

Supplemental Material

CBE—Life Sciences Education

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Thermodynamics Boxes Simulation Exercises Index of Assignments

Based on paper: Mayorga LS, López MJ, Becker WM. Molecular thermodynamics for cell biology as taught with boxes. *CBE Life Sci Educ.* 2012;11(1):31-8.

Use an updated and improved simulation (the one associated with the paper doesn't work with modern versions of Excel). Windows and Mac versions of the simulation download are available at:

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Notes: I have modified these exercises for use in general chemistry classes, physical chemistry classes, and biochemistry classes. In all cases, I assigned the **bioenergetics** hand-out from the CBE-Life Science Education supplementary material and pre-requisite reading.

Introduction to Thermodynamics:

1st Law of Thermodynamics. Conservation of Energy: The energy of the universe or a closed system is constant but can change forms. Energy (usually shown as U or sometimes E) is often converted to heat (usually shown as q). Enthalpy $\Delta H = q$ under constant pressure conditions. In biochemistry ΔH is given in units of kJ/mol, J/mol, cal/mol, or kcal/mol.

2nd Law of Thermodynamics. The entropy (called S) of the universe is increasing. This concept is often misunderstood as an increase in “disorder” which is inconsistent with many observations of the complexity of evolving life. Entropy is more accurately described as statistical probabilities being following – molecules tend to arrange into the most probably configurations. A drop of dye in a cup of water spreads out, it doesn’t become smaller over time causing diffusion.

Enthalpy: In biochemistry enthalpy, ΔH , is given in units of kJ/mol, J/mol, cal/mol, or kcal/mol. It is related to heat. A reaction with a positive enthalpy is endothermic (absorbs heat from environment), and a reaction with a negative enthalpy is exothermic (releases heat to environment). ΔH is often shown on the y-axis of reaction coordinate energy diagrams as in the boxes simulation example.

Entropy: Entropy, S, an absolute number directly related to the number of possible configurations (ways, W) and the Boltzmann constant ($k_b = 1.38 \cdot 10^{-23}$ J/K): $S = k_b \ln W$. Practically, however, usually the change in entropy, ΔS , is reported in units of J/(K*mol), or cal/(K*mol).

Gibbs Free Energy: Gibbs free energy is commonly used and reported in biochemistry. It relates enthalpy and entropy together to show what actually will happen to molecules in a reaction. It is shown as ΔG° under chemists’ standard state of 25°C, 1atm, and 1 unit of solute activity (usually close to 1M). Biochemists sometimes report under biochemists’ standard state, which is often buffered at pH = 7 with concentrations given in mol/L and water concentration not included in the calculation because solutions are aqueous so water concentrations (and activities) are assumed to be constant. Often, the biochemists standard state is reported as ΔG°

Important Thermodynamic Equations for biochemistry:

Note on units and constants: Calories are non SI unit often used in biology. Confusingly, 1 Calorie on food labels is actually 1kcal in science. $1 \text{ J} = 1 \text{ kg m}^2/\text{s}^2 \text{ K}$ in thermodynamics must always be in absolute temperature Kelvin (K) so that at 0K there is no movement of molecules, and the entropy term is 0. R is the gas constant = 8.31 J/ K*mol

$$\Delta G = \Delta H - T\Delta S$$

Note that

$$\Delta G = \Delta G^\circ + RT \ln K_{eq}$$

$K_{eq} = [\text{prod}] / [\text{reactant}]$ equilibrium concentrations.

The equivalent term Q is used under non-equilibrium conditions.

$$\Delta G^\circ = -RT \ln K_{eq}$$

$\Delta G < 0$ Spontaneous (equilibrium for reaction is forward)

ΔG , ΔH , ΔS are Path-Independent, and can be added and subtracted between reactions.

For instance: $A \rightarrow B$ ΔG_1 ; $B \rightarrow C$ ΔG_2 , $A \rightarrow C$ $\Delta G_1 + \Delta G_2$ (B cancels out when you add the reactions).

