Network Thermodynamic Curation of Human and Yeast Genome-Scale Metabolic Models

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Supporting Material

1 Calculation of Gibbs energy (ΔG)

The standard equation to estimate Gibbs energy of formation ($\Delta_f G$) is given by:

$$\Delta_f G_i = \Delta_f G_i^0 + RT \ln(c_i) \quad (1)$$

The standard Gibbs energy of formation ($\Delta_f G^0$) represents the Gibbs energy at standard conditions (pH 7, zero ionic strength, aqueous solution, 1 bar pressure and 25°C). However, biochemical reactions occur in non-standard conditions. Many factors affect the $\Delta_f G$, namely metabolite concentrations, ionic strength, pH, temperature and pressure. There is evidence to show that Gibbs energies at physiological pH and ionic strength significantly differ from $\Delta_f G^0$ (1, 2), therefore further adjustments are necessary.

1.1 Adjustment of the Gibbs energy of formation for physiological conditions

A more rigorous treatment when calculating the standard Gibbs energy of formation is to use activity (a_i) instead of the concentration (c_i) of species. The activity coefficient (y_i) correlates activity and concentration by the equation $a_i = y_i * c_i$. To account for ionic strength, the extended equation of Debye-Hückel is utilized to calculate the activity coefficient:

$$Log(y_i) = \frac{Az_i I^{1/2}}{1 + BI^{1/2}} \quad (2)$$

where z_i is the charge on ion i, I is the ionic strength, A=0.510651 $L^{1/2}mol^{1/2}$ and B=1.6 $L^{1/2}mol^{-1/2}$ at standard pressure and temperature. The equation is valid within the ionic strength range of 0.005 to 0.25 M. The activity coefficient is considered in the general equation of $\Delta_f G$, Eq.1:

$$\Delta_f G_i = \Delta_f G_i^{0*} + RT \ln(c_i) \quad (3)$$

Where a new standard Gibbs energy of formation ($\Delta_f G^{0*}$) is used, the notation * is used here to remind the reader that this is a function of ionic strength, expressed by:

$$\Delta_f G_i^{0*} = \Delta_f G_i^0 - \frac{2.91482 z_i^2 I^{1/2}}{1 + 1.6 I^{1/2}}$$

For biochemical reactions, pH is assumed to be constant due to the buffering capacity of proteins, so it is not necessary to balance hydrogen ions (3). For pH adjustment at constant pH, the standard transformed Gibbs energy of a specie i is defined as:

$$\Delta_f G_i^{'0} = \Delta_f G_i^{0*} - N_H \Delta G(H^+) \quad (4)$$

$$\Delta_f G_i^{'0} = \Delta_f G_i^{0*} - N_H \{\Delta_f G^{0*}(H^+) + RT \ln(10^{-pH})\} \quad (5)$$

where N_H is the number of hydrogen atoms in the species.

Finally, the combination of Eq.3 and Eq.5 produces an overall equation to estimate the standard transformed Gibbs energy of formation ($\Delta_f G_i'^0$) that accounts for physiological conditions (pH and ionic strength):

$$\Delta_f G_i'^0 = \Delta_f G_i^0 - N_H(i)RT\ln(10^{-pH}) - \frac{2.91482(z_i^2 - N_H(i))I^{1/2}}{1+1.6I^{1/2}} \quad (6)$$

1.2 Metabolites as group of species

From the point of view of thermodynamics, the main difference between biochemical and chemical reactions is that enzyme-catalyzed reactions are written in terms of reactants (e.g. ATP) that are made up of a sum of species (e.g. ATP^{4-},$HATP^{3-}$ and $MgATP^{2-}$) and chemical reactions are written in terms of species (3). Therefore, it is necessary to account for all involved species. To reduce the complexity of the calculations it is easier to work with reactants instead of individual species. The Gibbs energy of a reactant j is calculated by pseudo-isomers groups, whereby at chemical equilibrium all species (pseudo-isomers) have the same Gibbs free energy of formation, which is represented by $\Delta_f G_j$; and the concentration of the reactant (isomer group) c_j is the sum of the concentration of species:

$$c_j = \sum_{i=1}^{N} c_i \quad (7)$$

where N is the number of species i in the pseudo-isomer group. The Gibbs free energy of formation of the pseudo-isomer group is represented by:

$$\Delta_f G_j = \Delta_f G_j'^0 + RT\ln(c_j) \quad (8)$$

And the $\Delta_f G_i$ of the individual species at chemical equilibrium is given by:

$$\Delta_f G_i = \Delta_f G_i'^0 + RT\ln(c_i) \quad (9)$$

Finally using Eq.8 and Eq.9 in Eq. 7 and assuming that $\Delta_f G_j = \Delta_f G_i$ the equation to calculate $\Delta_f G_j'^0$ can be deduced (3):

$$\Delta_f G_j'^0 = -RT\ln\{\sum_{i=0}^{N}\exp\left[-\frac{\Delta_f G_i'^0}{RT}\right] \quad (10)$$

The notation (') is used to indicate that physiological conditions are assumed rather than the standard biological conditions (3). It is important to note that the standard transformed Gibbs energy of formation of a reactant is not the sum of the standard transformed Gibbs energy of its species.

