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Student Conceptions about Energy Transformations: Progression from General Chemistry to Biochemistry

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Abstract:

Students commencing studies in biochemistry must transfer and build on concepts they learned in chemistry and biology classes. It is well established, however, that students have difficulties in transferring critical concepts from general chemistry courses; one key concept is "energy." Most previous work on students' conception of energy has focused on their understanding of energy in the context of physics (including the idea of "work") and/or their understanding of energy in classical physical and inorganic chemistry contexts (particularly Gibbs Free Energy changes, the second law of thermodynamics, and equilibrium under standard conditions within a closed system). For biochemistry, students must go beyond those basic thermodynamics concepts of work, standard energy changes, and closed systems, and instead they must consider what energy flow, use, and transformation mean in living, open, and dynamic systems.

In this study we explored students' concepts about free energy and flow in biological chemical reactions and metabolic pathways by surveys and in-depth interviews. We worked with students in general chemistry classes and biochemistry courses in both an Australian and a US tertiary institution. We address three primary questions (i) What are

the most common alternative conceptions held by students when they explain energy-related phenomena in biochemistry?, (ii) What information do students transfer from introductory chemistry and biology when they are asked to consider energy in a biological reaction or reaction pathway?, and (iii) How do students at varying levels of competence articulate their understandings of energy in pathways and biological reactions? The answers to these questions are used to build a preliminary learning progression for understanding "energy" in biochemistry. We also propose crucial elements of content knowledge that instructors could apply to help students better grasp this threshold concept in biochemistry.

Introduction:

The topic of energy transfer and transformations is central to the sciences (Singer, 2013; National Research Council, 2012), however, it is one that presents serious difficulty in engineering, physics, chemistry, and biology classes. Previous studies in physics (reviewed in Ding *et al.*, 2013) and engineering (Prince *et al.*, 2012) have documented alternative conceptions around: conservation of energy; thermal radiation and the role of temperature; separating systems from surroundings; and differentiation of energy and work. In both chemistry and biology, it has been shown that students have conceptual difficulties around Gibbs Free Energy, the dynamic nature of equilibrium, and the relationship between equilibrium and free energy (reviewed in Piquette & Heikkinen, 2005; also see Johnstone *et al.*, 1977; Nakhleh, 1992; Banerjee, 1995; Quilez-Pardo & Solaz-Portoles, 1995; Teichert & Stacy, 2002; Shi *et al.*, 2010; Robic, 2010;Villafane *et al.*, 2011; Cooper & Klymkowsky, 2013).

Students are not “blank slates” when they begin to study a subject; rather, they have an established worldview that is firmly held and hard to shift (Thomas & Schwenz, 1998; National Research Council, 2000). Especially in the sciences, it has been repeatedly shown that pre-existing beliefs about the physical world dominate how students approach introductory courses, and that instruction needs to acknowledge and respond to pre-existing ideas by challenging alternate conceptions (Schmidt, 1997; Wieman, 2007; Childs & Sheehan, 2009; Prince *et al.*, 2012).

Biochemistry is a science that draws on concepts from both chemistry and biology. Hence, in the context of biochemistry, students who are struggling with fundamental energy concepts from chemistry will be ill-equipped to analyse more sophisticated processes such as protein folding and stability, metabolic flux, and ligand binding (Sears *et al.,* 2007; Robic, 2010). This assertion is backed by the findings of Ebenezer and Fraser (2001) who demonstrated the difficulty of transferring fundamental chemistry concepts to a different field; in their case, engineering.

Most of early work in the area of pre-existing beliefs and alternative conceptions was based in physics. The physics education community has developed a set of “concept inventories” (CIs) that test core understanding, as opposed to typical assessments that test familiarity with isolated facts and the ability to manipulate equations (Hestenes *et al.*, 1992; Duncan & Hmelo-Silver, 2009; Coll &Taylor, 2001) CIs have also been developed in Biology (Klymkowsky & Garvin-Doxas, 2008; Shi *et al.*, 2010), Chemistry (Mulford & Robinson, 2002), Geosciences (Libarkin & Anderson, 2005), Engineering (Prince *et al.*, 2012) and Astronomy (Sadler *et al.*, 2010). Wright *et al* have developed the Molecular Life Sciences Concept Inventory (MLS CI) for Biochemistry and Molecular

Biology (Wright & Hamilton, 2008, 2011; Howitt *et al.*, 2008). We have used the MLS CI as a starting point for this study in which we focus on students' conceptual understanding of energy transformations in biological systems.

We employ a sequential, mixed-methods approach (Towns, 2008) to implement a pilot review of the nature of these alternative conceptions, with a view to suggesting and developing methods to improve biochemistry students' understanding of energy in biological pathways. We are interested in the ways students express their emergent understanding of energy and equilibrium concepts when presented with a biology context, and we have classified and categorised the conceptual difficulties our pilot group of students encounter.

In this study, we collect preliminary data to address three primary questions

(i) What are the most common alternative conceptions presented by students when they explain energy-related phenomena in biochemistry?

(ii) What information do students transfer from introductory chemistry and biology when they are asked to consider energy in a biological reaction or reaction pathway?

(iii) How do students at varying levels of competence articulate their understandings of energy in pathways and biological reactions?

We use our data to propose models of emergent understandings about energy in a biological context, and contribute to the pedagogical content knowledge (PCK) (Shulman, 1986; 1987) in the area of energy in biological systems.

Methodology:

Overall methodology

A sequential, mixed-methods approach (Towns, 2008) comprising three stages was applied in this study, as shown in Figure 1. The results from Stage 1 (N=6) were used to determine which questions were asked of the larger groups in Stages 2 and 3. In Stage 2 we interviewed general chemistry students (N=12) in-depth about their understandings of energy in biological pathways. In Stage 3 we asked higher-level students (N=53) about their understandings of the same concepts presented in Stage 2.

Stage 1: Question development (Student Group 1)

This aim of this Stage of the study was to devise a set of questions that would probe students' alternative understandings about energy in metabolic pathways and reactions. Six students ("Group 1") were recruited at a private US tertiary college. The students were a mixture of sophomores (N=1) and seniors (N=5), who were completing Biology/Neuroscience (N=2), and Chemistry majors (N=4). They completed the five energy transformation-related questions from the MLS CI using an on-paper format (see Appendix 1 for abbreviated questions). They subsequently participated in a one-hour semi-structured interview on all of questions (see Table 1 for outline of interview prompts). The interview was recorded, transcribed, and deductively analysed.

The interview prompts were designed to differentiate between questions (or parts of questions) that students could readily answer correctly (according to our model answers) and sections that they found confusing. Explanations of *why* students found questions confusing were also sought, with the aim of modifying the prompts if students were confused by the form of the question, rather than by the concept itself. The semi-structured interview allowed participants to provide further explanations after they answered the scripted questions. The students' responses were used in construction of the interview and survey questions for Parts 2 and 3 of this study.

Specifically, MLS CI questions 1, 2, and 3 (about ATP hydrolysis) and 5 (about the energy diagram for glycolysis) were retained for use in interviews with general chemistry students (see Stage 2). (We noted that the y-axis on the energy diagram should have been labeled, "free energy," or "G," rather than "ΔG," as it was in the published MLSCI This was corrected for Stage 2.) Questions 1, 2, and 3 probed similar concepts and were therefore combined into one question for the interviews. Question 4 revealed difficulties with the concept of oxidation as well as energy transformations and was deemed too complex for this study. These modified questions were used to generate the content in Table 2.

Stage 2: Interview study with first year chemistry students (Student Group 3). Students ("Group 2", n = 12) recruited from the cohort of a second-semester general chemistry at a research-intensive Australian university were interviewed using the prompts developed in Stage 1. Six students were interviewed before the topic of free energy was formally introduced in their course, and six after. Interviews were approximately 30 minutes. The interview script, including figures, is presented in Table 2.

All interviews were recorded and transcribed. A series of correct statements about energy and equilibrium in biological pathways were developed based on text-books and expert opinions (shown in Table 3). Three researchers, working from either recorded or written transcripts, coded the interviews deductively and quantitatively, according to these statements and the levels of attainment described in Table 3. When subjects answered the interviewer's question related to one of the statements A-P, these were coded 1-7 depending on the level of student understanding. At least two of three researchers (AW, SR, and GL, working in pairs) coded each interview. Inter-rater reliability was established through changing the combination of researchers in pairs during the coding process with examination and confirmatory re-coding of material already coded by different pairs.

