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Lesson

Glycolysis Can Be Fun: Rediscovering Glycolysis as a Problem-Solving Introduction to Metabolism

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Abstract

A thorough understanding of glycolysis forms a foundation for students to analyze subsequent topics in metabolism, a core competency recognized by multiple national societies for biology and biochemistry. However, when confronted with the names of over ten chemicals and enzymes, along with various energy inputs and outputs, students can regard glycolysis as a daunting memorization task. Here we describe a card sorting activity in which small groups of students work out the steps of the glycolysis pathway **before** any lectures on the topic. They examine the chemical structures of glycolytic intermediates and deduce their logical order. Subsequent analysis of the reactions and the role of cofactors and substrates is reinforced with a POGIL®-inspired worksheet. In the process, the students engage in productive discussions of topics often introduced didactically in lecture. The activity was implemented at six different institutions in small (~12 students) and large classrooms (100+ students), and can be adapted to hybrid/online formats. This highly engaging exercise has been well-received by students and instructors in various undergraduate course contexts.

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Learning Goals

Students will:

◊ explore the metabolic pathway of glycolysis by comparing chemical structures of glycolytic intermediates and deducing their logical order.

◊ understand the connection between basic organic chemistry and biochemical pathways.

◊ appreciate how metabolic pathways were deduced historically.

◊ From the Biochemistry and Molecular Biology Learning Framework

- » What is the nature of biological energy?
- » How do enzymes catalyze biological reactions?
- » How is energy of chemical processes coupled in metabolic pathways?
- » What is the scientific process?
- » What skills are needed to access, comprehend and communicate science?
- » How is homeostasis controlled?

◊ From the Cell Biology Learning Framework

- » How do cells transform energy and cycle matter?

Learning Objectives

Students will be able to:

◊ predict a logical order of glycolytic intermediates by analyzing their molecular structures.

◊ classify the chemical reactions of glycolysis.

◊ analyze the molecular structures to deduce the roles of ATP, ADP, NAD^+, and NADH in glycolysis.

◊ recognize that enzymes catalyze single chemical steps in metabolic pathways.

◊ work productively in a team to produce a metabolic map of chemical reactions.

INTRODUCTION

Scientific societies recognize the importance of metabolism to biology education. Indeed, "biochemical pathway dynamics and regulation" has been identified as one of five threshold concepts in biochemistry—a concept that transforms a learner's understanding, without which they cannot progress to expertise (1). Moreover, *Vision and Change in Undergraduate Biology Education* identifies "pathways and transformation of energy and matter in living systems" as a core concept (2), while the American Society for Biochemistry and Molecular Biology includes energy coupling in metabolic pathways as one of its foundational concepts (3). While the importance of teaching metabolism is well-established, from the learner's perspective, metabolic pathways like glycolysis can seem like random sequences of steps that require rote memorization.

Typically, students study glycolysis in lecture-style biology courses as their introduction to metabolism. The history and central role of the pathway certainly justify its place in the curriculum. However, the teaching approach often focuses on the names of metabolic intermediates rather than their structures, and students are tested at lower levels of Bloom's taxonomy (4–6). This method omits the chemical foundation of the pathway, limits deeper biochemical understanding, and can lead to student anxiety about the study of metabolism (7). This suggests a need for more effective approaches to active learning of metabolism; as DiCarlo points out, "There is too much content, not enough thinking, and too little fun in many of our courses" (8).

Even though most textbooks and associated slide decks present the structures of the glycolytic intermediates, they are not designed to promote student discovery or reflection. When students are expected to learn glycolysis without structural exploration, they memorize without understanding and soon forget (8, 9). This is unfortunate, because the logical sequence of glycolytic intermediates can be foundational for understanding fundamental biochemical concepts, such as the energetics and regulation of glycolysis and biochemical pathways which students have not yet encountered. Not surprisingly, educational research suggests the need for improved pedagogical approaches to metabolism (10). Educators have taken steps toward this by introducing research articles in upper division courses (11), computer simulations (12), and interactive methods using movable intermediates on a magnetic board (13).

Similar to the magnetic board, card sorting activities involve physical objects that students can arrange based on characteristics they identify (14). Card sorting engages visual learning skills such as observation, reflection, classification, and interpretation of patterns—like recognizing similarities and differences—that pervade science practice (14, 15). This type of activity is familiar to students from a young age; for preschoolers, "One of these things is not like the other" appears often on the Sesame Street television program. In primary school science instruction, teachers often use active learning exercises that require observation, classification, and sorting of objects. The American Chemical Society recognizes sorting as an important chemical thinking skill and provides a number of inquiry activities that lack any chemical aspects for elementary school children (16).

