of this system involves 12 species, 6 acid dissociation constants, and 3 equilibrium constants for reference reactions. The level 2 discussion at specified pH involves six reactants and three apparent equilibrium constants. The level 3 discussion at specified pH and specified concentrations of ATP and ADP involves four reactants, but because these reactants are pseudoisomers under these conditions, the thermodynamic properties of the pseudoisomer group can be calculated. When the equilibrium concentrations of ATP and ADP are specified, the conservation equation for phosphorus atoms is eliminated, and the reactions between pseudoisomer groups can be represented by



The equilibrium calculations given here show how calculations become simpler as the values of more intensive variables are held constant.

When the partial pressure or concentration of a species is held constant, it is convenient to write the fundamental equation for  $G'$  using matrix notation.<sup>8,9</sup> The conservation matrixes and stoichiometric number matrixes are changed when the concentration of a species is held constant, and these matrixes help identify components and suitable sets of independent reactions. In making thermodynamic calculations it is important to know how many components there are and what different choices of components can be made. There are also questions as to how many and which variables need to be specified to describe the intensive state of the reaction system and the extensive state of the reaction system at equilibrium. Callen<sup>10</sup> has emphasized that “The choice of variables in terms of which a given problem is formulated, while a seemingly innocuous step, is often the most crucial step in the solution.” Matrix operations can be used to change the choice of components. The stoichiometric number matrixes, which can be derived from conservation matrixes, show how the reactions should be written at the successive levels.

The following sections show the importance of the fundamental equations and the Gibbs–Duhem equations in discussing a system of biochemical reactions and emphasize the use of conservation matrixes and stoichiometric number matrixes to identify components, to show the form of the reaction equations, and to calculate the numbers of degrees of freedom.

### The Fundamental Equation for $G$ for a System of Chemical Reactions

The fundamental equation for the Gibbs energy of a multi-reaction system can be written in matrix form as

$$dG = -SdT + VdP + \mu dn \quad (7)$$

where  $\mathbf{n}$  is the  $N \times 1$  column vector of amounts of  $N$  species and  $\mu$  is the  $1 \times N$  row vector of chemical potentials of the  $N$  species. In discussing the thermodynamics of biochemical reactions in terms of species, it is convenient to assume dilute aqueous solutions and to take the thermodynamic properties  $G$ ,  $S$ ,  $H$ , and chemical potentials  $\mu_i$  to be functions of ionic strength.<sup>11</sup> When this is done, the solutions can be discussed as

if they were ideal solutions. In other words, the chemical potential of an ionic species is expressed in terms of its concentration  $[i]$  by use of  $\mu_i = \mu_i^\circ + RT \ln[i]$ , where  $\mu_i^\circ$  is the standard chemical potential at specified  $T$ ,  $P$ , and ionic strength. Concentrations are expressed in moles per liter, but they are considered to be dimensionless so that this equation can be used. Thus equilibrium constant expressions are written in terms of concentrations of species, and equilibrium constants  $K$  are functions of ionic strength.

For a multireaction system, the amount of species vector  $\mathbf{n}$  is given by

$$\mathbf{n} = \mathbf{n}_0 + \nu \xi \quad (8)$$

where  $\mathbf{n}_0$  is the  $N \times 1$  column vector of initial amounts,  $\nu$  is the  $N \times R$  matrix of stoichiometric numbers (positive for products and negative for reactants), and  $\xi$  is the  $R \times 1$  column vector of extents of the  $R$  independent reactions. Thus eq 7 for a closed system can be written

$$dG = -SdT + VdP + \mu \nu d\xi \quad (9)$$

This equation indicates that the condition for equilibrium at constant  $T$ ,  $P$ , and ionic strength is  $\mu \nu = 0$ . This equation yields equilibrium constant expressions written in terms of concentrations of species, but it is not informative about the number of natural variables, which are the variables that must be specified to calculate the equilibrium composition. For a multireaction system, the stoichiometric number matrix is not unique, but the equilibrium composition can be calculated with any complete set of independent reactions.

A suitable set of independent reactions can always be calculated by use of the conservation matrix  $\mathbf{A}$  for the system, which is a  $C \times N$  matrix where  $C$  is the number of components. For chemical reaction systems the components are usually taken to be atoms of elements and electric charges, but groups or any combination of atoms can be used. The rows in the conservation matrix must be independent, but alternate choices can be made of components. The matrix product of the conservation matrix  $\mathbf{A}$  and the amount vector  $\mathbf{n}$  is the vector  $\mathbf{n}_C$  of amounts of the various components.

$$\mathbf{A}\mathbf{n} = \mathbf{n}_C \quad (10)$$

The stoichiometric number matrix is related to the conservation matrix for a system by  $\mathbf{A}\nu = 0$  and  $\nu^T \mathbf{A}^T = 0$ , where  $T$  indicates the transpose. The stoichiometric number matrix can be calculated from the conservation matrix by use of a computer program to calculate the null space. Alternatively, the transposed conservation matrix can be calculated from the transposed stoichiometric number matrix. A null space can be calculated by hand by row reducing a matrix, changing the signs of the elements to the right of the diagonal form, and putting a unit matrix of appropriate size below it.<sup>1</sup>