The statements were categorized as follows

Well Understood (where ≥80% of the responses were coded as correct with minimal prompting)

Reasonably well understood (where ≥50% of the responses were coded as correct with minimal prompting)

Problematic (where 50-80% of the responses were coded as incorrect or only correct with significant prompting)

Very problematic (where ≥80% of the responses were coded as incorrect or only correct with significant prompting)

Infrequently articulated (any of the above, where fewer than 2.5% of the coded responses related to the idea)

Stage 3: Online survey study with Australian intermediate biochemistry students and American pre-med students (Student Groups 3 and 4)

An online survey version of the interview questions was developed. It is shown in full in Table 5. Briefly, 8 multiple-choice questions were included. They used text and figures from Stage 1 and 2, and the answers were populated using a combination of the original MLS CI text and distractors from student responses during Stages 1 and 2.

One group ("Group 3", n=42) was enrolled in an intermediate-level biochemistry course at a research-intensive Australian university. They were surveyed in Week 2 of the course, before the topic of bioenergetics was addressed. These students had already completed freshman general chemistry in the previous semester in the same cohort from which the interview participants in Group 2 were drawn. It is not known if any of the interviewed participants also completed the survey.

The other group ("Group 4", n = 18) included third and fourth-year pre-med student enrolled in an advanced biochemistry class at a private US tertiary college. All of these students had completed general and organic chemistry as part of their program.

The survey results were analysed for correctness and choice of distractor answers. A significance limit of 90% correct for each question was established, as it has been suggested that important conceptual difficulties may be missed if a cut-off point greater than 10% incorrect is used (Gilbert, 1977 ; Caleon and Subramaniam, 2010; Treagust *et al.*, 2011).

Ethics: All protocols were reviewed and approved by the participating institutions' institutional review boards or boards for ethics in use of human subjects. Interview subjects were recruited by email to appropriate classes. Survey subjects were informed of the survey by their instructor. All students were told that the purpose of the study was to explore their understanding of some key concepts in chemistry, or to test what knowledge students beginning the study of biochemistry bring from their prior chemistry and biology courses. They were further informed that the results would allow instructors to adapt and improve courses. All signed consent forms, either at the beginning of interviews or as the first question on the survey.

Results:

Results from Student Group 1 and decisions on questions to be used for the remainder of the study

The students in Group 1 encompassed a range of advanced students (both juniors to seniors) who were completing degrees with majors in the biological and chemical studies. All students had completed at least two years of chemistry in their earlier years of tertiary study. Their responses to the survey and structured interview questions (Appendix 1 and Table 1) provided some unexpected insights into their conceptions about energy in metabolic pathways.

Before beginning this study it was anticipated, based on literature, that students would hold alternative conceptions around breaking the bond in ATP, particularly the idea that breakage of the “high-energy bond” will “release energy”. In fact, several students did say that breaking that bond released energy, but on reflection during the interview they understood that it is the overall energy difference between reactants and products that yields favorable ΔG, rather than the breakage of the bond per-se.

The most critical points of confusion were found to relate to the difference between standard and actual free energy changes, the role of enzymes, and the relationship of free energy change to flux through pathways. When asked directly, students almost always said that enzymes did not have an effect on ΔG, but they were often confused about why reactions move in one direction or another; they invoked the activity of enzymes when they had no other explanation. Several students exhibited confusion over the concept that reactions close to equilibrium can be controlled by small changes in reactants and products. It was surprising to find out how little students knew about biological conditions in solution compared to standard conditions. Consequently, explicit questions about each of these concepts were incorporated into later surveys and interviews (shown in Tables 2 and 5).

Students had some trouble with questions about strength of bonds and of “enzyme control” vs. “substrate control” of pathways. Otherwise, students said that they had already seen (in previous classes) the terms and types of diagrams that were presented to them in the interviews, and that they understood them.

The three questions on bonds and free energy (Q1, 2, and 3, Table 1) elicited the same information, so they were combined for the later interviews into the new question about ATP bond breakage. The term “control” was confusing, and since the concepts it addressed can be replaced by asking about changes in reactant and product concentration, these questions were eliminated from later interviews. The query about bond strength (Q2 part (v), Table 1) was also discarded because it became peripheral to the main study. Questions about ATP, phosphocreatine, and glucose-6P (Q3, Table 1) led the students into a discussion of muscle and other topics they vaguely remembered. It was decided that it was more efficient and clear to ask questions only about ATP hydrolysis and not try to include transfer of the phosphoryl group to molecules other than water. The question on mass, atoms in food, and energy (Q4, Table 1) distracted students from the energy focus of the study. It was also discarded.

The process of completing a pilot exercise enabled determination of the appropriate number of questions that could be reasonably included within the scope of a survey or an interview. Administration of the on-paper test and the structured interview took well over an hour and students were tired, and had lost focus, by the end. Consequently we decided to limit our questioning to shorter surveys *or* to half-hour interviews.

Stage 2: Interviews with general chemistry students.

During the interviews the twelve first-year general-chemistry students demonstrated a range of mastery in core concepts in their interviews.

All of the student responses (n=234 relevant statements) were analysed through application of the coding and categorisation system described in Tables 3 and 4. Because there were no clear differences between the groups interviewed before and after introduction of free energy in their course, responses from all 12 students were combined.

Figure 2 shows a comparison of students' ability to correctly articulate key concepts related to energy. Each horizontal bar represents one concept (A-P from Table 3), and the colours on the bar represent different levels of ability to correctly articulate the concept (as detailed in Table 4). Each bar is normalised to 100%, so that responses are compared as percentages, rather than as total numbers. The "N" at the right of each bar indicates the number of responses in each category.

The black line indicates the boundary between responses in category 3 (purple, where the student could correctly articulate the concept after mild prompting) and responses in category 4 (cyan, where the student could only correctly articulate after a large amount of prompting, a mini-lecture, or a full concept explanation from the interviewer). Responses categorised as level 5, 6, and 7 (on the far right of the figure) were completely incorrect; students who gave these responses may subsequently have given a correct response after reflection or prompting, so they would represented more than once in the data set for a particular response. The further to the left the black line is on the bar, the more difficulty students encountered when they articulated this concept.

Students' conceptual understandings from the Stage 2 interviews were categorized as follows:

Well Understood

F. At equilibrium there is no net change in reactants or products; forward and reverse reactions are going at the same rate.

H. ATP hydrolysis reaction is more favorable in cells than under standard conditions because ratio of ATP: ADP is higher than for equilibrium.

J. Energy diagrams provide information about the overall energy change for a reaction or pathway.

Reasonably well understood

G. Magnitude of ΔG depends on the particular reaction and how far from equilibrium the system is initially.

I. Reactions can proceed in either direction depending on relative concentrations of reactants and products.

K. ΔG is the difference in energy levels between reactants and products.

M. ΔG = 0 at equilibrium.

N. Reactions close to equilibrium can be controlled by small changes in reactant or product concentration.

O. Reactions far from equilibrium cannot be regulated by small changes in reactant or product concentration.

Problematic

A. Negative ΔG° indicates that a reaction is spontaneous under standard conditions.

B. A reaction can be spontaneous whether exothermic or endothermic; or, ΔG can be positive or negative for exothermic reaction.

C. Definition of standard conditions includes T, P, and concentration of reactants and products (in solution).

D. In biological systems, most reactants and products are in dilute aqueous solution.

E. Cells are isothermal systems that can’t use heat to do work.

Very problematic

L. ΔG, or equilibrium, not affected by catalyst (enzyme) or otherwise related to rate. Statement D is borderline for this category as well.

Infrequently articulated

Responses related to statements B, F, and H were very infrequent, suggesting that students did not often draw upon these concepts for their reasoning while they were answering the questions. We consider the data for these statements to be less reliable than the statements that garnered more frequent statements.

Specific examples of student responses relating to some of the key concepts and an ideal "expert" answer (which was considered the gold-standard for responses) are shown in Table 6. Students' answers have been categorized according to their similarity to the "ideal", and have identified components that are missing from the non-ideal student answers have been identified.

Section 3: Survey of second level biochemistry students (in Australia) and third and fourth year pre-med students (in the USA)

Responses to the survey instrument by second level biochemistry students and third/fourth year level pre-med students displayed very similar outcomes across both cohorts. The results and interpretations are displayed in Table 7.

