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Article

Identification of Threshold Concepts for Biochemistry

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Threshold concepts (TCs) are concepts that, when mastered, represent a transformed understanding of a discipline without which the learner cannot progress. We have undertaken a process involving more than 75 faculty members and 50 undergraduate students to identify a working list of TCs for biochemistry. The process of identifying TCs for biochemistry was modeled on extensive work related to TCs across a range of disciplines and included faculty workshops and student interviews. Using an iterative process, we prioritized five concepts on which to focus future development of instructional materials. Broadly defined, the concepts are steady state, biochemical pathway dynamics and regulation, the physical basis of interactions, thermodynamics of macromolecular structure formation, and free energy. The working list presented here is not intended to be exhaustive, but rather is meant to identify a subset of TCs for biochemistry for which instructional and assessment tools for undergraduate biochemistry will be developed.

INTRODUCTION

As a discipline, biochemistry has a set of concepts that most biochemists agree are essential for biochemistry undergraduates to master. For example, the American Society for Biochemistry and Molecular Biology (ASBMB) has recently completed the first phase of an effort to identify foundational concepts and skills for majors in biochemistry and molecular biology (Mattos *et al*., 2013; Tansey *et al*., 2013; White *et al*., 2013; Wright *et al*., 2013). Another group of biochemists has undertaken an effort to identify "big ideas" in the molecular life sciences as part of the development of the Molecular Life Science Concept Inventory (MLSCI; Howitt *et al*., 2008; Wright and Hamilton, 2008; see Table 1 for a complete list). Yet biochemistry instructors know from classroom experience that many students fail to master these concepts. A major barrier to achieving mastery is the fact that students bring scientifically inaccurate ideas from their prior experience to biochemistry classrooms, many of which are robust and therefore persistent (Anderson and Schönborn, 2008; Chi, 2008). This problem is further complicated by the fact that these inaccurate ideas may relate to threshold concepts (TCs; Meyer and Land, 2003).

TCs are concepts that, when mastered, represent a transformed and integrative understanding of a discipline without which the learner cannot progress. By focusing on student understanding of TCs in biochemistry, instructors can maximize the impact of classroom instruction toward learning achievement. Meyer and Land (2003), the originators of this educational term, posit that TCs can be identified for any discipline and provide a framework for linking student learning to curricular design. TCs have five defining characteristics:

Transformative: Once a TC is understood, a student's perception and comprehension of a subject are radically altered. In addition to stimulating cognitive development, learning of TCs can alter a student's self-perception or sense of identity. For example, students may shift from viewing themselves as students of biochemistry to recognizing that they have begun to think like biochemists.

Irreversible: Once a TC has been deeply understood, students are unlikely to forget it. The concept becomes central to

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Table 1. Previously published concepts for biochemistry

ASBMB foundational concepts in biochemistry and molecular biology[a]	Evolution, matter and energy transformation, homeostasis, biological information, macromolecular structure and function
MLSCI "big ideas" in the molecular life sciences[b]	Molecular evolution, self-assembly, compartmentalization, information, and communication, regulation, catalysis, energy and organization, complexity of molecular structures, and the aqueous environment

[a]Tansey *et al.*, 2013.

[b]Wright and Hamilton, 2008.

how students think about everything else in the field. Experts have difficulty remembering how they understood the discipline before deeply understanding TCs.

Integrative: TCs bridge concepts within a discipline and among disciplines. Once understood, previously hidden connections within a discipline, and perhaps even across disciplines, are apparent.

Troublesome: Most (but not all) TCs are troublesome for students. Concepts can be troublesome for a number of reasons, as described by Perkins (1999). Troublesome knowledge therefore falls into a number of different categories, including ritual knowledge (routine and therefore meaningless), inert knowledge (used only when specifically called on), conceptually difficult knowledge (complex and/or different from personal experience of the world), alien knowledge (conflicts with personal beliefs), tacit knowledge (understanding implicit and therefore often overlooked), and troublesome language (discipline-specific usage of terms; summarized by Meyer and Land, 2006). It is also important to remember, however, that although TCs tend to be troublesome, not all "troublesome knowledge" has a TC at its source.

Bounded: Bounded is a fifth category that has been used by educators in some disciplines to identify TCs. This slippery term refers to the fact that TCs sometimes act to define the academic territory of a discipline. The characteristic "bounded" was not used in this study, primarily because biochemistry is by definition interdisciplinary and therefore all concepts in biochemistry lie at the boundaries of academic disciplines (primarily chemistry and biology).

According to Ross and colleagues, who have studied TCs in biology, "we need to emphasize that while academics and teachers identify the content knowledge as troublesome or problematic, the threshold concepts which underlie the problematic difficult content knowledge *receive the least attention* in teaching" (Ross *et al.*, 2010, p. 170). Therefore, the primary purpose in identifying TCs for a discipline is to provide a starting point for focused curricular redesign, because an intentional approach to teaching TCs is likely to result in the greatest improvement in student learning (Entwistle, 2008; Perkins, 2008). Owing to the importance of TCs in mastery of a discipline, it is reasonable to expect that, if more time were spent developing student understanding of them, the learning of additional core concepts would happen more readily, deepening student understanding of the discipline. Land and colleagues suggest that TCs "be viewed as the 'jewels in the curriculum' insomuch as they can serve to identify crucial points in the curriculum that provide opportunities for students to gain important conceptual understandings" (Land *et al.*, 2006, p. 198).

TCs research is an iterative process involving disciplinary experts, students, and education experts. According to Cousin, research into TCs "offers an analytical framework for bringing into view conceptual and/or affective difficulties in the disciplines" (Cousin, 2009, p. 201). TCs research differs from other modes of qualitative research in that it places disciplinary experts at the center of the research endeavor. Furthermore, this approach places teachers and students in dialogue with the ultimate goal of improving teaching and learning. Therefore, according to Cousin, "research and pedagogy overlap" in this process of transactional curriculum research (Cousin, 2008, p. 269).