When a system is at chemical equilibrium, the form of the fundamental equation for  $G$  is obtained by using the equilibrium expressions  $\sum \nu_{ij} \mu_i = 0$ , where  $j$  is the number of the reaction, to eliminate  $R$  chemical potentials. This form of the fundamental equation is given by

$$dG = -SdT + VdP + \sum_{i=1}^C \mu_{ci} dn_{ci} \quad (11)$$

where the  $\mu_{ci}$  are the chemical potentials of species chosen as components, and the  $n_{ci}$  are the amounts of components. The matrix form of this equation is

$$dG = -SdT + VdP + \mu_c dn_c \quad (12)$$

where  $\mu_c$  is the  $1 \times C$  vector of chemical potentials of components and  $n_c$  is the  $C \times 1$  column vector of amounts of components. The chemical potentials of components are the chemical potentials of species taken as components. Because the components are independent variables for a chemical reaction system, the criterion for spontaneous change and equilibrium is  $dG \leq 0$  at constant  $T$ ,  $P$ , ionic strength, and specified amounts of  $C$  components. An especially good treatment of components is given by Beattie and Oppenheim.<sup>12</sup>

Equation 12 indicates that the corresponding Gibbs–Duhem equation is

$$-SdT + VdP - (d\mu_c)n_c = 0 \quad (13)$$

This equation can be derived by taking the complete Legendre transform of  $G$ . Because eq 13 provides a relation between the  $C + 2$  intensive variables for the system, the number of degrees of freedom is  $F = C + 1$ . This is in agreement with the phase rule for a one-phase system because  $F = C - p + 2 = C + 1$ , where  $p$  is the number of phases. To describe the extensive state of a system at equilibrium, an extensive variable must be specified for each phase, so that the number of extensive variables  $D$  for a one-phase system is given by  $D = F + p = C + 2$ . Note that this is in agreement with the number of terms in eq 11; the  $D$  independent variables for a system are referred to as its natural variables.

### The Fundamental Equation for $G'$ for a System of Biochemical Reactions at Specified pH

For a biochemical reaction system at specified pH, the criterion for spontaneous change and equilibrium is given by the transformed Gibbs energy  $G'$  that is defined by<sup>4,5</sup>

$$G' = G - n_c(H)\mu(H^+) \quad (14)$$

where the conjugate variables are  $n_c(H)$ , the amount of the hydrogen component in the system (total amount of hydrogen atoms), and  $\mu(H^+)$ , the specified chemical potential of hydrogen ions. Because  $G = \sum \mu_i n_i$  and  $n_c(H) = \sum N_H(i)n_i$ , eq 14 indicates that the transformed chemical potential of species  $i$  is given by

$$\mu_i' = \mu_i - N_H(i)\mu(H^+) \quad (15)$$

where  $N_H(i)$  is the number of hydrogen atoms in species  $i$ . As in the preceding section, thermodynamic properties are taken to be functions of the ionic strength. It can be shown that when proton transfers are at equilibrium in a system, the chemical potentials of species that differ only with respect to the number of hydrogen atoms are equal; therefore, the transformed chemical potential of a reactant (sum of species) is equal to the transformed chemical potentials of the species in the pseudoisomer group. Thus the system can be reconceptualized into  $N'$  pseudoisomer groups, rather than  $N$  species. The amount of a pseudoisomer group is represented by  $n_i'$ , which is the sum of the amounts of the pseudoisomers. The expressions for apparent equilibrium constants  $K'$  are written in terms of concentrations of pseudoisomer groups, which are referred to as reactants.

It can be shown<sup>4,5</sup> that the fundamental equation for  $G'$  is given by

$$dG' = -S'dT + VdP + \sum_{i=1}^{N'} \mu_i' dn_i' + \ln(10)RTn_c(H)d\text{pH} \quad (16)$$

where the transformed entropy of the system is given by

$$S' = S - n_c(H)\bar{S}(H^+) \quad (17)$$

where  $\bar{S}(H^+)$  is the molar entropy of hydrogen ions at the specified pH ( $-\log[H^+]$ ). There is a transformed enthalpy given by  $H' = G' + TS'$ . The number of terms in the summation (number of reactants) in eq 16 is represented by  $N'$  to distinguish it from the number  $N$  of species in the system. Because eq 16 has a term proportional to  $d\text{pH}$ , there are some new Maxwell equations.

The matrix form of eq 16 is

$$dG' = -S'dT + VdP + \mu' dn' + \ln(10)RTn_c(H)d\text{pH} \quad (18)$$

where  $\mu'$  is the  $1 \times N'$  row vector of transformed chemical potentials of reactants and  $n'$  is the  $N' \times 1$  column vector of amounts of reactants. When the pH is specified, reactions are written in terms of sums of species, as in  $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i$ , and the term biochemical reaction or overall reaction is used. When the pH is specified, eq 18 has the same form as eq 7; this is the reason that the thermodynamic treatment of a biochemical reaction at specified pH is so much like that for a chemical reaction, even though the interpretation of the thermodynamic properties is different.

For a system of biochemical reactions, the amount of reactants vector  $n'$  is given by

$$n' = n_0' + \nu' \xi' \quad (19)$$

where  $n_0'$  is the  $N' \times 1$  column vector of initial amounts of reactants,  $\nu'$  is the  $N' \times R'$  matrix of stoichiometric numbers, and  $\xi'$  is the  $R' \times 1$  column vector of extents of the  $R'$  independent biochemical reactions. Thus eq 18 can be written

$$dG' = -S'dT + VdP + \mu' \nu' d\xi' + \ln(10)RTn_c(H)d\text{pH} \quad (20)$$

This indicates that the condition for equilibrium at constant  $T$ ,  $P$ , ionic strength, and pH is  $\mu' \nu' = 0$ , but eq 20 is not informative about the number of natural variables, which are the variables that must be specified to calculate the equilibrium composition in terms of reactants. When there is single biochemical reaction, eq 20 can be used to show<sup>9</sup> that the change in binding of hydrogen ions in a biochemical reaction is given by  $\Delta_r N_H = -(\partial \log K' / \partial \text{pH})_{T,P,\xi}$ , where  $K'$  is the apparent equilibrium constant.