Comparison of our student groups and answers to our research questions

Students from Groups 3 and 4 who completed the online survey showed a strong grasp of the convention that a negative ΔG indicates a spontaneous reaction (Table 7 Q1), however interviews with Group 2 students, who are more junior than the Groups 3 and 4 students, uncovered significant confusion about this topic (Figure 2 line A and Table 6A). Students who incorrectly addressed the concept appear to confuse "spontaneous" reactions with single-molecule eliminations or rearrangements (SA4). They also appear to believe a reaction cannot be spontaneous if it involves bond breakage (Table 6 SA3). Other students demonstrate an understanding of a relationship between spontaneity and ΔG, however they do not know the convention for the sign (Table 6 SA2).

Students displayed a range of understandings of standard conditions, and found a description of biological conditions problematic (Figure 2 line C and D). They were unsure of the concentrations, temperature, and pressures that might be found in biological systems (Table 6C and D and Table 7 Q3). Table 6 shows that when students described the cellular conditions that contribute to reaction free energy changes and equilibrium, some attempted to incorporate ideal gas laws (SC4) and pH (SC3) while others omitted concentration (SC2). The students' very poor understanding of concentrations in the cell is typified by the comments from SD2 and SD3 (Table 6). These responses correlate with the high proportion of advanced students who believe that metabolite concentrations in cells could be higher than 1 M (Table 7 Q3D).

When students were asked to consider the relationship between ΔG°, ΔG, and reaction progress, (Table 7 Q4 and Table 6 G/H), they demonstrated some confusion. Group 2 students are able to address statements G and H "reasonably well" and "well" respectively (Figure 2), which indicates that, in principle, they understand that ΔG is influenced by concentrations of reagents and products in a system. However, when the more senior Group 3 and 4 students were presented with the idea that an enzyme could be the driving factor for a change in reaction ΔG, they chose this option preferentially over concentrations of reaction components as the driver (Table 7 Q4). In interviews, students who invoked the enzyme explanation frequently confused "spontaneity" with "rate of reaction" (Table 6 SG/H3–SG/H7) and/or thought that a catalysed reaction could not be spontaneous (Table 6 SL4).

More than 50% of students understood that reactions close to equilibrium could be pushed in either direction by changing the concentrations of reactants and products

(Table 7 Q7C and Figure 2 line N). A small majority of students also knew that reactions far from equilibrium cannot be reversed by changing reagent concentrations (Figure 2 line O). The results from Table 7 Q8D suggest, however, that many students think *no* reaction can be pushed backwards by changes in concentration – clearly there is confusion amongst our study subjects about the effect of concentration on reaction directions and equilibrium.

Graphical representations or visualizations are known to present particular difficulties in interpretation (Schönborn & Anderson, 2010). There was concern that students might misinterpret the glycolytic pathway energy diagram used in the second half of the survey and interview questions. In the most part students were able to identify that the energy diagram provided information about the overall reaction or pathway (Figure 2 line J). On occasion, interview subjects confused the energy diagram with depictions of electron transport or titration curves, which suggests that they were responding to overall or local shapes of the curves rather than the concepts involved.

The mislabeled y-axis of the energy diagram (as “ΔG,” rather than “G”) that was presented to students in the initial Stage 1 interviews may have led to some confusion on students’ part in regard to which step(s) in the pathway are closest to equilibrium; however, these data have not been included further. The corrected figure, used in Stages 2 and 3 of our study, reveal that this is, indeed, a difficult concept for students to grasp, with many incorrect responses observed. The particular energy diagram used for this study might have posed a particular difficulty for students because of the presence of a step, on interpretation, which results in a positive ΔG. Three of twelve students mentioned their observation that the energy change for one reaction in the sequence went "up" ("*There's a lot of intermediate reactions. Some of them going up and some of them going down.*"), but this observation did not seem to affect the students' ability to identify reactions close to equilibrium or to state that the overall pathway would have a favorable free energy change.

Discussion:

The transfer of core concepts between courses and contexts is critical in learning progressions as students move through the curriculum in college or university studies. Instructors make assumptions that students are able to recognize a concept in the new context and apply their prior understanding. The transition from chemistry to biochemistry is an example of one such articulation point.

This investigation explored student conceptions of energy and equilibrium in biological systems. The results confirm that the transition of energy-related concepts from chemistry to biochemistry is difficult, and that a structured learning progression may need to be developed to support student learning.

What are the foundations for an understanding of energy in biological systems?

The energy-related concepts we addressed in this study are underpinned by multiple anchoring concepts that are typically introduced in high school science and extended into general chemistry (Figure 3).

Inspection of related curricula and textbook resources reveals that the sequence in which these concepts are usually taught possesses an inherent hierarchy, with few texts introducing the concept of Gibbs free energy prior to the concepts of enthalpy and entropy. Figure 3 was developed as part of the current study to broadly represent the progression from lower level anchoring concepts (bottom) to the more complex abstract ideas (top). The proposed relationships were informed by Dixon and Emery's description of hierarchies in operational thermodynamics (Dixon & Emery, 1965) when applied to investigate students' conceptions of entropy and Gibbs free energy (Carson & Watson, 2002). Dixon and Emery proposed that the lowest level concepts encompass measurable quantities and that the highest level concepts included quantities that must be calculated, and are hence more abstract, including free energy. In Figure 3, these levels have been adjusted and anchoring concepts elaborated to reflect the large body of research investigating alternate conceptions that has been published since their original work (the details of this literature are beyond the scope of this study).

The complexity of the relationships between concepts is evident from Figure 3, particularly when we consider that the ideas of open/closed systems and system/surroundings are not illustrated. The statements for coding in Table 4 typically comprise more than one of these anchoring concepts.

What are the origins of issues in students' understanding in this study?

Two key components of Figure 3 (temperature and concentration) are deserving of special attention in our discussion for two reasons. First, there is a vast difference between open, living systems, and a test-tube in chemistry. Second, living systems *control* both of these variables; this skews the likelihood that a particular reaction will proceed, and largely removes the "T" modifier of ΔS in the Gibbs free energy equation (ΔS in biological systems is regularly increased by changes in the sizes and numbers of molecules, and changes in the freedom of electron movement, rather than by temperature-driven changes in kinetics).

Our results indicate strongly that students are confused about (or even unaware of) the concentrations of metabolites in living systems, and the small range of temperatures over which most living systems operate. This confusion about standard *vs*. cellular conditions means many students are left without a basis for understanding flux in metabolic pathways. Because they do not grasp the magnitude or relative amounts of metabolites in the cell, the only information they can call upon to explain the direction of steps in pathways is ΔG°. Lacking better explanations, they invoke enzyme activity, which suggests that they also do not have a clear understanding of what enzymes do. Even students who, in interviews, gave correct definitions of equilibrium or stated correctly that a catalyst has no effect on ΔG reverted to other explanations when faced with specific situations such as the hydrolysis of ATP or questions about energy diagrams. This reflects an incomplete reconciliation of distinct concepts, as described by Teichert & Stacy (2002).

The concept of ΔG° does provide students with a basis for describing reactions close-to or far-from equilibrium, but the students' main explanatory strategy continues to be Le Chatelier's principle – appropriate only to a limited extent – and enzymes – again, completely inappropriate. Similar types of confusion were observed in general chemistry students (Group 2), and those enrolled in later biochemistry and pre-med courses (Groups 1, 3, and 4), suggesting that (i) these conceptual difficulties are acquired very early in the study of chemistry and (ii) current teaching practice is not wholly effective in helping students transfer and broaden their understanding of chemistry into biochemistry.

These findings, particularly the persistence of incorrect ideas about equilibrium, spontaneity, and the role of enzymes, are consistent with earlier studies (Banerjee, 1995; Carson & Watson, 2002; Shi *et al.*, 2010) Although many of the alternative conceptions identified in this study have been reported previously, the fact that these misunderstandings are seen consistently in two very different settings (a small college in the USA and a large research university in Australia) and different student populations (those enrolled in general chemistry and those in intermediate- to advanced-level biochemistry) reinforces the depth of the problem.