Since the term was coined in 2002, TCs have been the subject of study across all major branches of higher education, with specific studies, for example, in biology (Taylor, 2008; Kinchin, 2010; Ross *et al.*, 2010) and the health sciences (Clouder, 2005), including dentistry (Kinchin *et al.*, 2011), occupational therapy (Rodger and Turpin, 2011), and nursing (Stacey and Stickley, 2012). Work has begun on identifying TCs in chemistry (Park and Light, 2009). As yet, however, TCs essential to biochemistry have not been characterized.

Work on TCs in biology provides a starting point for thinking about possible TCs in biochemistry. For example, submicroscopic events, energy transformations, and the ability to think correctly about scale are biology TCs that may also be relevant for biochemistry. Additionally, as part of their work to create the Biology Concept Inventory, Garvin-Doxas and Klymkowsky identified the role of randomness in processes such as diffusion and evolution as particularly troublesome for students (Garvin-Doxas and Klymkowsky, 2008). They suggest that a deep understanding of the role of emergent processes in biology and chemistry is fundamental and that, once students understand these processes, their view of the discipline changes dramatically. Investigation of these concepts in a biochemistry context may indicate that proper understanding of them is also transformational for students learning biochemistry.

In this study, we investigated two major research questions: 1) What potential TCs for biochemistry emerge from discussions with groups of faculty and students? 2) What knowledge statements related to each TC can be generated using an iterative process that considers both expert and student perspectives? Using TCs research in other disciplines as a starting point, we undertook a five-phase, national effort to engage faculty members and undergraduates in a process to identify and refine TCs for biochemistry. This study is part of a larger, systematic approach to improve student learning in undergraduate biochemistry courses nationwide through development of instructional and assessment materials focused on TCs. Therefore, the goal was not to produce an exhaustive list of TCs for biochemistry, but rather to identify a manageable number to move forward into the curriculum-development phase of the project. Finally, this study can act as a model for others wishing to investigate TCs in their area of interest.

METHODS

Project Context

This work is part of a National Science Foundation (NSF)-funded project to improve student understanding of TCs in biochemistry so instructors can maximize the impact of classroom instruction on student success. Because biochemistry is an interdisciplinary field taught in biology, chemistry, and medical departments, the project involves a diverse national community of experts to 1) identify and confirm TCs that are critical for learning in biochemistry, 2) develop assessment instruments to measure student achievement related to identified TCs, 3) design classroom activities to target TCs, 4) support a community of innovative biochemistry educators, and 5) disseminate classroom activities and assessment tools. The process for identifying TCs, Chapter 12: Transactional Curriculum Inquiry: Researching Threshold Concepts described below, is based on work by Cousin and others and involves input from both faculty experts and students (Cousin, 2009, p. 201–212).

Faculty Participants

More than 70 faculty members were involved in this process, primarily as participants in one or two of three workshops held in the Summer of 2013. The first was a 2-d interdisciplinary life sciences workshop held at the University of Minnesota in June 2013. Twenty faculty members with significant teaching experience and diverse expertise in the molecular life sciences attended. Some attendees were textbook authors and/or education researchers, and several had been involved in the ASBMB and MLSCI projects described above. This workshop was cofacilitated by four of the authors. The second was a 3.5-d biochemistry core collaborators workshop held at Seattle University in August 2013. Nineteen faculty members with biochemistry teaching experience attended. This workshop was cofacilitated by the two authors who are biochemists (J.L. and V.M.). The third was a 2.5-h dissemination workshop to introduce faculty to the idea of TCs and to solicit further feedback on potential biochemistry TCs, held as part of the ASBMB Symposium on Student-Centered Education in the Molecular Life Sciences at Seattle University in August 2013. The 38 attendees included high school teachers and faculty from a variety of colleges and universities. This workshop was cofacilitated by the two authors who are biochemists (J.L. and V.M.).

Ensuring diverse participation was a priority, especially for the interdisciplinary life sciences and biochemistry core collaborators workshops, where substantial work of the project was accomplished. Participation at these two workshops was by invitation, and attendees were recruited from a variety of institution types and departments. Faculty members from small colleges comprised the largest percentage of participants (44%), but faculty members from large research universities were also well represented (33%). Faculty members from master's-level universities (15%) and 2-yr colleges (8%) comprised the remainder of the participants. Gender distribution was reasonable, with 36% of participants being men and 64% being women. An effort was made to promote ethnic diversity among workshop participants and to include members from minority-serving institutions. Of the 13 faculty members representing these diversity characteristics who were invited, however, only four were able to attend either of the workshops. Diversity statistics were not compiled for the dissemination workshop, because it was organized in conjunction with an ASBMB event.

Student Interviews

Focus group interviews probing student understanding of potential TCs were conducted at five geographically dispersed institutions in Spring semester and Fall semester 2013. Two institutions were private, and three were public. One institution was a doctoral/research university, three were master's-granting universities, and one was a 2-yr college. Of the five participating institutions, two were minority-serving (one Hispanic-serving university and one historically black university). Students at four of the five interview sites were enrolled in biochemistry at the time of the interview or had recently completed a junior/senior undergraduate biochemistry course. Students at the remaining institution were enrolled in organic chemistry. We opted to include one group of students who had not yet enrolled in biochemistry based on a suggestion by two community college instructors who attended the interdisciplinary life sciences workshop. They asserted, and we agreed, that it is important to investigate student understanding of these concepts in prerequisite courses. This particular group of organic chemistry students was chosen because their instructor teaches a number of organic chemistry concepts using a biochemistry context. In compliance with human subjects protocols approved by all participating institutions, no further demographic data were collected about students.

A total of nine 1-h and three half-hour focus groups were held. Three to eight students participated in each interview, for a total of 56 students (10 in phase 1 and 46 in phase 4). Interviews at four of the five institutions were conducted in pairs by four researchers (J.L., D.G., S.L., and V.M.). Interviews at one institution were conducted by a single researcher (J.L.). The interviews followed a semistructured format (see the Supplemental Material) and were recorded and transcribed. Thematic analysis was performed on interview recordings using the following process. Immediately following interviews at each institution, the pairs of researchers who conducted the interviews debriefed on the experience and discussed themes that arose. Throughout the semester that interviews were being conducted, all four researchers (two biochemistry educators, a faculty developer with significant experience analyzing interview data, and a biochemistry student) met on an ongoing basis and engaged in iterative cycles of discussion related to major and minor themes. One researcher acted as the lead coder, listening to and taking notes on all interviews. As the semester continued, researchers also read the notes generated by the lead coder. The faculty developer and student played important roles in asking clarifying questions, especially those related to participant affect and to ways in which students may have interpreted questions differently than expected, as a consensus emerged through discussion. No major disagreements arose.