A suitable set of independent biochemical reactions for quantitative calculation can be derived from the apparent conservation matrix  $A'$ , which is  $C' \times N'$ . The number of components is represented by  $C'$  because it is the number of components other than hydrogen since pH is specified. The  $A'$  matrix is referred to as the apparent conservation matrix because the row for hydrogen has been deleted and redundant columns have been deleted so that the columns correspond with reactants (sums of species). The  $A'$  matrix has been referred to as if it is written in terms of atoms, but it can also be written in terms of groups of atoms and, as we will see below, in terms of reactants such as ATP. The matrix product of the apparent conservation matrix and the amounts of reactants vector  $n'$  is equal to the vector of the amounts  $n_c'$  of components other than hydrogen.

$$A'n' = n_c' \quad (21)$$

The apparent stoichiometric number matrix, which is  $(C' \times 1)$ , is related to the apparent conservation matrix for a system by



$\mathbf{A}'\mathbf{r}' = \mathbf{0}$  and  $(\mathbf{r}')^T(\mathbf{A}')^T = \mathbf{0}$ , where T indicates the transpose. Thus these matrixes can be interconverted as described above. These matrixes are not unique, but their row-reduced forms are unique for a given order of the reactant columns. It is important to recognize that we can change the choice of components (change the basis) by making a matrix multiplication.<sup>13</sup> Because components are really coordinates, we can shift to a new coordinate system by multiplying by the inverse of the transformation matrix between the two coordinate systems. The transformation matrix  $\mathbf{m}$  gives the composition of the new components (columns) in terms of the old components (rows). The new stoichiometric number matrix is obtained using  $\mathbf{A}(\text{new}) = \mathbf{m}^{-1}\mathbf{A}(\text{old})$ .

When a biochemical reaction system is at equilibrium, the form of the fundamental equation for  $G'$  is obtained by using the equilibrium expressions  $\sum v_{ij}'\mu_i' = 0$  to eliminate  $R'$  transformed chemical potentials. This yields the following fundamental equation:

$$dG' = -S'dT + VdP + \sum_{i=1}^C \mu_{ci}' dn_{ci}' + \ln(10)RTn_c(H)d\text{pH} \quad (22)$$

where  $C' = N' - R'$ . The  $\mu_{ci}'$  are the transformed chemical potentials of the reactants chosen as components, and the  $n_{ci}'$  are the amounts of apparent components. The matrix form of this equation is

$$dG' = -S'dT + VdP + \boldsymbol{\mu}_c' d\mathbf{n}_c' + \ln(10)RTn_c(H)d\text{pH} \quad (23)$$

where  $\boldsymbol{\mu}_c'$  is the  $1 \times C'$  row vector of transformed chemical potentials of apparent components and  $\mathbf{n}_c'$  is the  $C' \times 1$  column vector of amounts of apparent components. Because the apparent components are independent variables for a biochemical reaction system at specified pH, the criterion for spontaneous change and equilibrium is  $dG' \leq 0$  at specified  $T$ ,  $P$ , ionic strength, pH, and amounts of  $C'$  apparent components.

Equation 23 shows that the Gibbs–Duhem equation for this system is given by

$$-S'dT + VdP - (d\boldsymbol{\mu}_c')\mathbf{n}_c' + \ln(10)RTn_c(H)d\text{pH} = 0 \quad (24)$$

Because of this relation between the  $C' + 3$  intensive variables for the system, the total number of degrees of freedom  $F$  required to describe the intensive state of the system is given by  $F = C' + 2$ . This system has one less component than the corresponding chemical system; thus  $C' = C - 1$ , and  $F = C + 1$ , as in the preceding section. To describe the extensive state of a system at equilibrium, an extensive variable must be specified for each phase, so that the total number of degrees of freedom  $D$  required to describe the extensive state of the system is given by  $D = F + p = C' + 3$  in agreement with eq 22. Because  $F = C + 1$ ,  $D = C + 2$ , in the preceding section. This discussion corresponds with writing the phase rule as  $F = C - p + 2$  for a system of chemical reactions and as  $F = C' - p + 3$  for a system described in terms of biochemical reactions. Equation 24 shows that when the pH is specified,  $C' + 2$  intensive variables remain so that the number  $F'$  of intensive variables that must be specified to describe the intensive state of the system is given by  $F' = C' + 1$ . This is in agreement with the phase rule written as  $F' = C' - p + 2$ . This is another reason biochemical reactions at specified pH behave so much like chemical reactions written in terms of species. The corresponding number of variables  $D'$  required to describe the extensive state of a one-phase system is given by  $D' = F' + p = C' + 2$ .

The equilibrium concentrations of other species can be specified in the same Legendre transform as the hydrogen ion. An example of that is the specification of pMg in addition to pH, which has already been used.<sup>4,5</sup> But there is a limit on how many species concentrations can be specified at equilibrium. This limit is  $C - 1$ , where  $C$  is the number of components in the corresponding chemical system. When all  $C$  components are involved in the Legendre transform, it is referred to as complete, and the Gibbs–Duhem equation for the system is obtained.

