Combining Transformed and Constrained Gibbs Energies for Modeling Biochemical Systems

Peter B. A. Blomberg, Pertti S. Koukkari, and Risto Pajarre, VTT, Biologinkuja 7, Espoo, Finland

The application of thermodynamics in biology and biochemistry has recently gained increasing interest due to the general trend where substitutes for petrochemicals and entirely new, sustainable means of production are being sought. Biochemical pathways involved in biological production steps can be modified by metabolic engineering. The methodical improvement of these systems requires detailed analysis of the underlying reaction networks. While tedious and often costly experimental work is required to evaluate the molecular mechanisms of reactions and their kinetic parameters, advanced thermodynamic methods provide an alternative approach to look for the most promising pathways [1].

Transformed Gibbs energies are Gibbs energies for a standard state close to the real conditions of the system at interest. Solute concentrations within cells cannot currently be determined accurately enough for normal thermodynamic modeling. The regular method of dealing with these kinds of situations in chemistry is to assess the concentrations of the most influential compounds of the system and then evaluate all thermodynamic functions at those conditions. This assumes that the most influential compounds dominate the state properties of the system. Examples are conditional phase diagrams in material science or combustion chemistry and stability diagrams in geochemistry or aquatic chemistry. Their purpose is to give a rough overview of the chemistry in a wide range of experimental conditions.

Transformed Gibbs energies for biological systems are primarily determined for specific pH and ionic strength but can be extended to include any number of components [2]. An explicit pH can be accounted for by removing the chemical potential contribution of the hydrogen entity from all constituents in the system. Any constituents with identical assembly of entities are then grouped into an isomer group, which is thereafter treated as a single constituent. This transformation is reversible and no information is lost in either process. The removal of an entity will reduce the size of the transformed system. In the particular case of pH, different ionic forms of acids and bases are grouped into metabolite species.

Ionic strength is usually taken into account before any transformations occur that could group together constituents with different charges. Long range electrostatic interactions are typically described by the extended Debye-Hückel expression in lack of better models. The transform is straightforward and constitutes a change in the order of calculation of the terms in the chemical potentials. The activity coefficient term is transferred from the concentration term to the standard state term. The evaluation of the transformed standard state term is possible beforehand because the ionic strength is not determined by the system. This approach is justified because the systems under investigation are often very small subsystems of the whole. Transforms based on charges must not occur after a transform that group together constituents of different charges, because the latter transform has lost the relative amounts of each charge unless an average charge is calculated during the first transform.

Computation of global chemical equilibria in multiphase systems by minimization of the Gibbs free energy is an established technique with many applications. The method is principally a constrained optimization task where the system Gibbs energy is the objective function and entity conservation determine the constraints. The entity constraints may include mass balance, charge balance, enthalpy

balance, and reaction constraints. Due to the construction of the mathematical problem, both the Lagrange multipliers and the duality multipliers are the same. The mathematical solution may be obtained with any suitable constrained optimization tool. All constituents present at an optimum will have a chemical potential equal to a linear combination of the multipliers. The factors are directly determined by the matrix that describes the factors in the entity conservation relations.

Since equilibrium is defined in a limited timeframe with certain internal constraints that vary with time but not within the timeframe of inspection, some of these constraints can be included as a dynamic portion of a model to extend their frame of validity. Such dynamic constraints typically involve work terms of slow processes or kinetically limited chemical reactions. The potential corresponding to the added work term is then represented by a supplementary undetermined Lagrange multiplier. In addition, the maximum extents of selected catalyzed chemical reactions can be limited to any desired value and the related energy terms describe the thermodynamic affinities of the reactions. The method of Constrained Gibbs Energies thus adds kinetic reaction extent limitations to the internal constraints of the system [3].

The combination of reaction rates and multi-component Gibbs energy minimization enables direct calculation of the thermodynamic state properties during an irreversible chemical change. The obvious advantage of such modeling is the simultaneous and interdependent calculation of the chemical composition and thermodynamic quantities. For example, the co-dependent chemical composition changes and energetic/entropic changes are inherently followed, which can provide improved balance analysis of biochemical networks. The method may also be utilized for thermodynamic consistency analysis of a reaction path.

Thermodynamics can be applied for pathway feasibility evaluation in many ways. If concentrations are not available nor calculable, standard states may be transformed to reflect more realistic reference concentrations and crude evaluations of thermodynamic feasibility may be attempted. The (transformed) Gibbs energy of the system and the Gibbs energy changes for reactions can be calculated from any given composition. This may provide a rudimental chemical energy landscape for determining system tendencies. The concentrations are, however, rarely independent. Entity constraints limit the infinite search space to a simplex. This may already bring some insight of a path in comparison with other paths. Gibbs energy minimization may be used to determine equilibrium concentrations for any given set of entity amounts. This helps to establish thermodynamic driving forces. It also gives the relative importance of different constraints and places values on each reactive molecular group. Constrained Gibbs energies may be applied to determine system evolution from any given starting point towards equilibrium along any preferred path by dynamically altering the internal constraints. This provides not only all of the above but also the thermodynamic affinities of each kinetically constrained reaction at any point on the path.