A summary of findings with illustrative quotes from the interviews is presented in the results section of this document. All data presented are labeled with both a focus group number and a letter designating different individuals within a focus group. For example, Student G15-A is one student (Student A) from focus group 15 (G15).

Table 2. Summary of project phases

Phase	Participants	Goal(s)	Outcome(s)
1: Pilot student focus group interviews	10 students from one institution	Test focus group protocol on a small scale Investigate student understanding of a potential threshold concept (equilibrium)	Refined approach to writing semistructured focus group questions Insights about how visual representations can present barriers to student comprehension
2: Interdisciplinary life sciences workshop	20 experts in biology, biochemistry, and chemistry education	Generate a draft list of threshold concepts for biochemistry	List of provisional threshold concepts
3: Biochemistry core collaborators workshop[a] and dissemination workshop[b]	19 biochemistry teaching experts[a] 38 high school, college, and university educators in the molecular life sciences[b]	Revise draft list of threshold concepts for biochemistry Begin planning for student interviews	Refined list of provisional threshold concepts Draft protocols for student interviews
4: Student focus group interviews	46 students from five different institutions	Explore student understanding of three provisional threshold concepts (equilibrium; interactions; individual versus populations of molecules)	Insights about students' incorrect ideas related to potential threshold concepts Deeper understanding of specific concepts that pose barriers to student learning
5: Data analysis and determination of a working list of threshold concepts	Authors and biochemistry teaching experts	Produce a working list of threshold concepts using an iterative process of data analysis and feedback from experts	Working list of five threshold concepts to form the foundation for development of instructional and assessment materials

The a and b in columns 1 and 2 link workshop in column 1 to participants in column 2.

RESULTS

Our process for identifying TCs was composed of five phases (Table 2). The process and outcomes for each phase are described in considerable detail in the following sections. Our process warrants a rich description for two reasons. First, TCs research relies on dialogue among participating experts and students (Cousin, 2008), and the research process is therefore inseparable from the results. Second, a more detailed explanation of this complex process enables others to replicate it, so as to identify TCs in their own disciplines or to further explore additional TCs in biochemistry.

Phase 1: Pilot Student Focus Group Interviews

Before faculty workshops in Summer 2013, two focus group interviews were conducted with 10 students from one institution. All students were enrolled in biochemistry or had recently completed a biochemistry course at the time of the interviews. The purpose of these interviews was twofold. First we wanted to pilot focus group interview protocols on a small scale before undertaking large-scale interviews. More importantly, we aimed to explore student understanding of a potential TC before conducting faculty workshops. Starting the TCs research process by talking with students instead of faculty is not the approach that has been typically used in other disciplines (G. Cousin, personal communication). However, because our perspective is informed by our student-centered teaching practices, using student responses as formative feedback to guide our curriculum design and research process is natural to us. Reflecting on our process, we realize that starting the dialogue with students allowed us to keep conversations with faculty infused with a student perspective. This is important, because the irreversible nature of threshold concepts means that experts often forget how the discipline appeared to them when they were students (Meyer and Land, 2006).

The concept investigated in pilot focus groups was equilibrium, because it had already been identified as a TC in biology (Ross *et al.*, 2010) and was therefore also a strong candidate TC for biochemistry. Questions centered around examination of two figures: 1) a schematic showing steroid binding to a receptor and subsequent activation of transcription and 2) chemical reactions showing multiple linked equilibria (see the Supplemental Material). Analysis of student responses revealed two useful generalizations. First, students were confronted with the fact that biochemical schematics often obscure underlying chemical principles. Specifically, the steroid image seemed to communicate that steroid binding and transcriptional activation are unidirectional events that occur in simple sequential manner. Some students recognized that many of the processes depicted could be reversible, but others struggled to recognize that relative concentrations of molecules determine the direction of pathway flux at any given moment. Consultation with expert biochemists revealed that although these details are not explicitly depicted in schematic images, they are "seen" by experts who rely on tacit knowledge of cellular conditions and chemical principles. Second, we observed that all students struggled with the second scenario, in which multiple connected reactions were presented.

Table 3. Threshold concepts resulting from project phases

Phase 2	Phase 3	Phases 4 and 5
Individual versus populations of molecules	Individual versus populations of molecules	Steady state
Energy transfer and transformation	Energy transfer and transformation	Biochemical pathway dynamics and regulation
Reversibility of processes/reactions (equilibrium)	Equilibrium, including ideas related to steady state, open systems, reversibility of processes, and dynamic processes	Physical basis of interactions
Steady-state and open systems	Intermolecular and intramolecular interactions	Thermodynamics of macromolecular structure formation
Intermolecular and intramolecular interactions (how molecules interact)	Spatial/scale relationships	Free energy
Spatial/scale relationships	Entropy/enthalpy/free energy	
Entropy/enthalpy/free energy	pH, pK_a, and charge	
pH, pK_a, and charge	Biochemical visualization	
Moving between different structural representations of molecules	New concept added in phase 3: regulation	
Reading and understanding chemical formulas and structures		
Interpreting graphs		
Mental models and text representations		
Biochemical visualization		
Probabilistic thinking		
Dynamic/fluctuating processes		
Randomness versus directedness		

Reflection on our part led to the conclusion that we were not able to effectively construct a meaningful dialogue with students regarding these questions. Consequently, we recognized the importance of trying to anticipate what might sidetrack or confuse students when writing semistructured interview protocols for focus groups. Finally, data collected from these interviews were used to inform the faculty workshops described in the sections on phases 2 and 3. Specifically, we recognized the importance of using exploration of images as a means to investigate student and expert understanding of a concept.