The importance of sorting tasks extends to higher education, where higher levels of Bloom's taxonomy may be targeted (*e.g.,* analyze, evaluate). In one example, Smith et al. explored students' conceptual expertise in biology by how they arranged topics (novice understanding) compared to that of practicing scientists (expert) (17), demonstrating that card sorting does not require a single correct answer, nor must it use images. Regardless of the task and purpose, the sorting activity requires students, whether in groups or individually, to think analytically and has elements akin to concept mapping (14, 18).

Student-led exploration of the order of metabolic structures reinforces the historical context of metabolic study and the nature and process of science. Building on the discoveries of Pasteur (19), Buchner (20), and Harden and Young (21), it still took researchers several additional decades to deduce the glycolytic pathway as the chemical structures of its metabolic intermediates became known (22–24). Structural ordering came before the analysis of most enzymes, mechanistic details, and energetic and regulatory properties of glycolysis.

Here we present a visual card sorting activity as a problem-based learning exercise intended to provide a biochemical introduction to glycolysis (25). Students, working in groups, construct the glycolytic pathway as done by early researchers who had a limited understanding of enzymes, coenzymes, and metabolic regulation, but who knew the chemical structures of the pathway intermediates.

Intended Audience

This activity is appropriate in biochemistry, biology, and molecular biology courses for undergraduate students who have had a course in organic chemistry and can be implemented in a variety of class sizes and modalities (*i.e.,* face-to-face, online, or hybrid classrooms). It also can be used in graduate level courses and faculty workshops.

We introduced the activity to 20 undergraduate science majors (16 biochemistry majors and four biology majors) in a face-to-face introductory, non-survey biochemistry course at the University of Delaware. This biochemistry course uses classic research articles instead of a textbook and is taught in a problem-based learning format (26, 27). Students in this course ranged in undergraduate educational level from second-semester sophomore through senior class standing. All students had completed at least one semester of organic chemistry, and the majority of students had no prior background in biochemistry.

Instructors at Providence College, Saint Leo University, the University of Texas at Austin, Albright College, and Saint Mary's College of California also implemented this activity in their undergraduate biochemistry courses across face-to-face, hybrid, and online settings. While the following sections outline the context in which the activity was taught at the University of Delaware, adaptations employed by instructors at other institutions in their unique classroom settings are provided in the *Teaching Discussion* section.

Required Learning Time

This activity is designed for a single 75-minute class period and includes an out-of-class follow-up worksheet (see Table 1 for the progression through the lesson with approximate timing). However, we encourage the instructor to consider featuring the activity over two class periods to give students and the instructor sufficient time to discuss and debrief. In a 50-minute class setting, the activities could be distributed evenly over two class periods. Alternatively, if instructors wish to feature this exercise as part of a two- or three-hour lab block, students could complete both the card sorting activity (Activities 1–6, Table 1) and the follow-up POGIL®-inspired worksheet (Follow-up, Table 1) in the laboratory section. We highlight several alternate timelines in the *Teaching Discussion* section of the manuscript.

Prerequisite Student Knowledge

Students are expected to have completed courses in foundational biology, general chemistry, and at least one semester of organic chemistry prior to engaging in this activity. As such, students will have likely been exposed to glycolysis in the past; however, knowledge of the glycolytic pathway is not necessary to participate in this exercise. Students should be able to write and balance chemical equations and should be familiar with bond-line notation (where carbon and hydrogen atoms are not specifically depicted) and Fischer projections (and/or chair conformations and Haworth projections) for visualizing chemical structures of the glycolytic intermediates, cofactors, and substrates. Not all students in our class had seen Fischer projections before participating in the activity, so revisiting them in lecture before launching this activity or including the respective chair conformation or Haworth projection on the back of each corresponding Fischer projection card for the six-carbon sugars may be helpful for students' learning. Students should also be able to recognize key functional groups of molecules, as well as to identify the differences between the structures of oxidized and reduced organic compounds. It may be helpful for the instructor to review how to recognize oxidized and reduced structures, as such knowledge will aid students in the placement of cofactor and substrate cards during the activity. Classification of reaction types may also be reviewed, depending on the goals of the class.