Figure 1 shows some example energy diagrams for the glycolysis pathway. Pathway energy diagrams are created by evaluating the transformed Gibbs energies at different nodes in the pathway. They can be used to choose pleasing pathways in metabolic engineering or to find control points in metabolic control analysis. The red triangles show the unrealistic result when transformed standard state values are used. The grey squares accounts for mixing, which is an important improvement. The black diamonds further discards the assumption of on-off kinetics. Figure 2 shows the system composition at each node corresponding to the black diamonds. These calculations show that the Gibbs energy change of a reaction is not the sole determining factor when pathway bottlenecks are sought. A larger positive

energy change may be less restrictive if the mixing term is favorable as for the GAP-node below. Knowing the composition makes it possible to predict what metabolites to look for in single knockout studies where a specific enzyme is missing since the last intermediate before the missing reaction might not be prevalent. It may also aid in derivation of rate constants and to elucidate the metabolic map of new organisms.

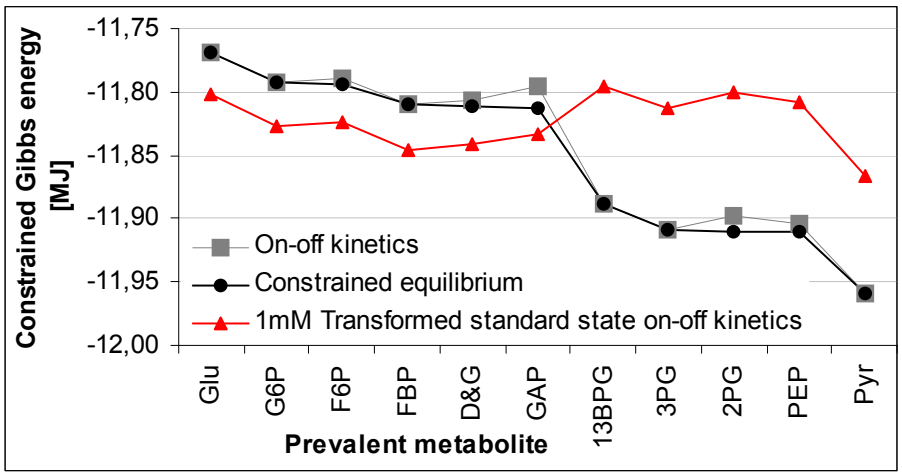

Figure 1.
The figure displays energy diagrams of the ten reactions of glycolysis. The red triangles show the result if transformed standard state values are used. This employs the biochemical convention of 1 mM standard state concentrations for solutes. On-off -kinetics assumes that enzymes are either perfect or inactive. The grey squares accounts for mixing, while the black diamonds replaces the assumption of on-off kinetics with constrained equilibrium.

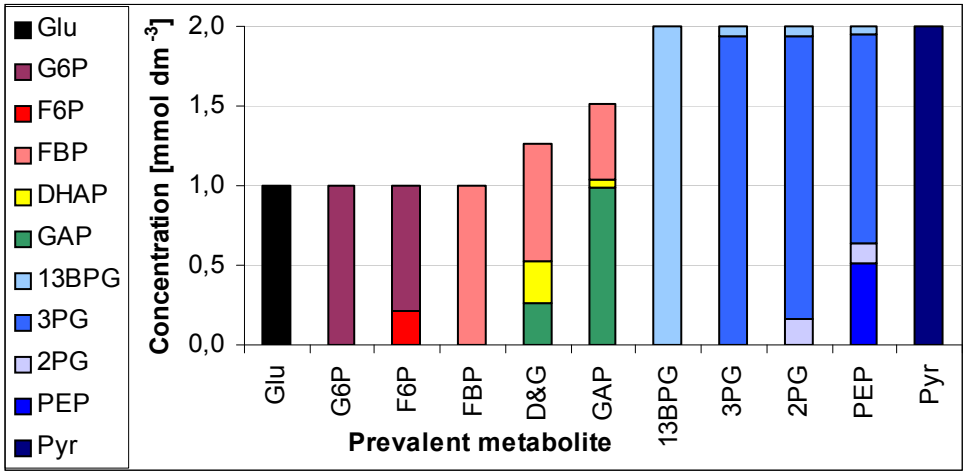

Figure 2.
The figure shows the composition of the system at each node in glycolysis at pH 6.5 and 0.2 M ionic strength. Nodes were calculated by assuming equilibrium of the first N reactions in the pathway. The black diamonds in figure 1 corresponds to these compositions.

Abbreviations

Glu	Glucose
G6P	Glucose-6-phosphate
F6P	Fructose-6-phosphate
FBP	Fructo-1,6-bisphosphate
D&G	Dihydroxyacetone phosphate and GAP
GAP	Glyceraldehyde-3-phosphate
13BPG	1,3-Bisphosphoglycerate
3PG	3-Phosphoglycerate
2PG	2-Phosphoglycerate
PEP	Phosphoenolpyruvate
Pyr	Pyruvate

References

[1] C. S. Henry, L. J. Broadbelt, and V. Hatzimanikatis, Thermodynamics-Based Metabolic Flux Analysis, Biophysical Journal 92 (2007) 1792–1805.

[2] R. A. Alberty, Thermodynamics of biochemical reactions, John Wiley & Sons, Inc., Hoboken, New Jersey, 2003.

[3] P. Koukkari, and R. Pajarre, Calculation of constrained equilibria by Gibbs energy minimization, CALPHAD 30 (2006) 18-26.