Phase 2: Interdisciplinary Life Sciences Workshop

The goal of the interdisciplinary life sciences workshop was to draw on the expertise of a diverse group of educators to develop a draft list of TCs for biochemistry. Workshop sessions were designed to provide enough structure to ensure productivity but were left sufficiently open to allow for creative contributions. Facilitators were careful not to influence the conversation with preconceived ideas, which position was supported by the presence of a non-scientist on the facilitation team, and made an earnest effort to allow every voice to be heard. Over the 2 d of the workshop, a draft list of 15 TCs (Table 3, first column) was generated through the four major activities described in the following sections.

Introduction to TCs. Participants were introduced to the idea of TCs by one of the authors (not a biochemist, but a faculty developer, D.G.) through wrestling with "signification," a known TC from literary studies (Meyer and Land, 2003). Through this activity, faculty members experienced the idea of TCs from a student perspective, because none of them had expert understanding of the term "signification." This term subsequently became a useful reference point for clarifying participants' definitions of terms and phrases.

Identification of TCs Using the Defined Characteristics. Participants brainstormed "troublesome" concepts in biochemistry individually and then discussed and refined their ideas in small groups. After reporting out small-group ideas and engaging in a whole-group discussion, individuals chose their top 10. The workshop facilitators compiled the individual top 10 lists over lunch, producing a list of 32 troublesome concepts. Analysis of the list of 32 troublesome concepts revealed a subset of 10 concepts that received a large number of votes. Next, small groups focused on one further characteristic of TCs (either irreversible, integrative, or transformative) and determined whether the 10 troublesome concepts also met their new criterion. Group members then rotated to other groups using a jigsaw approach in order to share ideas on the three additional TC characteristics and receive feedback. At the end of this activity, all 10 concepts had been determined to meet all four characteristics. The decision to start with "troublesome" before refining the list was informed, as mentioned previously, by wanting to activate participants' knowledge of the student perspective (as informed by teaching experiences) at the beginning of the process.

Image Analysis. A separate activity was introduced as a way of triangulating the previous findings by approaching biochemistry from a different perspective: that of visual representation. This decision arose out of our pilot focus groups, where we discovered images presented their own barriers to student learning and comprehension. Prototypical biochemical images were identified by the authors in collaboration with expert biochemistry educators before the workshop. The identified images related to a steroid hormone signaling pathway, the citric acid cycle, a molecular view of DNA polymerization, free-energy changes associated with glycolysis, a molecular view of hemoglobin binding 2,3-bisphosphoglycerate, and a molecular view of an enzyme–substrate interaction. Participants analyzed these images in

small groups to uncover the concepts embedded within each image that are obvious to experts but may be hidden to students. Each group was also asked to identify three to seven concepts or skills that an individual would need to have mastered in order to deeply understand the image. The concepts and skills identified were compiled and analyzed to determine whether they met all definitions for TCs. At the end of this process, 12 concepts had been determined to be potential TCs. Some of these overlapped with concepts identified in the above-described process, but others were new.

"Grain-Size" Activity. A combined list of concepts from the two above-mentioned activities was evaluated for "grain size." Participants were asked to determine whether the concepts were too broad, too narrow, or an appropriate size to be the subject of new instructional and assessment tools. Fifteen concepts were determined to be "just right." These are given in Table 3, column 1.

Phase 3: Biochemistry Core Collaborators Workshop and Dissemination Workshop

The major goal of both the biochemistry core collaborators workshop and the dissemination workshop was to investigate and refine the TCs list generated at the first workshop using the lens of biochemistry classroom teaching experience. Unlike attendees at the interdisciplinary life sciences workshop in phase 2, the primary teaching responsibility of the majority of participants at these workshops was biochemistry.

After one and a half days devoted to the following activities at the biochemistry core collaborators workshop, a refined list of nine potential TCs (Table 3, second column) was produced.

Introduction to TCs. Participants were introduced to the idea of TCs through wrestling with the concept of "signification" (as explained earlier).

Refinement of TCs Using the Defined Characteristics. Participants performed deep analysis of the draft TCs list to determine whether the items met all of the criteria of TCs (troublesome, transformative, integrative, irreversible).

Analysis of Additional TCs. Participants were given the opportunity to identify additional TC candidates. Small groups worked to determine whether these newly proposed TCs met all four criteria. At this point, regulation was added as a TC.

Ranking of Potential TCs. The process for choosing the final set of TCs focused on meeting three conditions: 1) Concepts are TCs for biochemistry. 2) Concepts relate to several foundational concepts in biochemistry. 3) Authors and faculty collaborators could envision developing effective assessment and classroom materials. Furthermore, because our ultimate goal in identifying TCs is to develop instructional and assessment tools, we needed a mechanism to prioritize the draft list of concepts in order to identify the three to five concepts to move forward into curriculum development. Participants therefore voted for their top five concepts in terms of perception of utility within their own teaching. Five concepts clearly rose to the top (given here in rank order): equilibrium, inter- and intramolecular interactions, pH and pK_a, regulation, and visualization.

To gain a broader perspective, participants at the much shorter dissemination workshop were also asked to perform the same ranking. After an orientation to the characteristics of TCs and an opportunity to comment on the existing list, the top five TCs from this workshop were (given in rank order) equilibrium, entropy/enthalpy/free energy, energy transfer, individual versus populations of molecules, and inter- and intramolecular interactions.

Phase 4: Student Focus Group Interviews

TC research relies on input from experts and students. Therefore, student focus group interviews were conducted in order to determine 1) whether concepts identified by faculty are indeed troublesome for students and 2) what, specifically, students think about each of the concepts. Findings related to the second point were used to refine the TCs and to define knowledge statements associated with each concept.

The first step in preparing for the focus groups was to determine which concepts to further explore with students, since nine was too many given the scope and goals of our project. Student understanding of candidate concepts was garnered from a variety of sources including 1) preliminary interviews; 2) the literature on learning in chemistry, biology, and biochemistry; 3) analysis of MLSCI data (T. Wright, personal communication); and 4) personal teaching experience. All of these factors were considered when deciding which concepts to explore using focus groups. The two authors who are biochemists (J.L. and V.M.) decided that equilibrium, intra- and intermolecular interactions, and individual versus populations of molecules would be the topics discussed in student focus groups. Interview protocols were developed based on known student alternate conceptions related to these concepts and on expert understanding of these concepts received from six participants in the interdisciplinary life sciences workshop (see the Supplemental Material).