### The Fundamental Equation for $G''$ for a System of Biochemical Reactions at Specified pH and Specified Concentrations of ATP and ADP

In discussing a system of biochemical reactions at specified pH, it may be of interest to calculate the equilibrium composition that can be reached at specified concentrations of reactants such as ATP and ADP. In a living cell, these reactants are produced and consumed by a large number of reactions, and so their concentrations tend to be in steady states. When their concentrations are constant, the criterion of spontaneous change and equilibrium is provided by the further transformed Gibbs energy  $G''$  that is defined by the following Legendre transform<sup>6</sup>:

$$G'' = G' - n_c'(\text{ATP})\mu'(\text{ATP}) - n_c'(\text{ADP})\mu'(\text{ADP}) \quad (25)$$

In this equation,  $n_c'(\text{ATP})$  and  $\mu'(\text{ATP})$  are conjugate variables, and  $n_c'(\text{ADP})$  and  $\mu'(\text{ADP})$  are conjugate variables. It may seem remarkable that ATP and ADP can be taken as components, but, as stated above, any group or combination of atoms can be taken as a component. A specific example is discussed in the next section. Because  $G' = \sum \mu_i' n_i'$ ,  $n_c'(\text{ATP}) = \sum N_{\text{ATP}}(i)n_i'$ , and  $n_c'(\text{ADP}) = \sum N_{\text{ADP}}(i)n_i'$ , the further transformed chemical potential of reactant  $i$  (sum of species) is given by

$$\mu_i'' = \mu_i' - N_{\text{ATP}}(i)\mu'(\text{ATP}) - N_{\text{ADP}}(i)\mu'(\text{ADP}) \quad (26)$$

where  $N_{\text{ATP}}(i)$  and  $N_{\text{ADP}}(i)$  are the numbers of ATP and ADP molecules required to make up the  $i$ th reactant. (Note that these numbers may be positive or negative.) The values of  $N_{\text{ATP}}(i)$  and  $N_{\text{ADP}}(i)$  can be obtained from the apparent conservation matrix (see eq 37). The Legendre transform in eq 25 leads to the following fundamental equation for  $G''$ :

$$dG'' = -S''dT + VdP + \sum_{i=1}^{N''} \mu_i'' dn_i'' + \ln(10)RTn_c(H)d\text{pH} - n_c'(\text{ATP})RT \ln[\text{ATP}] - n_c'(\text{ADP})RT \ln[\text{ADP}] \quad (27)$$

where  $N'' = N' - 2$  is the number of reactants for which equilibrium concentrations have not been specified. The further transformed entropy is given by

$$S'' = S' - n_c'(\text{ATP})\bar{S}'(\text{ATP}) - n_c'(\text{ADP})\bar{S}'(\text{ADP}) \quad (28)$$

where  $\bar{S}'(\text{ATP})$  is the molar transformed entropy of ATP. There is a further transformed enthalpy is given by  $H'' = G'' + TS''$ . When the reactions of ATP and ADP with reactants in the system are at equilibrium, the further transformed chemical potentials of some of the reactants are equal; these reactants form a pseudoisomer group with amount  $n_i''$ . Thus holding  $[\text{ATP}]$  and  $[\text{ADP}]$  constant allows us to reconceptualize the system into a smaller set of  $N''$  pseudoisomer groups. The expressions for apparent equilibrium constants  $K''$  are written in terms of concentrations of these  $N''$  pseudoisomer groups.

When [ATP] and [ADP] are specified, reactions are written without these reactants (see eqs 4–6).

With matrix notation, eq 27 can be written

$$dG'' = -S''dT + VdP + \mu''dn'' + \ln(10)RTn_c(H)d\text{pH} - n_c'(ATP)RT \ln[ATP] - n_c'(ADP)RT \ln[ADP] \quad (29)$$

where  $\mu''$  is the  $1 \times N''$  row vector of further transformed chemical potentials of reactants for which equilibrium concentrations have not been specified and  $n''$  is the corresponding  $N \times 1$  column vector.

For a system of apparent biochemical reactions at specified [ATP] and [ADP] (like eqs 4–6), the amount of reactants vector  $n''$  is given by

$$n'' = n_0'' + v''\xi'' \quad (30)$$

where  $n_0''$  is the  $N'' \times 1$  column vector of initial amounts of the reactants,  $v''$  is the  $N'' \times R''$  matrix of stoichiometric numbers (positive for products and negative for reactants), and  $\xi''$  is the  $R'' \times 1$  column vector of extents of the  $R''$  independent reactions. Thus eq 29 can be written

$$dG'' = -S''dT + VdP + \mu''v''d\xi'' + \ln(10)RTn_c(H)d\text{pH} - n_c'(ATP)RT \ln[ATP] - n_c'(ADP)RT \ln[ADP] \quad (31)$$

This indicates that the condition for equilibrium at constant  $T$ ,  $P$ , pH, [ATP], and [ADP] is  $\mu''v'' = 0$ . However, eq 31 is not informative about the number of natural variables, which are the variables that must be specified to calculate the equilibrium concentrations in terms of pseudoisomer groups.

A suitable set of apparent biochemical reactions can be calculated from the apparent conservation matrix  $A''$ , which is  $C'' \times N''$ . The number of components is represented by  $C''$  because it is the number of components other than hydrogen, ATP, and ADP. The  $A''$  matrix is referred to as an apparent conservation matrix because the rows for hydrogen, ATP, and ADP have been deleted. The matrix product of the apparent conservation matrix  $A''$  and the amounts of reactants vector  $n''$  is equal to the vector of the amounts  $n_c''$  of components other than hydrogen, ATP, and ADP.

$$A''n'' = n_c'' \quad (32)$$

The apparent stoichiometric number matrix is related to the apparent conservation matrix for a system by  $A''v'' = 0$  and  $(v'')^T(A'')^T = 0$ , where T indicates the transpose. Thus these matrixes can be interconverted as described above. These matrixes are not unique, but the row-reduced forms for a certain order of apparent reactants is unique.