Freezing Reactions

Consider the freezing of water to ice. $\text{H}_2\text{O} (\text{l}) \rightarrow \text{H}_2\text{O} (\text{s})$

- 1) Do you expect this to be an endothermic (takes energy) or exothermic (releases heat) process? What sign do you expect the ΔH to have?
- 2) Do you expect that this process has a positive entropy or negative entropy value (ΔS)?
- 3) For water freezing $\Delta H = -6.01 \text{ kJ/mol}$. $\Delta S = -22 \text{ J/ K*mol}$.
- 4) What temperature, in K, do you expect water to freeze at?
- 5) What is the change in Gibbs free energy at the freezing temperature? Does this make sense?
- 6) What is the sign of the Gibbs free energy at temperatures lower than freezing? Does this makes sense because we expect **spontaneous** freezing at these temperatures?

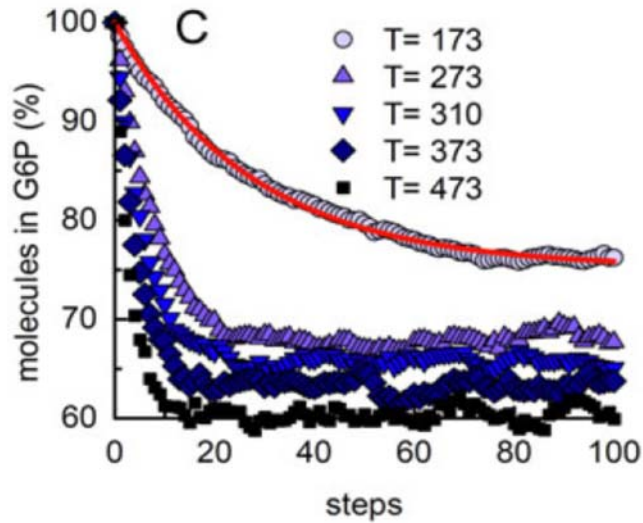
Open the boxes simulation on the computer. Use the $\Delta H = -6 \text{ kJ/mol}$ and $\Delta S = -0.022 \text{ kJ/mol}$ for the freezing of water. Set the temperature to freezing (remember to convert to K). Keep the activation energy at 0. Start with 100% of the water as liquid (% in box 1 = 100). Use the fast-forward button in the middle to speed up the reaction.

- a) Which side of the box is the liquid water, and which is the ice? Does depth (heat or ΔH) of the ice versus liquid box make sense?
- b) Based on the calculations of the program, what is relative width of the “ice” and the “water” boxes? Does this make sense based on what you expect for the relative entropy or number of configurations possible for water vs. ice?
- c) At what point does the reaction reach equilibrium? What are the equilibrium % of liquid and solid water at 0°C , or freezing temperature in a closed system?
- d) Another important thermodynamics equation in biochemistry is: $\Delta G^\circ = -RT \ln K_{\text{eq}}$
What aspects of the “boxes” analogy show the standard Gibbs free energy, and what aspects show the K_{eq} .
- e) Re-do the simulation with 0% in I, meaning 100% of the water starts as ice. What do you observe as you run the simulation?
- 7) Now, re-do the simulation using the same ΔH and ΔS starting with 100% water, but at a colder temperature. Then, play around with the temperature.
- How does the temperature change the equilibrium? Does your result make sense based on Le Chatlier’s principle?
 - How does the ΔG change with different temperatures? At what temperatures is water freezing spontaneous? How does the boxes simulation help you understand the spontaneity of the freezing reaction?

- 8) **Optional Extension Exercise:** Isopropanol freezes/melts at -89°C . At that temperature, the heat of fusion (melting) is 5.4 kJ/mol , and the entropy of fusion is $29.21\text{ J/K}\cdot\text{mol}$. Simulate the freezing of isopropanol at its freezing temperature using the boxes.
- What is the ΔG for the melting of isopropanol at 185.2K ?
 - Does all of the isopropanol freeze or melt at this temperature? Explain based on thermodynamics.
 - If you make the temperature higher, melting will be spontaneous. If you make the temperature lower, freezing will be spontaneous. Simulate these two situations.
 - If you switch to room temperature 25°C , does 100% of the isopropanol melt by the time it reaches equilibrium? If not, what is different about the situation where frozen isopropanol is placed in a warm room?
 - If you have 100% liquid isopropanol at room temperature in a closed adiabatic system, is some of that isopropanol expected to freeze? Explain.