As described in *Methods*, focus group interviews were conducted at five diverse institutions with a total of 46 students. Students from four institutions were enrolled in biochemistry at the time of the interviews, and students from one institution were enrolled in organic chemistry. As expected, wide variability in student understanding was observed, but a number of commonalities were also noted. Quotations are given to support key ideas summarized below when possible. Owing to the fact that focus group interviews led to dialogue among participants, it was sometimes challenging to capture brief snapshots of the conversations to use as examples.

In general, students tended to rely on contexts and concepts that were the focus of course work at the time of the interview—perhaps a matter of overzealous transfer (Schwartz *et al.*, 2012). For example, one class was just starting discussion of enzyme kinetics and therefore used enzyme kinetics as a context for many of their responses. Another group was in the midst of studying glycolysis and therefore discussed pathway flux and the role of rate-limiting steps in metabolism, concepts that students at other institutions did not mention. Yet despite the variety of perspectives, a number of common trends emerged. All groups discussed the complexity of biochemistry compared with systems studied in general and organic chemistry. Related to this, some students implied that the rules of chemistry used in previous course work are different or do not apply to chemistry in biological systems. Students also commented on the depth of understanding they needed to succeed in biochemistry; they acknowledged that memorization does not suffice in studying

biochemistry. Finally, students described the difficulties they encountered when moving from the molecular visualizations used in general and organic chemistry to new, often much more abstract, representations of biological macromolecules. Common incorrect or incomplete ideas related to each of the three targeted concepts are described in greater detail below.

Equilibrium. A major incorrect idea related to equilibrium was that biological systems are at equilibrium. This idea seemed to stem largely from an everyday use of the term *equilibrium* to mean "balanced" or "just right." When asked what comes to mind when they hear the term equilibrium, one student (Student G28-A) said the following: "Everything is happy, yeah that's the easiest way to say it." When asked whether reactions in the body are at equilibrium, a student in another focus group (Student G30-A) described equilibrium as meaning "normal": "I think it depends, you know, if you are sick . . . or if . . . I mean, like your body should be normal at equilibrium." Students also described an intuitive sense that concentrations of molecules are properly maintained within the body, and, therefore, they concluded that biological systems must be in equilibrium. An example is given here:

> Interviewer: Are reactions in the body at equilibrium?
>
> Student G13-A: I think it has to be at equilibrium. Like she said, if there's too much of something, it's gonna throw, you know, one of the systems off.

Another issue related to complexity and the ways in which biochemical systems appear to differ from systems studied in general chemistry. Students could describe the fact that multiple reactions in a pathway are required for net production of a given metabolite, but they could not reconcile net production with the idea of reactions going back and forth as they had learned when studying equilibrium in previous courses. This problem was complicated by the fact that students believed that biological reactions are in equilibrium. For example, one student (Student G21-A) discussed dissonance between prior ideas about equilibrium and net production of a substance in the body: "I think it's hard to grasp the concept of something that can be in equilibrium going back and forth, but also having to favor one side. Like, equilibrium, it has the word equal in it, so in my mind, they should be the same. So it's hard to have dynamic flow of something to favor something when they're in equilibrium. So it's just a difficult concept to grasp."

We also observed instances in which students concluded that the chemical rules related to equilibrium must apply differently, or even not apply at all to biological systems: Student G31-A said, "I think like it's not hard to understand equilibrium, but there's a key difference when you're looking at, like when you're studying equilibrium in gen chem or analytical chem versus in biochem." Finally, in cases in which students lacked a foundational understanding of chemical equilibrium, equilibrium constants, and Le Chatelier's principle, they were unable to apply these ideas to biochemistry.

Intra- and Intermolecular Interactions. Focus group interviews revealed that students had superficial, memorized, or incorrect understanding of the physical basis of noncovalent interactions such as hydrogen bonds, dipole–dipole interactions, and van der Waals interactions. Students could name the interactions, and some could discuss the role of polarizable electron clouds in these interactions, but they struggled to make generalizations about the electrostatic basis of the interactions. For example, when asked to list intermolecular interactions, several students in one focus group named van der Waals interactions, but when asked to explain the basis for van der Waals interactions, students struggled, as illustrated here:

> Student G14-A: Those are the ones that come in close contact with each other. They don't necessarily bond in a sense. They interact closely. I can show you a picture of that too.
>
> Interviewer: What is the basis of their interaction, why are they interacting?
>
> Student G14-A: Close proximity.
>
> Interviewer: Close proximity?
>
> Student G14-B: But what attracts them?
>
> Student G14-C: I think it's electronegativity. I don't want to . . . I think I might be wrong.

In another example, students again named van der Waals interactions as a noncovalent interaction, but then were unable to provide any detail about the physical basis of these interactions or how they would appear in biochemistry:

> Student G16-A: I'd just say like in my classes—I don't know about what you all had—but we never really talked that much about van der Waals interactions. Other than the fact that they said, you know, everybody has them and they keep it from floating away.
>
> Student G16-B: Yeah, I think I heard at one point in time that it just adds to stabilization, to, well, especially to protein structure. Yeah, it's not really touched on.

Students also did not feel confident in making predictions about interactions in complex macromolecules. For example, when shown an image of a protein, one student named two different types of interactions, but when asked for specifics, he was uncertain:

> Student G14-D: I said there was hydrogen, well I guess not, never mind. I said ionic interactions on the protein surface.
>
> Interviewer: Which part of the protein surface?
>
> Student G14-D: I'm not sure. I guess anywhere on the surface.