Like previous studies on student understanding of energy concepts (Carson & Watson, 2002; Sears *et al*., 2007; Hadfield & Wieman, 2010), our results indicate that these incorrect ideas have been formed before the students begin their tertiary education. They are not generally gained or developed through observation of the natural world. That is, unlike the models of force and motion in physics, or of changing seasons in earth science, or of plant growth in biology that children construct from their own personal experience, most students will not have pre-conceived ideas about topics such as glycolysis or ATP hydrolysis before entering school. Rather, these concepts are first encountered in formal classroom settings in high school or early college. The idea that some alternative conceptions are accumulated through teaching rather than direct experience is not new, having been proposed by Johnstone *et al*. (1977) and supported by identification of errors in texts (Aledo *et al.*, 2003; Orgill and Bodner, 2006; Aledo, 2007) and teachers' strategies (Piquette & Heikkinen, 2005). All of the students interviewed had at least one year of high school chemistry, and our interview subjects indicated clearly that they were drawing on their high-school chemistry to answer the questions. Although it would be ideal if students could arrive at college/university with correct ideas about biological energy already in place, this may be beyond the scope of a high-school education.

As noted by Ebenezer and Fraser (2001), teaching of fundamental concepts should aim for a consistent theory and make connections among related processes. This is not a simple task; it requires explicit design of progressions from course to course within and across disciplines. The typical "silo" structure of most science curricula, where biology, chemistry, and physics are treated as separate subjects and taught by separate teams (particularly at the high school level), means such integration is difficult (Schmidt, 1997; Dauphinee & Martin, 2000; Ratcliffe & Millar, 2009; Schmidt *et al.*, 2011; Ashgar *et al.*, 2012). Students may be exposed to very similar topics in different science courses, but they may not make explicit connections between the topics in the different contexts. Perhaps this task should fall to biochemistry teachers at the tertiary level, although there is not a single clear model for making these connections.

What are the implications for teaching in biochemistry?

If this is the case, what do we need to consider as biochemistry teachers? Results from this study, along with others, point to the need for structured learning progressions that focus on explicit transfer of concepts across courses and disciplines. In this section we make suggestions for teaching strategies that may help students transfer their energy understandings more easily from the pure disciplines of chemistry and biology to biochemistry.

(i) Adopting a learning progression for energy - Learning progressions represent a structured acquisition of general skills and knowledge (Perkins and Salomon, 1989; Salomon and Perkins, 1989) combined with the mastery of transformative concepts in the discipline (Cousin, 2006; Ross *et al.*, 2010). Students' movement through these pathways is dependent on both their engagement and their attachment to pre-existing conceptions (Salomon, 1988; Perkins, 2006). The language of learning progressions has entered the K-12 lexicon, and there have been some descriptions of learning progressions at the college level (Cooper *et al.*, 2012, Cooper *et al.*, 2013). The curriculum developed by Cooper and Klymkowsky (2013) for general chemistry is particularly relevant to the topics presented in this study, as their approach weaves the concept of "energy" throughout the two-semester sequence. Although their project is explicitly interdisciplinary, it does not address the question of "articulation" between chemistry and biochemistry and the need to restart the learning progression at a slightly higher level at the beginning of biochemistry courses.

Our participants appear to demonstrate a learning progression in which they bring correct ideas about Le Chatelier's principle to their understanding of the progress of metabolic reactions. They are not prepared, however, to explain phenomena where Le Chatelier's principle no longer applies (an irreversible metabolic reaction) – at this point they draw upon incorrect arguments (such as enzymes) to make their arguments.

We recommend that educators carefully consider whether their students understand the differences between biological systems and test-tube chemistry. In particular, we recommend that educators do not simply discuss standards conditions, but that they explicitly discuss with students the *range* of possible temperatures, pressures, reagent concentrations, and pH values in living systems. Since these ranges are limited in cells and organisms, we must then think differently about how organismal metabolism drives a reaction to proceed. Perhaps we should be asking our students explicitly about what happens when the limits of Le Chatelier's principle are reached and when certain parts of the Gibbs Free Energy Equation are made semi-redundant through homeostasis.

(ii) Explicit explanation of discipline specific language - Johnstone (1991) has written about the difficulties that students encounter in science because of the need to move expertly between macroscopic, microscopic, and symbolic thinking. These three levels of thought take different forms in the contexts of chemistry and biology (Johnstone, 1991; Bahar *et al.*, 1999a, Bahar *et al.*, 1999b). Students who are first encounter biochemistry have their opportunities for confusion exacerbated by the many types of symbolism used in the discipline. Biochemists use chemical symbols and mathematical descriptions, however they also use visualizations (such as energy diagrams), discipline-specific representations of very large chemical structures (such as proteins and nucleic acids), context-specific language, and non-standard abbreviations (or even uninformative "nicknames") for proteins, genes, and chemicals. In particular, the terminology associated with biochemical processes can reinforce existing alternative conceptions, for example, statements such as 'ATP contains an energy-rich bond' (Goedhart & Kaper, 2003). We recommend that chemistry, biology, and biochemistry curriculum designers explicitly cross-check their terminologies with one another when they are building their educational offerings, then work with their students to point out and explain any discipline-specific (or confusing) language and representations.

(iii) Active pedagogies and direct instruction – Active pedagogies that include collaborative work, peer teaching, hands-on activities, and formative assessment have all been shown to ameliorate conceptual difficulties and to foster deeper understanding (Kogut, 1996; Hake, 1998; Knight and Wood, 2005; Eberlein *et al.*, 2008; Regan *et al.*, 2011; Villafane *et al.*, 2011), although some educators believe that a constructivist approach that addresses alternative conceptions head-on is not needed for transmission of all concepts in chemistry (Coll & Taylor, 2001). Active pedagogy alone, however is not sufficient to overcome deeply-held alternative conceptions; specially-designed interventions that are integrated into course design can be successful but require intense attention that precludes wide "coverage" of material (Villafane *et al.*, 2011; Loertscher and Minderhout, personal communication). Some of the students' alternative conceptions appear to stem simply from lack of information (such as cellular conditions, difference between enthalpy and free energy), and students readily reversed these during the course of our interviews during their explanations and through provision of minor prompts by the interviewer. These "readily reversed" conceptions may be good candidates for specifically-designed direct instruction. Faculty could, for example, model the application of these principles to biological systems, while also consistently pointing out common alternative conceptions to their students (perhaps accompanied by the anonymised results from an concept inventory pre-test of their cohort). Concept inventories could be used for pre- and post-assessment so that educators can evaluate which approaches actually make a difference in student learning.

We believe that this study will assist practitioners in characterizing the levels of understanding that their students possess, with a view to developing learning progressions about “energy” in biological pathways and systems in biochemistry (National Research Council, 2007; Wilson, 2009; Duncan & Hmelo-Silver, 2009; Cooper *et al.*, 2012; Neumann *et al.*, 2013).

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AJW and SLR contributed equally to this work.

This research was supported in part by a grant from the Marion and Jasper Whiting Foundation to AJW.