When a biochemical reaction system is at equilibrium, the form of the fundamental equation for  $G''$  is obtained by using the equilibrium expressions  $\sum \nu_{ij}''\mu_{ci}'' = 0$  to eliminate  $R''$  further transformed chemical potentials. This yields the following fundamental equation:

$$dG'' = -S''dT + VdP + \sum_{i=1}^{C''} \mu_{ci}''dn_{ci}'' + \ln(10)RTn_c(H)d\text{pH} - n_c'(ATP)RT \ln[ATP] - n_c'(ADP)RT \ln[ADP] \quad (33)$$

where  $C'' = N'' - R''$ . The  $\mu_{ci}''$  are the further transformed chemical potentials of the pseudoisomer groups chosen as components, and the  $n_{ci}''$  are the amounts of these pseudoisomer

groups. The matrix form of this equation is

$$dG'' = -S''dT + VdP + \mu_c''dn_c'' + \ln(10)RTn_c(H)d\text{pH} - n_c'(ATP)RT \ln[ATP] - n_c'(ADP)RT \ln[ADP] \quad (34)$$

where  $\mu_c''$  is the  $1 \times C''$  row vector of further transformed chemical potentials of apparent components and  $n_c''$  is the  $C'' \times 1$  column vector of amounts of apparent components. Because the apparent components are independent variables for a biochemical reaction system at specified pH, [ATP], and [ADP], the criterion for spontaneous change and equilibrium is  $dG'' \leq 0$  at specified  $T$ ,  $P$ , ionic strength, pH, [ATP], [ADP], and specified amounts of  $C''$  components.

Equation 34 shows that the Gibbs–Duhem equation for this system is given by

$$-S''dT + VdP - (d\mu_c'')n_c'' + \ln(10)RTn_c(H)d\text{pH} - n_c'(ATP)RT \ln[ATP] - n_c'(ADP)RT \ln[ADP] = 0 \quad (35)$$

Because of this relation between the  $C'' + 5$  intensive variables for the system, the total number of degrees of freedom  $F$  required to describe the intensive state of the system is given by  $F = C'' + 4$ . This system has three fewer components than the corresponding chemical system described in terms of species. Thus  $C'' = C - 3$  and  $F = C + 1$ , as we saw earlier. The total number of extensive variables  $D$  required to describe the extensive state of the system is given by  $D = F + p = C'' + 5 = C + 2$ , as in the preceding two sections. This discussion corresponds with writing the phase rule as  $F = C - p + 2$  for a system of chemical reactions, as  $F = C' - p + 3$  for a system described in terms of biochemical reactions and as  $F = C'' - p + 5$  for a system described in terms of reactions of pseudoisomer groups. Equation 35 shows that when pH, [ATP], and [ADP] are specified,  $C'' + 2$  intensive variables remain so that the number of intensive variables  $F''$  that must be specified to describe the intensive state of the system is given by  $F'' = C'' + 1$ . This is in agreement with the phase rule written as  $F'' = C'' - p + 2$ . Thus again, we see that a system described in terms of pseudoisomer groups of reactants behaves very much like a system of chemical reactions. The corresponding number  $D''$  of variables that must be specified to describe the extensive state of the system after, pH, [ATP], and [ADP] have been specified is given by  $D'' = C'' + 2$ .

### Calculation of the Equilibrium Composition of a Biochemical Reaction System at Specified pH

At specified pH the first three reactions of glycolysis are described by reactions 1–3. The conservation matrix for this system is

$$A' = \begin{matrix} & \text{ATP} & \text{ADP} & \text{Glu} & \text{G6P} & \text{F6P} & \text{F16BP} \\ \text{Aden} & \begin{bmatrix} 1 & 1 & 0 & 0 & 0 & 0 \end{bmatrix} \\ \text{P} & \begin{bmatrix} 3 & 2 & 0 & 1 & 1 & 2 \end{bmatrix} \\ \text{Glu} & \begin{bmatrix} 0 & 0 & 1 & 1 & 1 & 1 \end{bmatrix} \end{matrix} \quad (36)$$

where the  $C'$  components are the adenine group, phosphorus atoms, and the glucose framework. Row reduction yields

$$A' = \begin{matrix} & \text{ATP} & \text{ADP} & \text{Glu} & \text{G6P} & \text{F6P} & \text{F16BP} \\ \text{ATP} & \begin{bmatrix} 1 & 0 & 0 & 1 & 1 & 2 \end{bmatrix} \\ \text{ADP} & \begin{bmatrix} 0 & 1 & 0 & -1 & -1 & -2 \end{bmatrix} \\ \text{Glu} & \begin{bmatrix} 0 & 0 & 1 & 1 & 1 & 1 \end{bmatrix} \end{matrix} \quad (37)$$

Note that the  $C'$  components are now ATP, ADP, and glucose. This shows that G6P and F6P each can be considered to be made up of 1ATP and  $-1$ ADP plus 1Glu and F16BP can be considered to be made up of 2ATP and  $-2$ ADP plus 1Glu.

Equation 37 indicates that there are three independent reactions. The row-reduced form of the transposed null space of this matrix is

$$(\mathbf{v}')^T = \begin{matrix} & \text{ATP} & \text{ADP} & \text{G} & \text{G6P} & \text{F6P} & \text{F16BP} \\ \text{Rx1} & \begin{bmatrix} 1 & -1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 1 & 0 & -2 & 1 \\ 0 & 0 & 0 & 1 & -1 & 0 \end{bmatrix} \end{matrix} \quad (38)$$

This same matrix is obtained by writing the stoichiometric number matrix for reactions 1–3 and row reducing it, and so this stoichiometric number matrix corresponds with reactions 1, 2, and 3. These matrix operations are carried out conveniently using Mathematica.<sup>14</sup>