Prior to engaging in this activity, groups of students at the University of Delaware had read and discussed Pasteur's 1858 article (19), as translated by Conant (28), in which Pasteur describes how he demonstrates that living cells are responsible for the fermentation of glucose to ethanol by yeast or to lactic acid by bacteria (29). The glycolysis card sorting activity served as a transition from Pasteur's article to the Harden and Young article (21) showing that phosphate stimulates the fermentation of glucose by cell-free extracts of yeast. While it is not required for students to have completed these readings before engaging in the activity, it may be helpful for students to understand that researchers had historically worked out the glycolytic pathway in the context of fermentation; we expand on our inclusion of fermentation products in the *Prerequisite Teacher Knowledge* section.

Prerequisite Teacher Knowledge

While the instructor and any teaching assistants should be familiar with the metabolic pathways of glycolysis and fermentation (both lactic acid and alcoholic) and chemical reactions at a level typically presented in introductory chemistry and biology textbooks, we highly recommend that they try the activity themselves to appreciate the issues students may confront. We would like to draw attention to the fact that the glycolytic pathways featured in this activity end with lactate or ethanol, not pyruvate, which may differ from many textbook representations and the way most instructors were taught. Because researchers historically worked out the glycolytic pathway in the context of fermentation (and, since we taught the lesson through this historical lens at the University of Delaware, with specific emphasis on lactic acid fermentation), we chose to include lactate in our main card deck (Supporting File S2). We include ethanol in an optional card deck (Supporting File S9); both lactate and ethanol are also featured in the glycolytic pathways in our follow-up POGIL®-inspired worksheet (Supporting File S4). During a post-activity discussion, the instructor may wish to emphasize that while glycolysis technically ends with pyruvate, pyruvate can then go on to enter a number of other pathways, including lactic acid and alcoholic fermentation.

In addition, the instructor may find it helpful to review the various ways molecular structures are depicted in organic chemistry: namely, bond-line notation and Fischer projections (and/or chair conformations and Haworth projections) for this activity. We also suggest that the instructor is aware of potential conceptual difficulties that students may have during the activity, especially in connection with understanding the molecular representation of structures and making the transition from six-carbon sugars to three-carbon sugars; more information is included in Supporting File S1. We offer suggestions to address these difficulties in the *Teaching Discussion* section of this manuscript.

Additionally, the instructor should feel comfortable using technology to administer the activity prompts included in Supporting File S1. Group management skills are also needed, as the instructor will circulate among student teams throughout the exercise. We encourage the instructor to give students sufficient time to engage in productive struggle (30) before stepping in, at which point they can facilitate student curiosity and thinking while being careful not to give answers.

SCIENTIFIC TEACHING THEMES

Active Learning

Students engage in a variety of active learning activities throughout this activity, including: examining chemical structures of glycolytic intermediates, cofactors, and substrates (printed on physical cards; Supporting Files S2, S3), arranging cards of glycolytic intermediates into a logical order, drawing reaction arrows to connect adjacent intermediates, placing cofactor and substrate cards beside appropriate reaction arrows, answering and asking questions, interacting in small groups, and completing a worksheet (Supporting File S4).

The worksheet associated with this activity is modeled after a specific form of active learning called Process Oriented Guided Inquiry Learning (POGIL®). POGIL® exercises are grounded in social constructivism, where students work together in groups to share ideas and solve problems (31–33). Our Energy for Life POGIL®-inspired worksheet consists of a series of scaffolded questions to guide students through their inquiries, thus helping them construct their own understanding. Specifically, it guides students through an exploration of issues typically discussed in lectures such as coupling of reactions, coenzyme structures, and types of enzymatic reactions.

During the card sorting activity and POGIL®-inspired worksheet, the instructor and any teaching assistants are encouraged to circulate among groups of students to facilitate discussions and identify potential conceptual difficulties that they may wish to bring to everyone's attention via a whole class discussion, either between phases of the card sorting activity or after activities conclude, should time permit. The POGIL®-inspired worksheet has directed prompts in contrast to the preceding card sorting activity, where students are presented with a problem and must decide how to proceed among themselves.

Assessment

Formative assessment of students' understanding takes place at several stages throughout the activity. After students have agreed on the arrangements of the white glycolytic intermediate cards, the instructor checks each group's glycolytic pathway (Phase 1 of the card sorting activity, Table 1 and Figure 1). If time allows, instructors can conduct additional formative assessments by checking on each group after students have agreed on: (i) the identities of atoms and/or molecules gained or lost in each reaction to balance each equation (Phase 2 of the card sorting activity, Table 1 and Figure 1) and (ii) the arrangements of the colored cofactor and substrate cards (Phase 3 of the card sorting activity, Table 1 and Figure 1). Between each phase of the card sorting activity, the instructor can hold a whole class discussion to ask students to identify aspects of the compounds and/or reactions in their pathways, as well as to clarify and address potential conceptual difficulties. Additional formative feedback (34) in the forms of peer dialogue and self-assessment are expected to occur throughout the card sorting activity based on the provided prompts and tasks students must complete within their groups. We further encourage the instructor to photograph each group's card arrangements between phases of the sorting activity and/or at the end of class before cards are collected; such documentation can serve as a record for students and the instructor for future reference.