Glucose-6-Phosphate Isomerization

- 9) Glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) are isomers of each other: they have the same atoms arranged in different ways. An isomerase enzyme catalyzes the conversion of G6P to F6P. $\Delta H^\circ = +2\text{kJ/mol}$ and $\Delta S^\circ = 0 \text{ kJ/ mol}\cdot\text{K}$.
- a) Draw the structures of this reaction: $\text{G6P} \rightarrow \text{F6P}$.
- b) Set up a simulation using the following information for the reaction above: Δ Run at $T=310\text{K}$ (body temperature) and $T = 273\text{K}$ (freezing temperature). After each run reaches equilibrium, click on “view results” to pull up results in Excel.
- c) Calculate the ΔG° at $T = 273\text{K}$ and $T = 310\text{K}$ and 500K .
- d) Calculate the K_{eq} values at 273K and 310K and 500K . Which temperature leads to the most product at equilibrium?
- 10) Set up a simulation of the G6P isomerization reaction. Run the simulation at 500K , $T=310\text{K}$ (body temperature) and $T = 273\text{K}$ (freezing temperature). After each run reaches equilibrium, click on “view results” to pull up results in Excel.
- a) Plot the results from each temperature on one graph as shown in the example from the paper Mayorga, L. S., López, M. J., & Becker, W. M. (2012). Molecular thermodynamics for cell biology as taught with boxes. *CBE—Life Sciences Education*, 11(1), 31-38.



b) Looking just at the final concentration of starting material and product, do the results match your calculated K_{eq} values at each temperature?

11) Add an activation energy of 4kJ/mol to your simulation at 310K.

a) Does the activation energy change the final equilibrium concentrations? (Set to fast-forward to help get to equilibrium).

b) Does the activation energy change the TIME it takes to get to equilibrium?

c) In a test tube, even at body temperature, it would take a very long time for a significant amount of G6P to isomerize to F6P. What does the enzyme phosphoglucose isomerase do to accelerate the isomerization reaction in the body? This enzyme is the second enzyme important in the metabolic process of glycolysis which breaks down glucose to produce energy.

- d) 5mL of 0.5M G6P and 0.5M F6P were mixed in test tube #1 and left for 100 years in tropical climate (310K) next to tube #2 with 5mL 1M F6P, and tube #3 with 5mL of 1M G6P. What do you expect the concentrations of G6P and F6P will be in each tube, assuming they have approached equilibrium by this time?

12) In the body, non-equilibrium conditions exist.

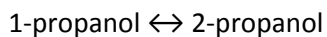
a) The reaction $G6P \rightarrow F6P$ occurs in the body during glycolysis, even though it is thermodynamically unfavorable with a positive ΔG° . Construct an explanation for how a thermodynamically unspontaneous reaction could still go forward in biology. Hints: Think about Le Chatelier's principle and how you could use it by coupling unfavorable with thermodynamically favorable reactions? The mechanism of phosphoglucose isomerase is given in your textbook chapter 15, section 2 (fig 15-3). In fact, depending on whether the body needs to make (glycolysis) or break down (gluconeogenesis) glucose, the isomerase enzyme can catalyze the reaction going in EITHER direction.

- c) In Red Blood Cells the concentration of G6P was measured to be $83\mu\text{M}$ and the concentration of F6P was $14\mu\text{M}$. Are these the concentrations of the starting material and product at equilibrium (at 310K)? Source: <http://www.chembio.uoguelph.ca/educmat/chm452/lecture2.htm>

- d) The starting material and product concentrations measured in cells are STEADY STATE concentrations constant because of many biological processes constantly running to keep them at a certain level; they are NOT equilibrium concentrations that would exist due to chemical equilibrium being reached in the system. Is **the G6P isomerization reaction expected to be spontaneous** (negative ΔG) in the body ($T = 310\text{K}$) if the concentration of G6P was $83\mu\text{M}$ and the concentration of F6P was $14\mu\text{M}$? Use the non-equilibrium ΔG equation (with $Q = [F6P]/[G6P]$).