1.3 Inter-compartment reactions

A reaction that occurs between two compartments is considered a transport reaction; an example is the reaction of ATP synthase in the oxidative phosphorylation pathway, where protons are transported across the mitochondrial membrane (for eukaryotes) or the plasma membrane (for prokaryotes). To calculate the Gibbs energy of a transport reaction, the reaction is first divided between the part that takes place in one compartment and the

transmembrane transport portion in order to separately calculate their $\Delta_r G'^0$, finally the $\Delta_r G'^0$'s are added up (4)

$$\Delta_r G'^0 = \Delta_r G'^0_{comp} + \Delta_r G'^0_{transport} \quad (11)$$

The calculation of $\Delta_r G'^0$ of the transport term should take into account both the pH gradient between compartments, and the membrane potential, if the molecule is charged. This calculation is expressed as:

$$\Delta_r G'^0_{transport} = \Delta_{\Delta\Psi} G + \Delta_{\Delta pH} G \quad (12)$$

Under physiological conditions (pH $\neq$ 7 and ΔpH$\neq$0, ionic strength $\neq$0 and $\Delta\Psi\neq$0 (the difference in membrane potential between compartments)), $\Delta_r G'^0_{\ transport}$ is not zero and must be considered in the estimation of $\Delta_r G'^0$. It is dependent on $\Delta\Psi$ and ΔpH (5) :

$$\Delta_{\Delta\Psi}[KJ/mol] = nF\Delta\Psi \quad (13)$$

$$\Delta_{\Delta pH} G[KJ/mol] = \sum_{i=1}^{N} s_i \Delta_f G'^0_j - 2.3RT \sum_{i=1}^{N} s_i N_H(i) pH_i \quad (14)$$

where n is the net charge transported through the membrane, F is the Faraday constant, s_i is the stoichiometric coefficient of the transported specie i and $\Delta_f G'^0_j$ is the transformed Gibbs energy of formation of transported metabolite j.

1.4 Combining $\Delta_f G^0$ from literature and estimated from group contribution method

In order to increase the coverage of reactions with calculated Gibbs energy of reaction, $\Delta_f G^0$ from literature was complemented with $\Delta_f G^0$ estimated by the group contribution method recognizing the need for a common reference state (6). The group contribution method used here use the same general reference state as used for the experimentally estimated $\Delta_f G^0$ from Alberty (7). Alberty, however, specifies a zero value reference $\Delta_f G^0$ for one of members in cognate co-factor and redox carrier pairs and use this as reference for the other (e.g. NAD with $\Delta_f G^0$ of zero and NADH with $\Delta_f G^0$ of 22.65 KJ/mol, relative to $\Delta_f G^0$ of NAD). These artificial reference points yield valid reaction Gibbs energies as long as the cognates are found on either side of the reaction, hereby cancelling out the reference. In the de novo synthesis of cofactors/redox carriers, however, this is not the case.

In the current study, the artificial zero references were replaced with group contribution estimates of $\Delta_f G^0$ wherever possible (e.g. $\Delta_f G^0$ of NAD was changed to -2214.72 KJ/mol and $\Delta_f G^0$ of NADH to -2192.07 KJ/mol). A group contribution estimate could not be produced for two redox pairs, ferricytochrome C/ferrocytochrome c and thioredoxin oxidized/reduced. In the two models studied, however, these metabolites are always present as pairs hereby cancelling out the effect of using a different reference point.

Table S1 Cell compartment volume fractions

	Percentage of total cell volume	
	Human	**Yeast**
Cytosol	0.63 [a]	0.53 [a]
Nucleus	0.10 [b]	0.07 [c]
Mitochondria	0.09 [d]	0.09 [e]
Mitochondrial intermembrane space	0.01 [e]	0.01
Golgi apparatus	0.06 [b]	0.04 [e]
Lysosome	0.01 [b]	0
Peroxisome	0.01[b]	0.01 [e]
Endoplasmic reticulum	0.09 [b]	0.05 [e]
Endoplasmic reticulum membrane	0	0.01 [e]
Cell envelope	0	0.15 [f]
Vacuole	0	0.02 [e]
Vacuolar membrane	0	0.01 [e]
Lipid particle	0	0.01 [e]

References: a. the reaming volume was considered cytosol, b. (8), c. (9), d. (10), e. assumed values, f. (11)

2 Error analysis of new irreversibility constraints

NExT currently does not consider uncertainties in the $\Delta_f G^0$ estimates and their propagation into the final $\Delta_r G$ estimates. Previous thermodynamics studies have observed that these errors can affect conclusions (12, 13). Accordingly, each irreversibility identified by *NExT* with an extreme $\Delta_r G$ within ± 5 [Kcal/mol] of zero was manually analysed. Using the standard error, SE, from (14), we found that eight out of the 41 reactions identified as irreversible had a confidence interval (lower limit ± 2 SE) including zero (Table S11). The ΔrG SE of the two reactions involved in vitamin A metabolism (RADH2 and RADH4) was not reported in (14). However, the reactions have been previously stated irreversible when a narrower physiological nadp/nadph ratio range was specified (15).