The equilibrium composition of this reaction system at 298.15 K, pH 7, and ionic strength 0.25 M can be calculated because the standard transformed Gibbs energies of formation of the six reactants are known.<sup>15</sup> To calculate the equilibrium composition of this system, it is necessary to specify  $D = C' + 3$  variables, at least one of which has to be extensive, as indicated by the discussion after eq 24. These can be taken to be  $T$ ,  $P$ , pH, and the amounts of the adenine, phosphorus, and glucose components. Suppose that the system initially contains 0.01 M glucose and 0.01 M ATP. It may appear that this only specifies the initial amounts of two components, but this is not true because matrixes 36 and 37 indicate that the components can be chosen to be Aden, P, and Glu or ATP, ADP, and Glu. If the system with a volume of 1 L initially contains 0.01 M glucose and 0.01 M ATP, the amount of the adenine component per liter is 0.01 mol, the amount of the phosphate component is 0.03 mol, and the amount of the glucose component is 0.01 mol. Thus, there are three equilibrium equations and three conservation equations. Mathematica<sup>14</sup> has been used to solve these six simultaneous equations, and the equilibrium composition is [ATP] =  $2.7 \times 10^{-7}$  M, [ADP] = 0.00999 M, [glucose] = 0.0024 M, [glucose 6-phosphate] = 0.0124 M, [fructose 6-phosphate] = 0.00395 M, and [fructose 1,6-biphosphate] = 0.0024 M.

### Calculation of the Equilibrium Composition of a Biochemical Reaction System at Specified pH and Specified Concentrations of ATP and ADP

An alternative way to make equilibrium calculations on the first three steps of glycolysis is to specify the equilibrium concentrations of ATP and ADP and calculate the resulting equilibrium concentrations of the other four reactants, a level 3 calculation. When the equilibrium concentrations of ATP and ADP are specified, the first two rows and first two columns of the apparent conservation matrix in eq 37 are deleted, so the apparent conservation matrix becomes  $A'' = [1, 1, 1, 1]$ . This indicates that these four reactants form a pseudoisomer group; in other words, the system consists of one pseudoisomer group. The reactions within the pseudoisomer group are represented by eqs 4–6.

To calculate the equilibrium concentrations of these four reactants, their further transformed Gibbs energies of formation are calculated using eq 26. In discussing the fundamental equation, chemical potentials are used, but in making calculations standard transformed Gibbs energies are used so that:

$$\Delta_f G''(i) = \Delta_f G'(i) - N_{\text{ATP}}(i) \Delta_f G'(\text{ATP}) - N_{\text{ADP}}(i) \Delta_f G'(\text{ADP}) \quad (39)$$

where

$$\Delta_f G'(i) = \Delta_f G'^{\circ}(i) + RT \ln[i] \quad \text{and} \quad \Delta_f G''(i) = \Delta_f G''^{\circ}(i) + RT \ln[i] \quad (40)$$

so that

$$\Delta_f G''^{\circ}(i) = \Delta_f G'^{\circ}(i) - N_{\text{ATP}}(i) \Delta_f G'(\text{ATP}) - N_{\text{ADP}}(i) \Delta_f G'(\text{ADP}) \quad (41)$$

The numbers of ATP molecules and ADP molecules involved in these four reactions are shown in the row reduced conservation matrix (eq 37). When the equilibrium concentration of ATP is 0.0001 M and the equilibrium concentration of ADP is 0.01 M, the standard further transformed Gibbs energies of formation of the remaining four reactants are as follows: (in kJ mol<sup>-1</sup>) glucose,  $-426.71$ ; glucose 6-phosphate,  $-439.73$ ; fructose 6-phosphate,  $-436.55$ ; fructose 1,6-biphosphate,  $449.98$ . Because these reactants are pseudoisomers, the standard further transformed Gibbs energy of formation of the pseudoisomer group can be calculated by using<sup>1</sup>

$$\Delta_f G''^{\circ}(\text{iso}) = -RT \ln \sum_{i=1}^{N_{\text{iso}}} \exp(-\Delta_f G''^{\circ}(i)/RT) = -450.03 \text{ kJ mol}^{-1} \quad (42)$$

where  $N_{\text{iso}}$  is the number of pseudoisomers in the group, that is 4. The equilibrium mole fractions of glucose, glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-biphosphate can be calculated by using

$$r_i = \exp[(\Delta_f G''^{\circ}(\text{iso}) - \Delta_f G''^{\circ}(i))/RT] \quad (43)$$

Thus the equilibrium concentrations of glucose, glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-biphosphate are  $8.21 \times 10^{-7}$ ,  $1.56 \times 10^{-4}$ ,  $4.34 \times 10^{-5}$ , and  $9.8 \times 10^{-2}$  M. The specification of the equilibrium concentrations of ATP and ADP has simplified this equilibrium calculation. When all 10 reactions of glycolysis are considered and ATP, ADP, NAD<sub>ox</sub>, NAD<sub>red</sub>, P<sub>i</sub>, and H<sub>2</sub>O are held constant, the system is represented by two pseudoisomer groups, as indicated by  $C_6 = 2C_3$ . The expression for the apparent equilibrium constant is  $K'' = [C_3]^2/[C_6]$ . When a large system of biochemical reactions, like glycolysis, is considered, more than three levels of thermodynamic treatment can be made.