Isomerization of Propanol

13) Consider the following isomerization reaction:



$$\Delta H^\circ = -3630 \text{ cal mol}^{-1}$$

$$\Delta S^\circ = -3.85 \text{ cal mol}^{-1} \text{ K}^{-1}$$

- a) Draw the structures of the starting material and product molecules.
- b) Simulate the isomerization reaction at a) 25°C, b) the boiling temperature of isopropanol (82.3°C), and c) 700°C. Start your simulation with 100% 1-propanol. What do you observe about the % of product formed after equilibrium is achieved at each of the 3 temperatures? Write down the equilibrium % of 1 and 2-propanol below.
- 25°C
- 82.3°C
- 700°C
- c) Calculate the $\Delta G = \Delta H - T\Delta S$ for each temperature you tested above. You can use units of cal/mol.
- d) Use these ΔG values to calculate the expected K_{eq} for the reaction. You will need to convert ΔG into J/mol or R the gas constant into cal/mol*K. Do your calculated numbers match what you observe at equilibrium using the boxes simulation model?

Biodiesel and Catalysts

15) Triglycerides can be converted into biodiesel (methyl esters) using the following reactions: For this process:

$$\Delta H^\circ = 544 \text{ cal mol}^{-1}$$

$$\Delta S^\circ = -5.2 \text{ cal mol}^{-1} \text{ K}^{-1}$$

Reference: Pogaku, R., Raman, J. K., & Ravikumar, G. (2012). Evaluation of activation energy and thermodynamic properties of enzyme-catalysed transesterification reactions. *Adv Chem Eng Sci*, 2, 150-154.

- a) Is this reaction **spontaneous** (negative ΔG) at room temperature?

- b) The reaction is multi-step with activation energies associated with each step. For the final step at $T = 563^\circ\text{C}$ the activation energy was determined to be 10 200 cal/mol. Do you expect triglycerides to react much with methanol if they are left on a lab bench for a week?

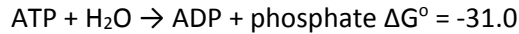
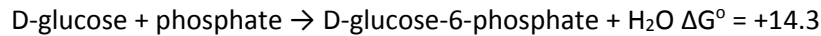
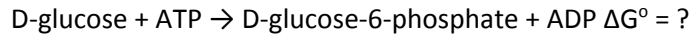
- c) Catalysts often work by breaking reactions into many steps with lower activation energies than the uncatalyzed reaction. In one study cited, a catalyst was able to lower one of the activation energies to 2670 cal/mol at 230°C . Simulate this activation energy into the boxes simulation with the other parameters set for biodiesel. Reference: Sanjel, N., Gu, J. H., & Oh, S. C. (2014). Transesterification Kinetics of Waste Vegetable Oil in Supercritical Alcohols. *Energies*, 7(4), 2095-2106.
 - a. Does the catalyst decrease the activation energy enough for you to be able to watch the reaction proceed toward equilibrium?

 - b. Run the reaction on fast-forward using the information in "c" till equilibrium appears to be achieved. Next, run with 0 activation energy. After each run, click on "view results" to make the results pop up in Excel. For each situation, make a graph of Q and K_{eq} (called Q before equilibrium is reached) over time.

 - c. How does the activation energy effect the equilibrium concentrations of reactant and product? How does the activation energy effect **the time** to reach equilibrium? Note that time is in arbitrary units in the simulation, not real reaction times.

Concept and Review Questions:

17) You want to calculate the ΔG° for the following reaction, but only know the ΔG° for the reactions given below. Because ΔG° are path-independent, you can add or subtract the reactions to get the ΔG° of the new reaction. This example shows how ATP energy is often harnessed to drive thermodynamically unfavorable reactions.



18) The enzyme aldolase catalyzes the conversion of fructose 1,6-diphosphate (FDP) to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). The standard free energy change for the reaction is **positive** 23.8kJ/mol.



The effective free energy change is based on non-equilibrium conditions using this equation:

$$\Delta G = \Delta G^\circ + RT \ln Q \quad \text{where } Q = \frac{[\text{DHAP}][\text{GAP}]}{[\text{FDP}]}$$

Remember you must convert the concentrations into M = mol/L.