In seven of the eight reactions, changing the lower concentration limit from 10^{-4} to 10^{-3} mM (keeping upper limit at 10 mM) was sufficient to restore irreversibility. The last reaction, dCMP aminohydrolase in both yeast and human models (DCMPDA and r_0326), requires a minimum concentration of 0.0058 mM to claim irreversibility assuming that the estimate is off by 2 SE. We should point out that many thermodynamics papers (12, 16) use 10^{-2} mM as their minimum and all the reactions identified by *NExT* would be irreversible under those conditions.

Table S11 Error analysis of the new identified irreversible reactions with an extreme DrG within zero and ± 5 [Kcal/mol]

ID	Reaction	ΔrG min [KJ/mol]	ΔrGmax [KJ/mol]	Pathway	Standard Error [KJ/mol] [a]	Minimun Conc [mM]	New Extreme ΔrG [KJ/mol]	Extreme Minimun Conc [mM]	New Extreme ΔrG [KJ/mol]	**Observations**
AIRCr**	[c]**air**+co2tot = **5aizc**+h+h2o	3.17	71.67	IMP Biosynthesis	3.07	$1x10^{-3}$	8.88	0.00033	6.14	
DPGM	[c]13dpg = **23dpg**+h	-70.55	-13.47	Glycolysis/Gluconeogenesis	1.4					
DCMPDA	[c]**dcmp**+h+h2o = **dump**+nh4	-87.76	-2.14	Pyrimidine Catabolism	6.11	$1x10^{-3}$	-7.85	0.00583	-12.22	
L_LACtm	h[c]+lac_L[c] = h[m]+lac_L[m]	-31.85	-7.66	Transport, Mitochondrial	0*					
GTHRDt	atp[c]+**gthrd**[c]+h2o[c] = adp[c]+**gthrd**[m]+h[c]+pi[c]	-108	-8.72	Glutathione Metabolism	3.0**					
RADH2	[c]h2o+nadp+**retinal** = (2)h+nadph+**retn**	-86.86	-15.97	Vitamin A Metabolism	Not found					Previously reported [b]
RADH4	[c]h2o+nadp+**retinal_cis_13**=13**_cis_ret**n+(2)h+nadph	-86.86	-15.97	Vitamin A Metabolism	Not found					Previously reported [b]
34DHPLACOX_NADP	[c]**34dhpac**+h2o+nadp = **34dhpha**+(2)h+nadph	-83.54	-12.65	Tyrosine metabolism	4.9					
34DHXMANDACOX_NADP	[c]**34dhmald**+h2o+nadp = **34dhoxm**and+(2)h+nadph	-83.54	-12.65	Tyrosine metabolism	4.9					
3MOX4HOXPGALDOX_NADP	[c]**3m4hpga**+h2o+nadp = **3mox4hoxm**+(2)h+nadph	-83.54	-12.65	Tyrosine metabolism	4.9					
4HOXPACDOX_NADP	[c]**4hoxpacd**+h2o+nadp = **4hphac**+(2)h+nadph	-83.54	-12.65	Tyrosine metabolism	4.9					
FACOAL161	[c]atp+coa+**hdcea**=amp+**hdcoa**+ppi	-119.89	-0.65	Fatty acid metabolism	5.16	$1x10^{-3}$	-17.78	0.00040	-10.96	Due to AEC constraint
r_0356	[c]13dpg = **23dpg**+h	-71.96	-14.88	Glycolysis/Gluconeogenesis	1.4					
r_0911	[c]**air**+co2tot = **5aizc**+h+h2o	3.34	71.84	IMP Biosynthesis	3.07	$1x10^{-3}$	8.88	0.00033	6.14	
r_0073	[c]**4ppmip**+h2o=(2)h+**minohp**+pi	-69.16	-0.67	Inositol Phosphate Metabolism	3.00	$1x10^{-3}$	-6.38	0.00086	-6.01	
r_0092	[c]**6ppmip**+h2o=(2)h+**minohp**+pi	-69.16	-0.67	Inositol Phosphate Metabolism	3.00	$1x10^{-3}$	-6.38	0.00086	-6.01	
r_0767	[n]h2o +**NAD** =**ADP-rib** +h +**ncam**	-89.44	-3.82	NAD Metabolism	4.28	$1x10^{-3}$	-9.52	0.00068	-8.56	
r_0204	[c]**ap4a**+h2o=(2)adp+(2)h	-102.97	-17.35	Nucleotides	3.1					
r_0326	[c]**dcmp**+h+h2o = **dump**+nh4	-90.02	-4.4	Pyrimidine Catabolism	6.11	$1x10^{-3}$	-10.11	0.00234	-12.22	

References: a.(14), b. (15)

* Transport reaction, therefore, it does not create or destroy groups.

** SE of ATP hydrolysis.

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