In a different focus group, one student (Student G20-A) noted that molecules are much more complex in biochemistry as compared with those studied in prerequisite courses: "So in gen chem it is just thinking about molecules separately, not the overall picture of, like, if we kept folding this in on itself again and again." Furthermore, when we did hear students making the statement that hydrophobic amino acid residues move to the inside of folded proteins and hydrophilic residues remain on the outside, no further explanation for this phenomenon could be produced. When asked to explain, for example, students claimed that hydrophobic groups *want* to be on the inside of the protein, erroneously attributing intentionality to proteins: Student G20-B said, "So polar things want to interact with polar things and the nonpolar things don't want to interact with the polar so they are going to hide somewhere else." The fact that student understanding of the enthalpic and entropic factors affecting an

event like protein folding was limited to what appeared to be memorized statements strongly suggests that more needs to be done to develop deep conceptual understanding of the energetics that drive interactions and folding of macromolecules in an aqueous environment.

Individual versus Populations of Molecules. Examination of student responses related to the concept of individual versus populations of molecules revealed no uniform TC. Students acknowledged that they had not previously considered the term "population" in a biochemical context. For example, during one focus group, students discussed the fact that they had never previously considered that there is more than one molecule of hemoglobin in each red blood cell:

> Student G29-A: I was unaware that there were so many hemoglobin molecules in a red blood cell. I wasn't aware of that for the longest time until I got to this class.
>
> Student G29-B: Did you think there is just one?
>
> Student G29-A: I thought there was only a certain number of hemoglobin molecules per red blood cell. [*Whispers*] But there's millions.
>
> Student G29-B: I'm learning this right now [as we speak].
>
> Student G29-C: Honestly, when I thought of a red blood cell, I saw pictures of how big they were—but I honestly, I thought, mechanism-wise, I thought it was one hemoglobin [until this conversation].
>
> Student G29-A: Yeah, 'cause I was reading about sickle cell anemia and how they can form these polymerizing rods of hemoglobin and so I thought, there must be lots. So I looked it up on Google. But no one ever specifically said, like, there's a lot of hemoglobin, this is just one small chemical.
>
> Student G29-B: I can see—if you think about—hemoglobin is this, like, protein—a proteinous thing. And proteins are small compared to a cell. So it makes sense, but I just never made that association before.

Furthermore, students sometimes struggled to move back and forth between representations of individual molecules and measurable properties arising from average characteristics of a population of molecules. However, while we recognized that these ideas are transformative for students, we found that understanding these concepts, once presented, did not seem particularly troublesome or integrative for students. Instead of retaining this concept as a TC, we decided to add explicit treatment of individual versus populations of molecules as a guiding principle in our future instructional materials design process.

Phase 5: Data Analysis and Determination of a Working List of Threshold Concepts

Ideas collected from disciplinary experts and students were compiled and analyzed in order to identify the specific concepts that are troublesome for students and to discern how deep understanding of these concepts leads to a transformed and integrated understanding of biochemistry. Using an iterative analytic process, described in greater detail below, the authors worked in collaboration with project advisory board members and participants from the interdisciplinary life sciences workshop to produce a detailed description of five TCs that will be used as foundation for future development of instructional and assessment materials.

Careful analysis of all student focus group data was essential in developing the finalized list of TCs. Interestingly, the three concepts used for the interviews (equilibrium, intra- and intermolecular interactions, and individual versus populations of molecules) appear to be significantly different from the finalized list given in Table 4. Yet all of the ideas described in Table 4 originated from analysis of student conceptions and comparison of student perspectives with those of experts. Equilibrium serves as a good illustration. Throughout the faculty workshops, equilibrium appeared as a relevant concept, but the term elicited different meanings for different people. Some thought of equilibrium constants and Le Chatelier's principle, others thought of dynamic processes in general, still others focused on the fact that biological systems are not in a state of chemical equilibrium. Going into the interviews, we were interested in discovering from students what exactly about equilibrium was problematic or important. As mentioned previously, talking with students revealed several common issues. First, students recognize that biological systems are much more complex than systems they studied in general chemistry, but instead of considering how to apply equilibrium concepts to the more complex system, many concluded that the chemical "rules" must be different for biochemistry. Second, when asked whether biological systems are at equilibrium, most students relied on everyday usages of the term to mean "just right," "stable," or "balanced." As a result, they concluded that biological systems must be in equilibrium. This colloquial use of the term *equilibrium* had not been apparent to us previously, but after hearing it from students, we were able to see how the misuse of language could be the root of alternate conceptions that we had previously recognized. Finally, related to the issue of terminology, students were upfront about the fact that they did not know what "steady state" means in a biological context. When we compared these student conceptions with the expert understanding we had collected before interviews, a new vision of the TCs related to equilibrium began to emerge. Similar to students, experts indicated that the concept we had called "equilibrium" actually contained a number of different concepts, including biological steady state, reversibility of chemical and physical phenomena, and the complexity of multiple, interconnected equilibria commonly observed in biological systems. As a result, two concepts—steady state and biochemical pathway dynamics and regulation—were ultimately determined to be TCs (Table 4). A process similar to that described for equilibrium was used with other initial concepts to arrive at all of the concepts shown in Table 4.

After deciding on the five major concepts, we next sought to define the specific characteristics that make each of the five identified concepts TCs. To accomplish this task, we sought to clarify how or why understanding a given concept is troublesome, transformative, integrative, and irreversible. The first step in this process was to define knowledge statements that present a more detailed picture of each TC (Table 4). We started the process of producing knowledge statements by systematically analyzing the interview data for incorrect or incomplete ideas held by students. Such ideas were considered to be troublesome. We then generated the knowledge