At the end of the card sorting activity, students complete additional formative assessments: they answer feedback questions on the card sorting activity both as a class (Supporting File S1, slide 8) and individually (Supporting File S5), and they work on a follow-up POGIL®-inspired worksheet (Supporting File S4). Such tasks are designed to further help students self-evaluate their learning. We did not collect or grade the worksheet responses, but instructors may collect the worksheet for credit should they wish. Suggested answers for the card sorting activity and follow-up POGIL®-inspired worksheet are provided (Supporting Files S6, S7).

The following are additional ideas for possible questions that the instructor could pose to assess student understanding of the learning goals and objectives for this activity:

- Provide the structures of two intermediates in another pathway with the intervening intermediate omitted. Ask students to draw the structure of the missing intermediate and describe the two reactions that connect it to the preceding and following intermediates, identify any missing reactants or products, and identify a cofactor requirement, if needed.
- The preceding could be simplified as multiple-choice questions with various options available for selection.
- Provide an intermediate and ask students to predict the product with different enzyme reactions. For

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example, pyruvate can be converted to a variety of other metabolites.
- Ask students to trace the fate of 1-[^{14}C]-glucose to ethanol and CO_2 in the Embden-Meyerhof glycolysis fermentation pathway.
- Ask students to trace the fate of 1-[^{14}C]-glucose to ethanol and CO_2 in the related Entner-Doudoroff glycolysis fermentation pathway.
- Ask students to trace the fate of radiolabeled inorganic phosphate ($H^{32}PO_4^{2-}$) on its way to glucose-6-phosphate.

Example questions are provided along with suggested answers in Supporting File S8.

Finally, two classes outside of the University of Delaware (namely, the University of Texas at Austin and Providence College) administered short pre- and post-tests before and immediately after the card sorting activity to examine changes in student understanding (Supporting Files S13, S14). The design, administration, and results are provided in the *Teaching Discussion* section of the manuscript.

Inclusive Teaching

This activity relies heavily on small group work, varied active learning strategies, and instructor monitoring of student participation, all of which are equitable teaching practices to promote inclusive classrooms (35). In the University of Delaware implementation, students were placed into heterogeneous discussion groups of five at the beginning of the semester that varied in terms of GPA, race/ethnicity, gender, graduation year, and pre-semester survey responses on questions probing prior biochemical knowledge and personal interests (27, 29). This method helped the instructor balance the groups to accommodate students' diverse learning preferences and needs throughout the semester (29). Students worked collaboratively in these small groups throughout the activity, which provided them with the opportunity to draw on each other's strengths, assist each other in problem solving, and build classroom community. During the activity, the instructor and teaching assistants rotated between groups to support peer discussion and, when appropriate, encouraged participation from group members in an effort to give all voices the opportunity to be heard. While groups are presented with the same series of prompts throughout this activity, discussions are led by the students, allowing them to focus on the concepts and questions they find most compelling to address. For the card sorting portion of the activity, students were encouraged to assign roles within their groups (namely, at least one scribe to manage the markers, and at least one assembler to manage the tape) and were able to rotate these roles as the activity progresses; such a practice further promotes participation and collective engagement (36). Neither the card sorting activity nor the accompanying POGIL®-inspired worksheet were graded for correctness. This grading policy encourages students to view their peers as collaborators rather than competitors and can serve to motivate students and promote learning (37).

To be mindful of individual differences in prior knowledge and levels of comfort in organic chemistry, we carefully designed the cards featuring Fischer projections of the six-carbon glycolytic intermediates to include alternative representations (namely, chair conformations and Haworth projections) when printed double-sided (Supporting File S2). Students' socioeconomic diversity was also an important consideration in our activity design. Students were not required to purchase any materials to participate in the activity; rather, the instructor provided all necessary materials (namely, print-outs of the cards and POGIL®-inspired worksheet, markers, masking or painter's tape, and cardboard-backed flip chart paper).