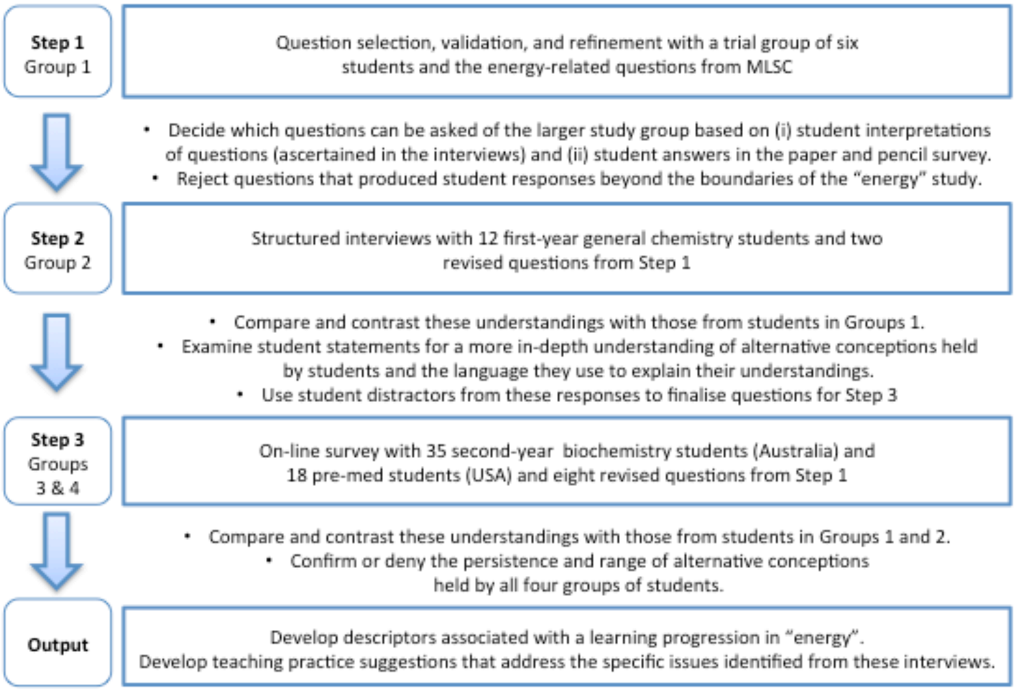


Figure 1: Methodology for this study

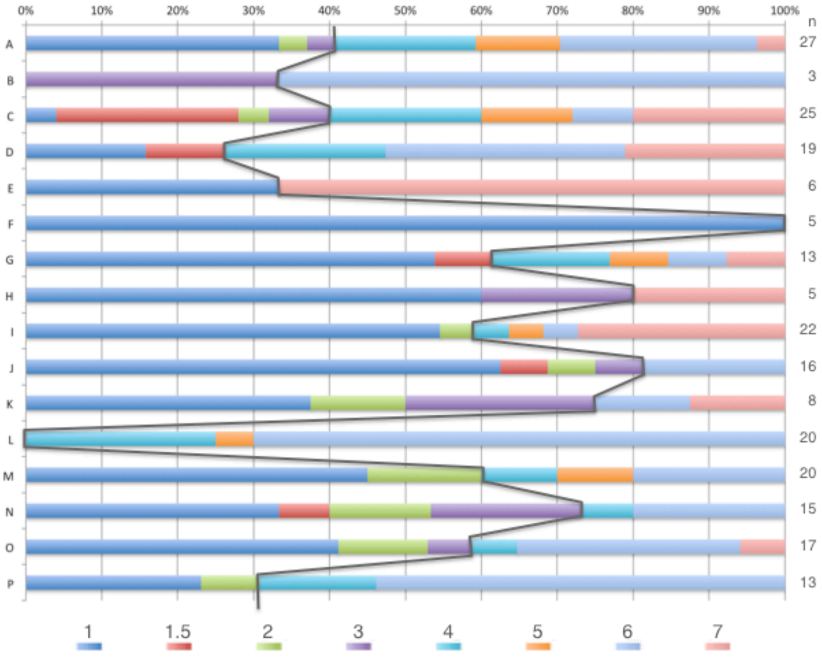


Figure 2: Analysis of responses by general chemistry students to statements related to energy transformations. **See Table 3 for a description of statements A-P on the Y axis.**

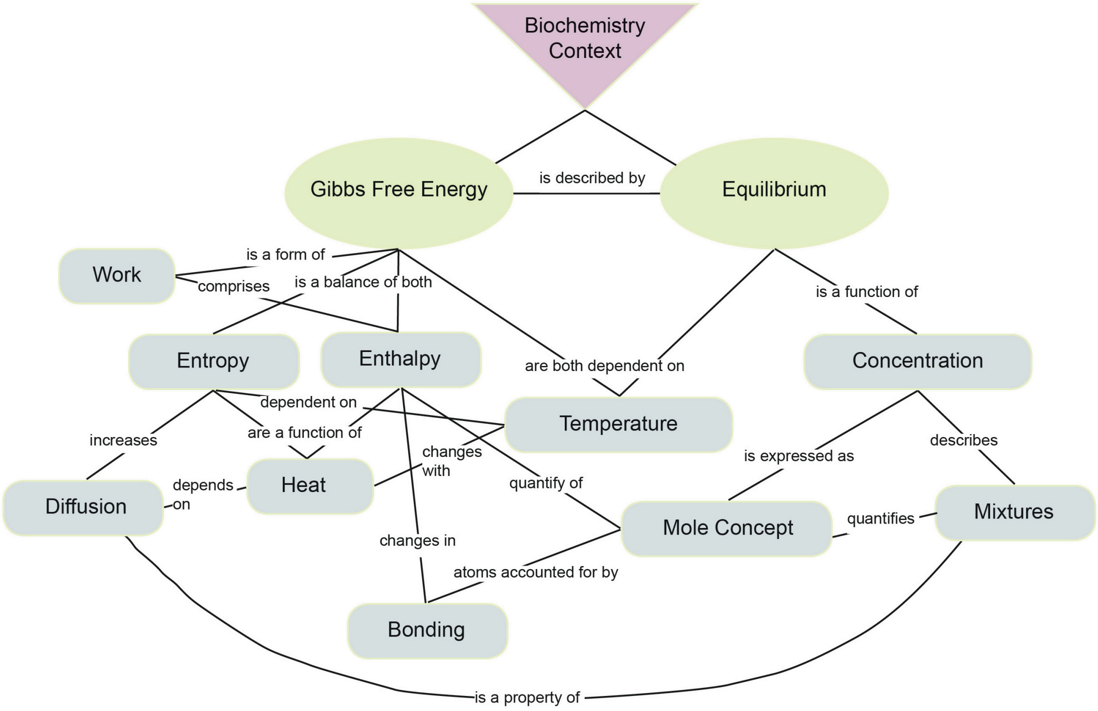


Figure 3: Relationship between anchoring concepts and the conceptions of energy and equilibrium in a biochemistry context.

Table 1: Scheme for Stage 1 interview questions on MLS CI "Energy" Module 4 (Group 1)

I would like start out by asking some general questions about the material on this survey and then move into some specific questions. How familiar are you with the material that was in these questions? Was any of this completely unknown to you?

Let's go through some of the questions one by one. I'm not going to ask about every single part of every question but I am interested in the overall concepts that you saw.

As you look at question 1, (i) what's your general understanding of what ΔG tells you about a reaction?; (ii) What is meant by the standard free energy of a reaction?; (iii) What do we mean by standard conditions?; (iv) How is that related to rate?; (v) You notice that the enzymes are asked about. What is the effect of enzymes on ΔG?

Let's look at question 2. (i) Can you describe what happens when ATP is hydrolyzed?; (ii) Does that reaction release or require energy?; (iii) In general if you think about bond breaking, would that require or release energy?; (iv) Is there anything else about the products and reactants in the ATP going to ADP reaction that yield that differences in energy at the end?; (v) In part c of this question they ask you about strong P-O bonds formed in the reaction. What do you think they are asking about there?; (vi) Which bond do you think they are talking about as the strong P-O bond?

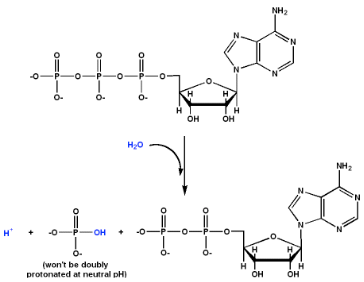
Let's look at question 3. You might want to keep referring back and forth to the previous one as well since this one relates to ATP also. (i) So in this question, how does this reaction relate to the one you saw about ATP?; (ii) In the previous question we just said that there is a favorable ΔG going from ATP to ADP, and here is favorable to form ATP. How can you reconcile those two?; (iii) What's your understanding about what's happening in this system at equilibrium?; (iv) If it's going to move away from that equilibrium, what's going to have to happen?; (v) What would cause it to move away from equilibrium?; (vi) Again they mention ΔG° and they give you the equilibrium constant that is related to ΔG°, under cellular conditions what does that ΔG° tell you?; (vii) How would the conditions in the cell likely be different from standard conditions?

Question 4 is asking you about what happens to food when you eat it. Another way to say that is that they are asking you about metabolism. (i) Have you studied metabolism at all?; (ii) What's your understanding of what happens to the atoms of the food you eat in the end?; (iii) What kind of reaction is that?; (iv) What do you expect are going to be the end products for this athlete who is not actually gaining any weight in the process?; (v) They say the athlete is not gaining any weight, but for someone who is not running every day, what is going to happen to those atoms in food?