Table 4. Refined threshold concepts and knowledge statements

Name	Knowledge statement(s)	Biochemical ideas that are unlocked once this concept is understood	Connections that were invisible before deep understanding of the concept
Steady state	Living organisms constitute open systems, which constantly exchange matter and energy with their surroundings, yet net concentrations remain relatively constant over time. This dynamic, yet outwardly stable condition is referred to as a *steady state*. "Steady" is not synonymous with chemically "stable." Concentrations are determined by kinetic, rather than thermodynamic, factors. Hence, biological systems do not exist in a state of chemical equilibrium. If an organism reaches chemical equilibrium, its life ceases. Consequently, organisms have evolved extensive regulatory systems for maintaining steady-state conditions.	Steady state is an emergent process that results from regulation of numerous biological reactions. Steady state is a metastable condition that can be maintained only because of constant input of energy from the environment. Steady state defines the conditions of life under which chemical reactions take place in cells and organisms. Therefore an understanding of steady state is necessary in order to correctly contextualize all of biochemistry.	Once the condition of steady state is recognized, the purpose of complex regulatory systems in maintaining steady state and their connections to each other become apparent. Once the metastable nature of steady state is recognized, the importance of multi-tiered energy storage systems (starch, glycogen, triglycerides, etc.) becomes apparent.
Biochemical pathway dynamics and regulation	Reactions and interactions in biological systems are dynamic and reversible. Directionality of processes depends on the free energy and relative concentrations of reactants and products available. Observable flux is the net result of forward and reverse processes. Enzymes control rates of forward and reverse reactions. Enzyme activity is highly regulated.	Chemical drivers result in bulk (emergent) properties observed in biological systems. Enzyme-mediated regulatory mechanisms allow pathways to be sensitive and responsive to the needs of the organism. Enzymes act as gatekeepers rather than drivers of chemical change.	Once these concepts are understood, predictions can be made about 1) how biochemical pathways are likely to respond to changes environmental conditions and 2) cause and effect of fluctuations in biochemical pathways.
The physical basis of interactions	Interactions occur because of the electrostatic properties of molecules. These properties can involve full, partial, and/or momentary charges.	Once this concept is understood, similarities between different types of interactions become clear. Although interactions are given different names, they are all based on the same electrostatic principles.	A core biochemical principle is that structure governs function. Correct understanding of noncovalent interactions is essential in integrating structure and function.
Thermodynamics of macromolecular structure formation	Interactions in biological systems almost always take place in aqueous solution. Bulk interactions in an aqueous system have an entropic component. Enthalpic and entropic contributions are responsible for biological structure.	Protein folding, the assembly of lipids into micelles and bilayers, the association of polypeptide subunits to form oligomeric proteins, base pairing of DNA and RNA molecules, and all other biological interactions are driven by a common set of thermodynamic forces. The aqueous environment of the cell plays an active and essential role in biochemical structure formation.	When the entropic and enthalpic forces that drive processes like protein folding and binding are understood, predictions can be made about the conditions under which these events will occur and what effect perturbations, like mutations will have.

statements shown in Table 4 by comparing student alternate conceptions with expert conceptions generated from a variety of sources, including the faculty workshops, the literature, textbooks, and the authors' personal knowledge. We next addressed the characteristics of transformative and integrative by asking ourselves what biochemical ideas are unlocked once the concept is understood (transformative) and what previously hidden connections are made visible once the concept is understood (integrative). These ideas (shown in the last two columns on Table 4) were produced through iterative conversations among authors and two project advisory board members. It is important to note that one of

Table 4. Continued

Name	Knowledge statement(s)	Biochemical ideas that are unlocked once this concept is understood	Connections that were invisible before deep understanding of the concept
Free energy	The tendency toward equilibrium drives biological processes. Differences in free energy drive the chemical transformations underlying biological function. By providing a direct link between a thermodynamically favorable reaction with a thermodynamically unfavorable one, enzymes enable biological systems to drive a normally unfavorable reaction by coupling it to one with a large and favorable free-energy change. Enzymes affect reaction rate, yet do not affect equilibrium position.	Biological systems use favorable processes to drive less-favorable processes, which allows for maintenance of steady state.	Once this concept is understood, the relationship among free energy, equilibrium, and steady state becomes apparent.

the authors was trained as a physical chemist and was therefore able to contribute a perspective that extended beyond biochemistry. The characteristic of "irreversible" was impossible to define given available data. Table 4 was reviewed in its entirety by two project advisory board members to determine whether the statements were correct and relevant for learning in biochemistry. Modifications were made based on their feedback.

One unanticipated result of this process was that we decided to break the intra- and intermolecular interactions concept into two distinct but related categories: the physical basis of interactions and thermodynamics of macromolecular structure formation. This decision was based on our analysis of student interviews and reflects our vision of how these concepts could be better taught in biochemistry courses. Although students should have a fundamental understanding of Coulomb's law and intermolecular interactions from general chemistry, responses given during interviews made clear that the students do not have a solid understanding of how these two concepts are related. Student interviewees were unable to make the generalization that all noncovalent interactions are based on charge–charge interactions. They were able to produce apparently memorized lists of interaction types, and some could even describe the polarization of electron clouds. However, when questioned further, they were largely unable to connect the events they had previously described to simple electrostatic interactions. The result of this superficial understanding of intermolecular forces was that they subsequently went on to describe a superficial understanding of these concepts in a biochemical context. For example, many students could list the types of interactions one might find in each level of protein structure, and they could state that hydrophobic groups go to the inside of a protein and hydrophilic to the outside. However, most were unable to identify the atoms involved in interactions in proteins. Furthermore, most students explained the hydrophilic/hydrophobic characteristics by attributing desires to molecules ("they *want* to be on the inside or outside"). On the basis of these observations, we concluded that classroom strategies aimed at understanding the electrostatic basis of interactions (enthalpic contributions) needs to precede higher-level discussions of structure that rely on enthalpic and entropic contributions. The more basic discussions should still happen in the context of biological macromolecules.

Finally, a survey was sent to all participants from the interdisciplinary life sciences workshop asking for feedback on the knowledge statements and labels we had given to each set of ideas. Nineteen of 20 participants responded. Although one respondent was concerned that none of the five proposed TCs was narrow enough to fit the definition of a concept, no other participants raised this concern, and no other major concerns were raised. The majority of respondents agreed with or were neutral regarding our choice of names for the five different TCs, and their feedback was used to further refine the names of three of the five TCs to improve clarity and focus.