### Discussion

When a Legendre transform is used to define a transformed Gibbs energy  $G'$  that provides the criterion for spontaneous change and equilibrium at specified concentrations of one or more species, the reaction system is reconceptualized as being made up of pseudoisomer groups, like ATP and ADP, which are referred to a reactants. When a second Legendre transform is used to define a further transformed Gibbs energy  $G''$  that provides the criterion for spontaneous change and equilibrium at specified concentrations of one or more species and one or more reactants, the reaction system is reconceptualized again as being made up of pseudoisomer groups of reactants. Thus the equilibrium composition of a biochemical reaction system can be calculated at three levels, in terms of species (level 1), in terms of reactants (sums of species) at specified pH and



specified concentrations of other ions (like  $\text{Ca}^{2+}$ ) that are bound (level 2), and in terms of pseudoisomer groups of reactants (sums of reactants) at specified pH and specified concentrations of reactants such as ATP and ADP (level 3). At each level it is important to know the number of components and what they are chosen to be for a particular equilibrium calculation. Components are important because their amounts are conserved and are used in the statement of the criterion for spontaneous change and equilibrium. Matrix operations are useful in identifying components and stoichiometric number matrixes for complicated reaction systems. It is useful to write the fundamental equations for  $G$ ,  $G'$ , and  $G''$  using matrix notation because these equations contain important information about the independent intensive and extensive variables before and after equilibrium and the relations between them obtained by taking partial derivatives and using Maxwell equations.

Higher level calculations give more global views of a reaction system, and higher level equilibrium calculations are also simpler to carry out because there are fewer variable concentrations and fewer conservation equations, as illustrated in this article. At each level, there is a full set of thermodynamic properties, so that superficially the equations at each level have the same general form. However, the similar properties have different symbols, different values, and different interpretations. At each level there is a different way to write reactions. At Levels 2 and 3 there are new types of terms in the fundamental equation and new types of Maxwell equations. As shown in this article, the thermodynamic properties at the three levels are connected by mathematical equations. It is suggested that further levels may be useful in discussing large systems of biochemical reactions, like glycolysis.

Conservation matrixes play a central role in considering a reaction system at various levels. The conservation matrix  $\mathbf{A}$  for a reaction system in terms of species is simplified when the equilibrium concentrations of species such as  $\text{H}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$  are held constant. The conservation matrix  $\mathbf{A}'$  in terms of reactants is further simplified when the equilibrium concentrations of reactants such as ATP, ADP,  $\text{NAD}_{\text{ox}}$ , and  $\text{NAD}_{\text{red}}$  are specified. The limit on the number of components for which equilibrium concentrations can be specified is  $C - 1$ , where  $C$  is the number of components in the chemical system.

More discussion of new partial derivatives, Maxwell equations, and Gibbs–Helmholtz equations could have been given, but the important role of Gibbs–Duhem equations in identifying degrees of freedom for describing the intensive state of a system at equilibrium has been discussed. It is shown that the specification of equilibrium concentrations of species or reactants does not change the total number  $F$  of degrees of freedom for describing the initial state of the system or the total number  $D$  of degrees of freedom for describing the extensive state of the system. However, when concentrations of species are specified, it is convenient to use the phase rule in the form  $F' = C' - p + 2$ , and when additional concentrations of reactants such as ATP and ADP are specified, it is convenient to use  $F'' = C'' - p + 2$ .

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## Glossary

$\mathbf{A}$	conservation matrix ( $C \times N$ )
$\mathbf{A}'$	apparent conservation matrix when the concentrations of one or more species are held constant ( $C' \times N'$ )
$\mathbf{A}''$	apparent conservation matrix when the concentrations of one or more species and one or more reactants are held constant ( $C'' \times N''$ )
$C$	number of components in a reaction system
$C'$	apparent number of components in a reaction system when the concentrations of one or more species are held constant
$C''$	apparent number of components when the concentrations of one or more species and one or more reactants are held constant
$D$	number of variables needed to describe the extensive state of a system
$D'$	apparent number of variables needed to describe the extensive state of a system when the concentrations of one or more species have been specified
$D''$	apparent number of variables needed to describe the extensive state of a system when the concentrations of one or more species and the concentrations of one or more reactants have been specified
$F$	number of variables needed to describe the intensive state of a system
$F'$	apparent number of variables needed to describe the intensive state of a system after the concentrations of one or more species have been specified
$F''$	apparent number of variables needed to describe the intensive state of a system when the concentrations of one or more species and the concentrations of one or more reactants have been specified
$G$	Gibbs energy of a system at specified $T$ , $P$ , and ionic strength
$G'$	transformed Gibbs energy of a system at specified $T$ , $P$ , ionic strength, and concentrations of one or more species
$G''$	further transformed Gibbs energy of a system at specified $T$ , $P$ , ionic strength, and concentrations of one or more species and one or more reactants
$\Delta_f G_i^\circ$	standard Gibbs energy of formation of a species at specified $T$ , $P$ , and ionic strength
$\Delta_f G_i'^\circ$	standard transformed Gibbs energy of formation of a reactant at specified $T$ , $P$ , ionic strength, and specified concentrations of one or more species
$\Delta_f G_i''^\circ$	standard further transformed Gibbs energy of formation of a pseudoisomer group of reactants at specified $T$ , $P$ , ionic strength, and specified concentrations of one or more species and one or more reactants
$H$	enthalpy of a system at specified $T$ , $P$ , and ionic strength
$H'$	transformed enthalpy of a system at specified $T$ , $P$ , ionic strength, and concentrations of one or more species
$H''$	further transformed enthalpy of a system at specified $T$ , $P$ , ionic strength, and concentrations of one or more species and one or more reactants
$K$	equilibrium constant written in terms of concentrations of species at specified $T$ , $P$ , and ionic strength
$K'$	apparent equilibrium constant written in terms of concentrations of reactants (sums of species) at specified $T$ , $P$ , ionic strength and concentrations of one or more species
$K''$	apparent equilibrium constant written in terms of concentrations of pseudoisomer groups (sums of reactants) at specified $T$ , $P$ , ionic strength, and concentrations of one or more species and one or more reactants
$\mathbf{m}$	transformation matrix
$n_i$	amount of species $i$
$n_i'$	amount of reactant $i$ (sum of species)
$n_i''$	amount of pseudoisomer group $i$ (sum of reactants)
$n_{ci}$	amount of component $i$
$n_{ci}'$	amount of apparent component $i$ at specified concentrations of one or more species
$n_{ci}''$	amount of apparent component $i$ at specified concentrations of one or more species and one or more reactants