Finally, let's look at question 5, which is again a metabolic pathway. (i) Have you seen energy diagrams?; (ii) This is glycolysis, but it could be any biological pathway. Just looking at this energy diagram and forgetting that we are using the words glycolysis or glucose or pyruvate what can you say about the overall reaction and the energy change that is happening here from start to finish?; (iii) What can you say about the energy changes that are occurring in this reaction pathway; (iv) Did you understand what the question meant by the “flow of materials” in this pathway?; (v) Looking at that step with a small ΔG, are you going to be able to push or pull a reaction in terms of the concentrations?; (vi) Do you have a sense of what they mean by enzyme regulation of a pathway or step?; (vii) This series of reactions going from glucose to pyruvate is what we might call an oxidative pathway or a catabolic pathway. In some situations we might want to do the opposite, say if you have a lot of pyruvate you might want to make it into glucose. What’s your feeling about how you could get from here to here? *(interviewer points to the intermediates joined by step G, and those joined by step F)*; (viii) Do think that these steps are going to be just exactly the opposites all the way up?; (ix) Would you be able do the same for those first steps, step C for example with a big negative ΔG, could you do the same thing there?

Table 2: Script for Stage 2 interviews for general chemistry students (Group 2)
Note: in the actual interview the diagrams are reproduced in a larger form, with each diagram being the width of one A4 page.

Thank you for participating in this study. Just to reassure you, I am not grading you on your performance. I am just trying to find out what you have learned about the specific topics around energy transformations and the kinds of language you use to describe what you've learned. The more you can say about your thinking, the better. There are two sections with a set of questions for each.

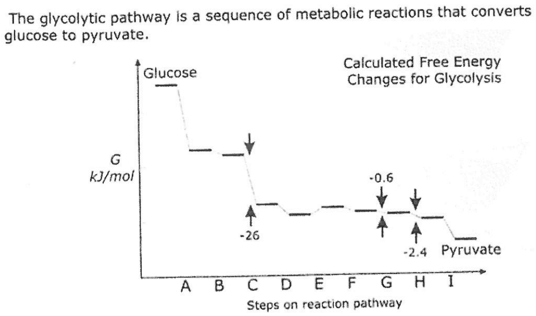


This reaction has an overall ΔG° of about -30 kJ/mol.

The first section has to do with the reaction on the right, the hydrolysis of ATP. *(Students are given 1-2 minutes to examine the diagram).*

Questions: (i) Is this reaction spontaneous under standard conditions?; (ii) What is your understanding of what ΔG tells you about a reaction?; (iii) Can you describe what is meant by the "standard free energy of reaction"?; (iv) What might be different in biological systems?; (v) If ΔG for the reaction in a red blood cell is -57 kJ/mol, how is this possible?; (vi) And what does it mean about the direction of reaction?

The second section has to do with the energy diagram on the right.


The glycolytic pathway is a sequence of metabolic reactions that converts glucose to pyruvate.

It asks about glycolysis but we could draw such a diagram for any pathway. *(Students are given 1-2 minutes to examine the diagram).*

Questions: (i) Have you seen an energy diagram before?; (ii) Looking at the energy diagram, what can you say about the overall pathway and energy change?; (iii) Which steps are close to equilibrium?; (iv) How can you tell?; (v) Can you describe how a reaction might go one way or the other in response to changes in concentration of substrate (reactant) or product?; (vi) Thank you for your time and insights. Do you have any questions for me?

Table 3: Propositional knowledge statements for coding of Stage 2 interview responses (Group 2)

A. Negative ΔG ° indicates that a reaction is spontaneous under standard conditions.
B. A reaction can be spontaneous whether exothermic or endothermic; or, ΔG can be positive or negative for exothermic reaction.
C. Definition of standard conditions includes T, P, and concentration of reactants and products (in solution).
D. In biological systems, most reactants and products are in dilute aqueous solution.
E. Cells are isothermal systems that can't use heat to do work.
F. At equilibrium there is no net change in reactants or products; forward and reverse reactions are going at the same rate.
G. Magnitude of ΔG depends on the particular reaction and how far from equilibrium the system is initially.
H. ATP hydrolysis reaction is more favorable in cells than under standard conditions because ratio of ATP: ADP is higher than for equilibrium.
I. Reactions can proceed in either direction depending on relative concentrations of reactants and products.
J. Energy diagrams provide information about the overall energy change for a reaction or pathway.
K. ΔG is the difference in energy levels between reactants and products.
L. ΔG, or equilibrium, not affected by catalyst (enzyme) or otherwise related to rate.
M. ΔG = 0 at equilibrium.
N. Reactions close to equilibrium can be controlled by small changes in reactant or product concentration.
O. Reactions far from equilibrium cannot be regulated by small changes in reactant or product concentration.
P. Free energy includes both enthalpy and entropy terms.

Table 4: Coding scheme for understanding of concept statements in Stage 2 (Group 2)

1	Student volunteers correct (or close to correct) version of the statement without prompting*
2	Student comes to that correct version on reflection**
3	Student comes to that correct version after minimal prompting***
4	Student comes to that correct version after much prompting****
5	Student never comes to that correct version
6	Student says something opposite or in contradiction to that statement
7	Student fails to see that concept is same in chemical and biological context

* Statements were coded as “1.5” if they were correct but incomplete.

** Statement was coded as “2” if repetition of the student’s response led to a change to the correct version

*** Statement was coded as “3” if a restatement of the question and clarification led to a change to the correct version

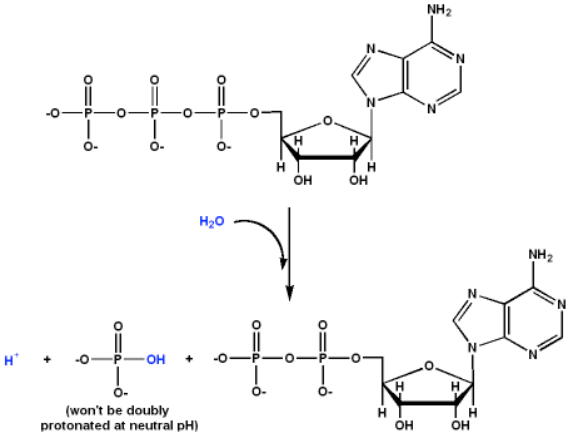
****Statement was coded as “4” if the student required additional information and or a mini-lecture in order to come to the correct version.

Table 5: Stage 3 survey questions (Groups 3 and 4)

ATP hydrolysis questions

Please answer the first four questions while you look at this ATP hydrolysis image.

Assume that standard conditions include:
T = 25° C; P = 1 atm; concentration of solutes = 1 M



$\Delta G^\circ = -30$ kL/mol

1. Is this reaction spontaneous under standard conditions?
a) Yes
b) No
c) Cannot be determined from the information given

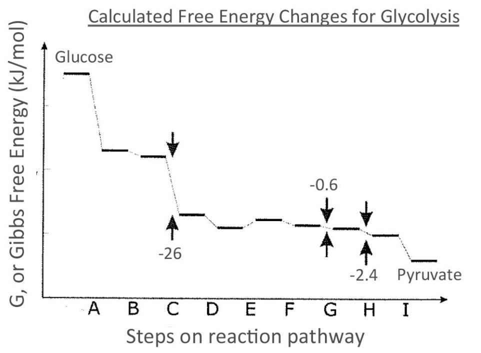
2. What was the reason for your answer in (1) above?
a) Because ΔG° is negative.
b) Because ATP is unstable.
c) Because I know that this process happens in biological systems.
d) We cannot determine if the reaction is spontaneous because the catalyst (enzyme) is not included.

3. What differences in concentration occur between "standard conditions" and those that occur in a cell in your body?
a) Concentrations of all solutes are the same as for standard.
b) Concentrations of all solutes are lower than for standard.
c) Concentrations of all solutes are higher than for standard.
d) Concentrations of all solutes can be either higher or lower than for standard.

4. In the red blood cell, ΔG for the reaction shown is -57 kJ/mol. This is different from ΔG° because:
a) An enzyme catalyzes the reaction.
b) ATP is constantly required by other cellular processes.
c) The ratio of ATP/ADP is higher than for standard conditions.
d) Hemoglobin binds to ATP.

Glycolysis Energy Diagram

Please answer these four questions while you look at the glycolysis energy diagram
Consider the figure below, an energy diagram for the glycolytic pathway, in answering questions 5-8:



5. Which of the following is true of the overal
pathway:
a) It is spontaneous under standard conditions
b) It is not spontaneous under standard
c) The information given is not sufficient to determine spontaneity under standard conditions.

6. Which of the following reactions/steps in th
pathway is/are closest to equilibrium?
a) A and C
b) F and G
c) I
d) They are all similarly close to equilibrium.