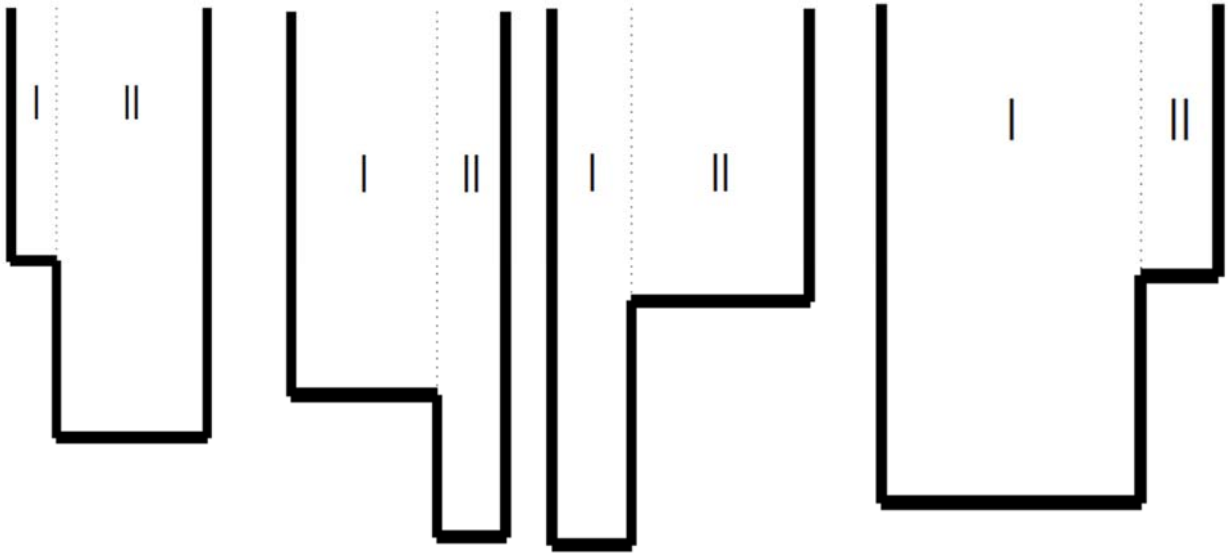
a) Draw out the structures for this reaction:

b) Under physiological conditions the concentrations of these species in red blood cells (erythrocytes) are $[\text{FDP}] = 35 \mu\text{M}$, $[\text{DHAP}] = 130 \mu\text{M}$ and $[\text{GAP}] = 15 \mu\text{M}$. Will the conversion occur spontaneously under these conditions?

19) Give an example of a real reaction where ΔH is negative and ΔS is positive.

a) Write out the reaction and **label reactant and product** for this real reaction on the correct shaped box below. Reference: "Bioenergetics" handout from: Mayorga LS, López MJ, Becker WM. Molecular thermodynamics for cell biology as taught with boxes. *CBE Life Sci Educ.* 2012;11(1):31-8.

b) Is this reaction spontaneous (negative ΔG) at all temperatures? Explain.



20) In the document I gave you on “bioenergetics” the authors discuss why the life does not spontaneously arise from a pool of nucleotides because it is statistically almost impossible to form a functional string of DNA (only a small subset of ALL possible combinations) in a test tube. However, millions of cells divide accurately and efficiently in a seemingly statistically impossible way every day. Explain in your own words.

Common Reactions that work with the Thermodynamics Boxes Simulation

Reaction	ΔH , kJ/mol	ΔS , kJ/mol	Temp K	Ref.
Freezing				
$\text{H}_2\text{O (l)} \rightarrow \text{H}_2\text{O (s)}$	-6.01	-.022	273	1,2
Ethanol (l) \rightarrow ethanol (s)	-4.973	-0.0313	159	3
Al (l) \rightarrow Al(s)	-10.8	-0.0115	932	4
Zn (l) \rightarrow Zn (s)	-6.69	-0.0133	693	4
Sn (l) \rightarrow Sn (s)	-20.0	-0.0184	505	4
Na (l) \rightarrow Na (s)	-2.64	-0.0071	371	4
Reactions				
1-propanol \rightarrow 2-propanol	-15.19	-0.0161	(298)	5
Glucose-6-phosphate \rightarrow Fructose-6-phosphate	1.67	~ 0	(298)	5
2,4 pentadione keto \rightarrow enol tautomerization (gas phase)	-8.8	-0.0071	(298)	6
C (graphite) \rightarrow C (diamond)	1.88	-0.0034	(298)	7

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