DISCUSSION

As has been observed previously with biology TCs, TCs for biochemistry have conceptual connections to each other and to additional biochemical concepts and skills, resulting in a web of ideas (Ross *et al.*, 2010). For example, intermolecular interactions are involved in the vast majority of biochemical events and therefore connect many concepts in biochemistry. Likewise, free-energy changes can be characterized for any biochemical event, and understanding biochemical energetics allows one to make predictions across biochemical contexts. Because of their centrality in biochemistry, the five TCs characterized in Table 4 connect to and underlie the foundational concepts and "big ideas" described in Table 1. This integration emphasizes the central importance of the TCs in learning biochemistry and reaffirms our decision to focus future instructional materials on a small number of knowledge statements related to these concepts.

When a provisional list of TCs was circulated to workshop participants at the conclusion of phase 4, several were concerned by the focus on what they consider to be general chemistry concepts. What has become clear through this undertaking is that we, as biochemistry educators, can no longer act as though students develop deep, correct understandings of key concepts in prerequisite courses. Decades

of discipline-based education research on alternate conceptions provide ample evidence that students move through chemistry and biology programs with major gaps in understanding (for examples, see Mulford and Robinson, 2002; Shi *et al.*, 2010; Parker *et al.*, 2012). Unfortunately, these alternate conceptions are often regarded by biochemistry educators as a problem that others need to fix. We assert that this approach is not constructive and that we must develop methods that simultaneously teach new biochemistry concepts, and also help students refine and strengthen their understanding of foundational concepts. Furthermore, student interview data suggest that even when students understand concepts in prerequisite courses, they struggle to apply their understanding in new contexts. Separating scientific course work into discipline-specific silos (e.g., chemistry and biology) may act as an additional barrier to student learning of TCs.

Identification of TCs is meaningful only insofar as it is used to inform effective changes in teaching and learning. Therefore, the critical next step is to work with a community of biochemistry educators to produce instructional and assessment materials related to these concepts. For a number of reasons, we are committed to working with a large, nationwide community of biochemistry educators to accomplish this goal. First, there is historical precedent in the TC community to involve a diversity of faculty voices in the process (Cousin, 2009). Furthermore, we have had previous success in developing and disseminating assessment and teaching materials in collaboration with a community of faculty members (Murray *et al.*, 2011; Villafañe *et al.*, 2011). Finally, Vision and Change (American Association for the Advancement of Science [AAAS], 2011) and other projects have created national momentum to work collaboratively to reshape teaching and learning in the molecular life sciences. Indeed, the *Vision and Change* report demands that we engage in regular conversations resulting in a coordinated national effort to change how undergraduates learn in the life sciences.

In our work through the phases of this project, we listened to faculty ideas about the production of teaching materials related to TCs. As a result, we plan to prioritize several guiding principles to support future production of instructional materials. For example, whenever possible, classroom activities should contain components that explicitly develop the visual literacy skills needed for expert interpretation of biochemical representations. These skills were initially considered as a TC in their own right, and the case could readily be made. However, for the purposes of this project, the developing consensus is that it will be more powerful to weave the teaching of visual literacy into as many activities as possible. The literature on visualization in biochemistry will be considered in production of any teaching materials (Schönborn and Anderson, 2006, 2009, 2010; Towns *et al.*, 2012). Another guiding principle is that research skills be included whenever appropriate. These skills could include experimental design and critical interpretation of the literature. There is precedent for including experimental approaches as a cross-cutting concept in course curriculum design. For example, Rowland and colleagues placed experimental approaches at the center of their concept lens diagram to acknowledge that they underlie all knowledge in biochemistry and that developing these skills supports student understanding of the process of science, a key component of Vision and Change (AAAS, 2011; Rowland *et al.*, 2011). Finally, although the relationship between individual and populations of molecules was not determined to be a TC, an effort should be made to make explicit connections between molecular characteristics and observable macroscopic properties.

Curricular changes that support learning of TCs in biochemistry are likely to be multifaceted. Land and colleagues suggest that curricular changes that attempt to focus on TCs should be systematically reviewed to consider three related elements: 1) the sequence in which concepts are introduced and connected, 2) the process by which students recognize and internalize TCs, and 3) the ways in which students and teachers will recognize when TCs have been internalized (Land *et al.*, 2006). They go on to describe nine considerations that are important in curriculum redesign and assessment. Interestingly, their recommendations focus less on *what* is taught (although discipline-specific concepts and skills are certainly central to any curriculum redesign process) and more on *how* TCs are taught. They emphasize, among other things, the importance of listening to students, engaging them in the learning process, and teaching students to tolerate uncertainty. Furthermore, they claim the learning of TCs often involves a change in students' sense of personal identity. Therefore, a curriculum designed around TCs should actively recognize and support attitudinal changes and emotional challenges that could accompany transformational learning. For example, students could be asked to write meta-cognitive self-reflections and receive feedback on these documents from instructors.

In addition to the five concepts described here, the other provisional TCs (Table 3), as well as still-unidentified concepts, are also likely to be instrumental for learning in biochemistry. Therefore, although we are confident that the five targeted areas are indeed TCs for biochemistry, they by no means represent an exhaustive list and may not even be the five most important TCs for biochemistry. Rich opportunities remain to further refine the provisional list using the process described in this paper. A long-term goal would be to reimagine how content is organized in undergraduate biochemistry courses, such that course structure aligns with deep learning of TCs. A possible model comes from Rowland and coworkers, who rearranged their course content using concept lenses, a model that arose from the "big ideas" in the molecular life sciences (Rowland *et al.*, 2011). This model discards traditional organizational schemes for biochemistry courses, which are often based on textbook organization and content choices, and replaces them with a student-centered, concept-based structure that seeks to build deep conceptual understanding and habits of mind used by professional biochemists.

CONCLUSIONS

We have undertaken a nationwide effort to identify TCs for biochemistry with the goal of improving student learning in undergraduate biochemistry courses. Five TCs—steady state, biochemical pathway dynamics and regulation, the physical basis of interactions, thermodynamics of macromolecular structure formation, and free energy—will be the target of future work to produce instructional and assessment materials with the aim of improving learning in undergraduate biochemistry courses. The targeted TCs are integral to deep

understanding of biochemistry but also relate strongly to foundational concepts from general chemistry and biology. Our inability to fully disaggregate learning in biochemistry from foundational concepts in prerequisite courses emphasizes the importance of holistic curriculum design both within and among disciplines.

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