7. Reactions that are close to equilibrium can be shifted right or left by which of the following:
a) Addition of substrate (reactant) or product
b) Removal of substrate (reactant) or product
c) (a) or (b)
d) Addition of a catalyst (enzyme)
e) (a), (b), or (d)

8. The reaction pathway sometimes proceeds in the opposite direction as shown (ie from pyruvate to glucose). Which of the following is likely to be true of the reverse pathway?
a) All reactions will be shifted in the reverse direction by small changes in reactant or product.
b) None of the reactions will be able to run in reverse unless additional energy is provided to the system.
c) The reverse pathway will be slower than the forward pathway.
d) Reactions with large energy change in the forward direction will not be readily shifted to the reverse by changes in reactant or product.

9. Think about your answers to the questions on this survey. Did you change any answers after reading further into the survey or after further consideration? If so, which ones did you change? What made you reconsider your original answer?

Table 6: Some specific examples of progression through concepts
(The level of understanding within each topic decreases from top to bottom. The ideal "expert" answer is followed by the bracketed letters (IEA). The ideal answers actually given by students are shown in ***bold, italic.*** Other answers given by students are shown in plain text. Prompts or additional questions asked by interviewers are shown in *[italics in square brackets].* The designations "SA1", "SA2" etc indicate individual students).

<table>
<tr><td colspan="2">A (The relationship of ΔG° to spontaneity under standard conditions): The hydrolysis of ATP is spontaneous under standard condition. We know this from the data because ΔG° is negative, indicating an exergonic reaction (release of energy), indicating that the products are more stable than reactants. (IEA)</td></tr>
<tr><td>At this level the student -</td><td>Typical student answer for this level of understanding (quote)</td></tr>
<tr><td><i>Has a clear understanding that the ATP hydrolysis reaction is spontaneous under standard conditions (even if language is not always precise)</i></td><td><i>SA1: I think it is because you've got the negative free energy.</i>
<i>SA2: Because it has a negative ΔG value.</i></td></tr>
<tr><td><i>Has not learned the convention for ΔG° and spontaneity, but understands that there is a relationship between the two</i></td><td>SA3: I may have it round the wrong way, but if Gibbs energy is more than zero it will occur spontaneously and that's a negative value so it won't.</td></tr>
<tr><td><i>Doesn't use the information presented about ΔG°; reasoning from other knowledge</i></td><td>SA4: Not spontaneous; you have to break the bond between the oxygen and phosphate. It would take a lot of energy to break it.</td></tr>
<tr><td><i>Doesn't use the information presented about ΔG°; misunderstands spontaneity</i></td><td>SA5: Not spontaneous; because there's been water added to it. So it's not happening by itself.</td></tr>
<tr><td colspan="2">C (The definition of standard conditions in solution): Standard conditions in solution are commonly taken to be: temperature 298 K (25° C), 1 atm pressure, 1.0 M all reactants and products, although the formal (IUPAC) definition is: temperature (273 K) 0° C and absolute pressure of 100 kPa along with 1.0 M concentrations. (IEA)</td></tr>
<tr><td>At this level the student -</td><td>Typical student answer for this level of understanding (quote)</td></tr>
<tr><td><i>Has a clear knowledge of T, P and concentration conditions (even if language is not precise)</i></td><td><i>SC1: So standard pressure is usually 1 atmosphere, temperature would be 25° C, and I'm guessing concentration would be a mole</i></td></tr>
<tr><td><i>Includes only T and P in the explanation</i></td><td>SC2: Standard temperature and pressure - 25° Celsius and 1 atmosphere.</td></tr>
<tr><td><i>Includes pH in the explanation</i></td><td>SC2: I'd say it's going to be a neutral pH</td></tr>
<tr><td><i>Confuses standard conditions with ideal gases</i></td><td>SC3: I think it means when things are under the ideal gas law.</td></tr>
<tr><td colspan="2">D (The difference between standard conditions and biological conditions): Temperature may be different from standard (for humans it will be 37° C) and there may be local variations in pressure, but the main difference is that solutes are present at dilute</td></tr>
</table>

concentrations, nowhere near 1.0 M. (IEA)	
At this level the student -	**Typical student answer for this level of understanding (quote)**
Has a clear idea about T and P, understands that solutes will be in dilute solution	***SD1: Temperature. Wouldn't it be operating at body temperature? And I'm guessing pressure would be close. Would not always be 1 atmosphere and concentration would certainly vary a fair bit. I doubt it would be as much as 1 molar.*** 10^{-5} ***milli?molar or something like that***
Has no idea about concentrations of solutes in biological systems	SD2: *[Would concentrations be more, less, or the same as for standard conditions?]* Probably more. Because the cell would want whatever reactions it's trying to have, take place when there's a surplus of things to react with SD3: The concentrations would be wildly different in both ways I guess, for different chemicals.
Understands that pH will be around neutral but does not equate neutral pH with $\sim 10^{-7}$ *M protons until asked to do the calculation*	SD4: Concentration of protons? Maybe about 1 M SD5: *[What's the concentration of protons at pH 7?]* 10,000? I don't know. SD6: *[Do you remember the definition of pH?]* Not really.
G/H (The dependence of ΔG on reaction conditions, either generally or specifically for ATP reaction): $\Delta G°$ is a constant. ΔG depends on both $\Delta G°$ and concentrations of reactants and products: $\Delta G = \Delta G° + RT \ln Q$, where $Q = [P]/[R]$. When $Q >$ the K_{eq}, ΔG will be greater than (less negative than) $\Delta G°$ and the reaction will have less tendency to go in the forward direction than under standard conditions. The opposite is true when $Q < K_{eq}$. In the case of the ATP hydrolysis reaction, under cellular conditions [ATP]/[ADP] is greater than under standard conditions, so that $Q < K_{eq}$ and the reaction is more favorable. (IEA)	
At this level the student -	**Typical student answer for this level of understanding (quote)**
Understands that reaction will be more favorable when reactants are greater than for standard conditions or products less (although none explained in terms of reaction quotient or relationship of ΔG *to* $\Delta G°$	***SG/H1: The reason why it might be more? Concentration is probably perhaps higher… of ATP…or take away the ADP.***
Understands that reaction will be more favorable when reactants are greater than for standard conditions but focuses on water as reactant to be increased in concentration	SG/H2: You have to give it the motivation to go from one side to the other. Increasing the number of ATP molecules and increasing the number of water molecules would do that. *[How can the reaction be more spontaneous?]* Maybe if you drink more water
Confuses "rate of reaction" with "spontaneity" (invoking	SG/H3: *[under standard conditions* ΔG *is -30. In the red blood cell the* ΔG *for the reaction is -57kJ/mol. So*

either a catalyst or a change in temperature as the driving force)	*how is that possible?]* Would it be with enzymes? SG/H4: *[does thermodynamics have anything to do with enzymes?]* An enzyme functions as a catalyst so it will help to lower the energy profile in itself. But in terms of thermodynamics, I'm really not sure. SG/H5: Maybe that they have an enzyme or a catalyst that helps? SG/H6: Well, you've got the higher temperature; in most reactions the higher temperature will speed up the reaction.
J (The interpretation of the energy diagram and the explanation of the energetics and equilibrium of reversible and irreversible reactions): Energy diagrams give a visual representation of the relative stability of reactants and products and the size of ΔG for individual steps or for the overall pathways. Reactions close to equilibrium can be seen as those with reactants and products at close to the same energy levels, whereas those far from equilibrium are seen as those with large steps between reactants and products. Reactions that are close to equilibrium can be shifted right or left by addition or removal of reactant or product. Reactions with large energy changes in one direction cannot be readily reversed by small changes in reactant and product. These are understood to be metabolically irreversible. (IEA)	
At this level the student -	**Typical student answer for this level of understanding (quote)**
Understands that ΔG of zero is indicated by products and reactants at same energy level	***SJ1: I would just say whenever the graph is flat or horizontal would be when it's at a sort of equilibrium and then it requires some sort of input to move to the next one.***
Understands release of energy but thinks only of enthalpy change	SJ2: In total it would be an exothermic sort of release of energy from the high initial going down to the low. SJ3: It's exothermic. It's giving out energy to the system. It's spontaneous.
Thinks about an absolute value of G (rather than ΔG)	SJ4: I'm guessing the bottom of the graph is zero. So that would mean pyruvate has the lowest or is most like an equilibrium. SJ5: Well it's sort of a bit hard to tell from this. I don't think you can really actually tell from this diagram [which steps are at equilibrium] because there's no indication of the free energy on the side and it's just giving you the changes here. So that could be, for example, a thousand kJ/mol and it's going down this much and it goes down this much to 25 and it never actually, in fact actually reaches 0 which is equilibrium.
Does not know how to draw conclusions from energy diagram about reactions at equilibrium	SJ6: *[can you see if any of the steps are close to equilibrium?]* I've got no idea… So like maybe from *[one step to the next]* there's not very much change in the amount of energy. But that doesn't necessarily