$\mathbf{n}$	column vector of amounts of species ( $N \times 1$ )
$\mathbf{n}'$	column vector of amounts of reactants (sum of species) ( $N' \times 1$ )
$\mathbf{n}''$	column vector of amounts of pseudoisomer groups of reactants ( $N'' \times 1$ )
$\mathbf{n}_c$	column vector of amounts of components ( $C \times 1$ )
$\mathbf{n}_c'$	column vector of amounts of apparent components at specified concentrations of one or more species ( $C' \times 1$ )
$\mathbf{n}_c''$	column vector of amounts of apparent components at specified concentrations of one or more species and one or more reactants ( $C'' \times 1$ )
$N$	number of different species in a system
$N'$	number of different reactants (sums of species) in a system
$N''$	number of different pseudoisomer groups of reactants in a system
$N_{\text{iso}}$	number of isomers in an isomer group or pseudoisomers in a pseudoisomer group
$N_{\text{H}}(\text{i})$	number of hydrogen atoms in species i
$N_{\text{ATP}}(\text{i})$	number of ATP in a reactant (can be positive or negative)
$\Delta_r N_{\text{H}}$	change in binding of hydrogen ions in a biochemical reaction at specified $T, P$ , ionic strength and concentrations of one or more species
$p$	number of different phases in a system
$P$	pressure
pH	$-\log[\text{H}^+]$ at specified $T, P$ , and ionic strength
pMg	$-\log[\text{Mg}^{2+}]$ at specified $T, P$ , and ionic strength
$r_i$	mole fraction of isomer i within an isomer group or pseudoisomer within a pseudoisomer group
$R$	gas constant ( $8.31451 \text{ J K}^{-1} \text{ mol}^{-1}$ )
$R$	number of independent reactions in a system described in terms of species
$R'$	number of independent reactions in a system described in terms of reactants (sums of species)
$R''$	number of independent reactions in a system described in terms of pseudoisomer groups of reactants
$S$	entropy of a system at specified $T, P$ , and ionic strength
$S'$	transformed entropy of a system at specified $T, P$ , ionic strength, and concentrations of one or more species
$S''$	further transformed entropy of a system at specified $T, P$ , ionic strength, and concentrations of one or more species and one or more reactants
$\bar{S}(\text{i})$	molar entropy of species i
$\bar{S}'(\text{i})$	molar transformed entropy of reactant i
$T$	temperature in Kelvins
$\nu_{ij}$	stoichiometric number of species i in reaction j
$\nu_{ij}'$	stoichiometric number of reactant i in reaction j
$\nu_{ij}''$	stoichiometric number of pseudoisomer group i in reaction j
$\mathbf{v}$	stoichiometric number matrix in terms of species ( $N \times R$ )
$\mathbf{v}'$	stoichiometric number matrix in terms of reactants ( $N' \times R'$ )
$\mathbf{v}''$	stoichiometric number matrix in terms of pseudoisomer groups of reactants ( $N'' \times R''$ )
$\mu_i$	chemical potential of species i at specified $T, P$ , and ionic strength
$\mu_i'$	transformed chemical potential of reactant i at specified $T, P$ , ionic strength, and concentrations of one or more species

$\mu_i''$	further transformed chemical potential of pseudoisomer group i at specified $T, P$ , ionic strength, and concentrations of one or more species and one or more reactants
$\mu_i^\circ$	standard chemical potential of species i at specified $T, P$ , and ionic strength
$\boldsymbol{\mu}$	vector of chemical potentials of species at specified $T, P$ , and ionic strength ( $1 \times N$ )
$\boldsymbol{\mu}'$	vector of transformed chemical potentials of reactants at specified $T, P$ , ionic strength, and concentrations of one or more species ( $1 \times N'$ )
$\boldsymbol{\mu}''$	vector of further transformed chemical potentials of pseudoisomer groups of reactants at specified $T, P$ , ionic strength, and concentrations of one or more species and one or more reactants ( $1 \times N''$ )
$\mu_{\text{ci}}$	chemical potential of component i at specified $T, P$ , and ionic strength
$\mu_{\text{ci}}'$	transformed chemical potential of component i at specified $T, P$ , ionic strength, and concentrations of one or more species
$\mu_{\text{ci}}''$	further transformed chemical potential of component i at specified $T, P$ , ionic strength, and concentrations of one or more species and one or more reactants
$\mu_c$	vector of chemical potentials of components at specified $T, P$ , and ionic strength ( $1 \times C$ )
$\mu_c'$	vector of transformed chemical potentials of components at specified $T, P$ , ionic strength, and concentrations of one or more species ( $1 \times C'$ )
$\mu_c''$	vector of further transformed chemical potentials of components at specified $T, P$ , ionic strength, and concentrations of one or more species and one or more reactants ( $1 \times C''$ )
$\xi$	extent of reaction column vector at specified $T, P$ , and ionic strength ( $R \times 1$ )
$\xi'$	extent of reaction column vector at specified $T, P$ , ionic strength, and concentrations of one or more species ( $R' \times 1$ )
$\xi''$	extent of reaction column vector at specified $T, P$ , ionic strength, and concentrations of one or more species and one or more reactants ( $R'' \times 1$ )

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