	mean that it's at equilibrium, does it?
Confuses reaction coordinate with time	SJ7: Wouldn't the gradient between each bold line be the rate of that particular reaction?*[does rate have anything to do with equilibrium? With free energy?]* I want to say it does have something to do with equilibrium, but not so much with free energy.
L (equilibrium position and ΔG unaffected by catalyst): Enzymes (catalysts) speed up reactions in both forward and reverse direction but cannot affect the final equilibrium position or, therefore, ΔG. (IEA)	
At this level the student -	**Typical student answer for this level of understanding (quote)**
States role of enzymes appropriately but still invokes catalysis as way to shift equilibrium	***SL1: Enzymes reduce activation and energy needed. So it makes it easier to change from reactants to products.*** *[Is that going to change the equilibrium position?]* ***No.*** But it can help to catalyze the next reaction so it can keep going. *[Will that change whether or not it is spontaneous?]* I don't know.
Invokes catalyst as way to move shift equilibrium	SL2: When it's at a sort of equilibrium and then it requires some sort of input to move to the next one… it could be energy or a catalyst
Confuses "spontaneous" with "uncatalyzed"	SL3: *[Is the ATP hydrolysis reaction spontaneous under standard conditions?]* No. I know from my previous studies that it has to be mediated by various enzymes and things like that.

Table 7: Results of survey instrument

See Table 5 for full text of survey questions. Correct responses are in bold

When asked:	Response (Group 3)	Response (Group 4)
Q1. Is this reaction spontaneous under standard conditions? **A) Yes** B) No C) Cannot be determined	A) 88% B) 7% C) 5%	A) 100% B) 0% C) 0%
Q2. What was the reason for your answer above: **A) ΔG° is negative** B) ATP is unstable C) I know this happens in biological systems D) Can't determine because enzyme not included	A) 90% B) 2% C) 2% D) 5%	A) 94% B) 0% C) 6% D) 0%
Our Interpretation: Most students know the convention that negative free energy change is associated with spontaneous reaction. There is some confusion over the relevance of this data in the biological setting (not signficant according to our criteria).		
Q3. What differences in concentration occur between "standard conditions" and those that occur in a cell in your body? A) Same as for standard (1 M) **B) Lower than for standard** C) Higher than for standard D) Could be higher or lower	A) 10% B) 24% C) 12% D) 56%	A) 6% B) 6% C) 17% D) 72%
Our Interpretation: The majority of students have no idea of the order of magnitude of cellular concentrations of metabolites (significant).		
Q4. In the red blood cell, ΔG for the reaction shown is -57 kJ/mol. This is different from the ΔG° because: A) An enzyme catalyzes the reaction B) ATP is constantly required by other processes **C) The ratio of ATP/ADP is higher than for standard conditions** D) Hemoglobin binds ATP	A) 44% B) 10% C) 36% D) 10%	A) 44% B) 22% C) 28% D) 6%
Our Interpretation: Almost half the students believe an enzyme will change the ΔG of a reaction and do not understand the relationship between ΔG and ΔG° (significant).		
Q6. Which of the reactions/steps in the [glycolytic] pathway energy diagram is/are closest to equilibrium? A) A and C **B) F and G** C) I D) All are similarly close	A) 9% B) 46% C) 43% D) 3%	A) 0% B) 72% C) 28% D) 0%
Our Interpretation: Up to 43% of students chose the last reaction of the pathway, which actually has a larger ΔG than steps F and G. This suggests that students (i) may not be able to interpret the diagram or (ii) may be choosing the point in the pathway that they believe is closest to equlibrium (the end), rather than the "step" as requested (significant).		
Q7. Reactions that are close to equilibrium can be shifted right or left by which of the following:		

A) Addition of reactant or product B) Removal of reactant or product **C) Either of the above options** D) Addition of catalyst (enzyme) E) Any of the above	A) 3% B) 0% C) 66% D) 6% E) 26%	A) 0% B) 0% C) 67% D) 0% E) 33%
Our Interpretation: Almost all knew that changing substrate or product concentration would shift the equilibrium, but one-quarter to one-third thought that an enzyme could contribute to this shift (significant).		
Q8. The reaction pathway sometimes proceeds in the opposite direction as shown (ie from pyruvate to glucose). Which of the following is likely to be true of the reverse pathway? A) All reactions can be shifted by small changes in reactant and product B) None can go in reverse without input of additional energy C) The reverse reactions will be slower **D) Reactions with large energy changes in forward direction cannot be readily reversed by small changes in reactant and product**	A) 9% B) 63% C) 3% D) 26%	A) 11% B) 56% C) 11% D) 22%
Our Interpretation: Most students do not understand the difference between reactions close to equilibrium and those that are metabolically irreversible (significant).		

Note that Question 5 of the survey has been eliminated from analysis because it referred to standard, rather than actual, conditions.

Appendix 1: Part 1 abbreviated version of MLS CI module on energy transformations (Wright and Hamilton, 2008, 2011) used with Student Group 1

The statements in parentheses did not appear in the survey as written but are added here because figures are not included. All questions contained images. Full inventory available as link from (Wright and Hamilton, 2011).

Q1 The standard free energy of hydrolysis of ATP (ΔG°) is -35.7 kJ/mol at pH 7.
Indicate which of the following statements is **true** or **false** or you **don't know**:

a) The reaction is spontaneous under standard conditions.
b) An enzyme will catalyse the reaction by reducing the ΔG°.
c) From this value of ΔG° we can tell that the hydrolysis will occur rapidly at pH 7.

Q2: The conversion of ATP to ADP in the cell is able to drive thermodynamically unfavourable processes such as glucose to glucose-6-phosphate:
ATP + glucose -> ADP + glucose-6-phosphate
In the reaction, bond A *(between b and g phosphate)* breaks.
Indicate which of the following statements is **true** or **false** or you **don't know**:

a) ATP is able to drive the reaction because energy from the conversion of ATP to ADP makes the overall reaction favourable.
b) The breaking of bond A releases energy.
c) The ATP molecule is a good source of energy due to the strong P-O bonds formed in the reaction.

Q3: During the first few seconds of vigorous exercise ATP is consumed rapidly by contracting muscle cells, yet the concentration of ATP does not decrease significantly. This is because the reaction below is able to maintain the concentration of ATP in the cell. The reaction is catalyzed by an enzyme. (Students are provided with a diagram showing phosphocreatine + ADP reversibly going to creatine + ATP)
In resting muscle the reaction is effectively at equilibrium and the K_{eq} is 160. The standard free energy for the reaction is ΔG° = -12.5 kJ/mol at pH =7.
Indicate which of the following statements is **true** or **false** or you **don't know**:

a) In resting muscle there is no net reaction in either direction.
b) The reaction to give phosphocreatine will occur spontaneously if the ratio of ATP:ADP becomes sufficiently large.
c) The reaction to give phosphocreatine has a ΔG° = +12.5 kJ/mol and therefore is not spontaneous in muscle cells.

Q4: Athletes eat large meals containing carbohydrate and fat when they are in training but their body weight (mass) remains nearly constant. What happens to most of the mass consumed?

Indicate which of the following statements is **true** or **false** or you **don't know**:

a) It is converted to faeces and eliminated from the body.
b) It is converted to energy.
c) It is released as carbon dioxide and water.
d) It is converted into adenosine triphosphate, ATP.

Q5: The glycolytic pathway is a sequence of metabolic reactions that converts glucose to pyruvate. *(Step C has ΔG value shown of -26 kJ/mol; step G of -0.6 kJ/mok, and step H of -2.4 kJ/mol.)*

Indicate which of the following statements is **true** or **false** or you **don't know**:

a) Step G is far from equilibrium.
b) The small value of ΔG in step H means that the flow of material at this point in the pathway can be regulated by changes in substrate concentration.
c) Enzyme regulation of the flow of material in the pathway will be less effective at Step C than at Step G.

References

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