Finally, two zero-cost, virtual alternative formats for the card sorting activity are provided to accommodate the diverse needs of both students and instructors, to support different classroom modalities, and to provide students with accessible ways to access the cards after the activity has concluded, such as for review before an exam (Supporting Files S11, S12).

LESSON PLAN

Students are familiar with arranging words in alphabetical order or putting numbers in numerical order. This card sorting activity asks groups of students to arrange the intermediates of the glycolytic pathway, each presented by their chemical structure on a different card in a shuffled deck and **unnamed**, in a chemically sensible order. Completion of the task depends on students' basic familiarity with visual representations of molecules from organic chemistry. Alternative tasks (such as assigning cofactors and substrates to specific reactions) come after establishing the pathway. Ideally, the card sorting activity and follow-up POGIL®-inspired worksheet (Figure 1) should be conducted **before, not after**, glycolysis is formally discussed

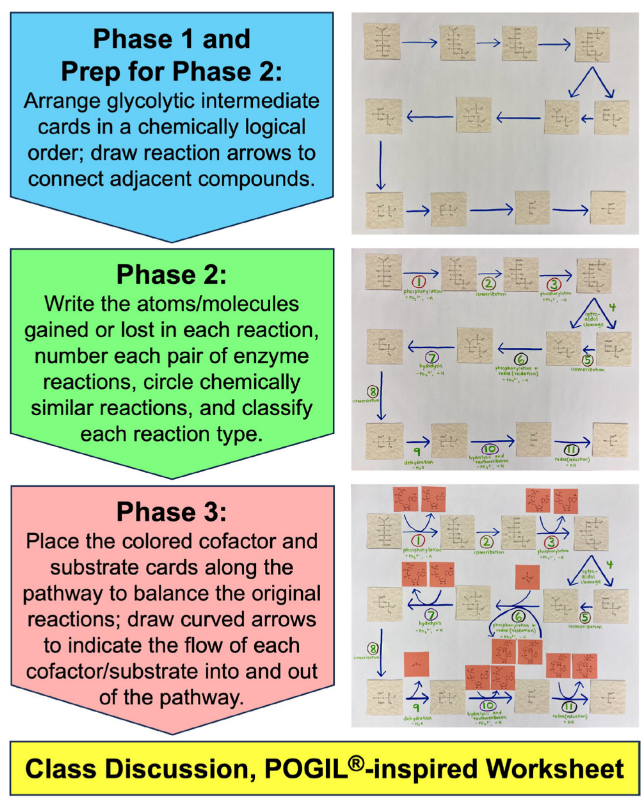

Figure 1. Overview of the activity workflow, with representative photographs corresponding to each card-sorting phase.

in class so that students focus on the chemistry as a problem, not something to bring up from memory. During the activity, instructors may provide helpful hints when groups get stalled, but should not give direct answers. Such practices are consistent with social constructivist pedagogies, in which learners create knowledge through a shared experience (33).

Pre-Class Preparation

For the first time through the activity, the instructor needs to prepare sets of cards for the card sorting portion of the activity. Each group of students (we recommend 4–5 students per group) will need one set of glycolytic intermediate cards (Supporting File S2) and one set of cofactor/substrate cards (Supporting File S3). Each card contains a molecular structure but does not include the corresponding chemical name. The reasoning behind this choice is to encourage students to use chemical structures to deduce logical placements of the intermediates, cofactors, and substrates, as opposed to relying on their memory of the glycolytic pathway.

To help the students and instructor quickly differentiate the sets of cards for this activity (and to facilitate post-activity collection of the card sets), we encourage the instructor to use white commercial cardstock or paper (8.5 in x 11 in) to print the molecular structures of the glycolytic intermediates, and to use colored commercial cardstock or paper (8.5 in x 11 in) to print the molecular structures of the cofactors and substrates. Alternatively, different colored manila file folders pre-cut to 8.5 in x 11 in dimensions could be used for printing, although the instructor should first check that such a paper type is supported by their printer.

We recommend the instructor selects the "two-sided (long-edge binding)" printing option for glycolytic intermediate cards so alternate representations of molecular structures are printed on the reverse side of the select cards. In our provided template Supporting File S2, we depict the glycolytic intermediates using Fischer projections commonly used for sugars because all atoms are represented, making it easier for students to balance atoms in the equation for each reaction. However, students may not be familiar with Fischer projections, so they may prefer other representations. We provide alternate chair conformations for glucose and glucose-6-phosphate, as well as Haworth projections for fructose-6-phosphate and fructose-1,6-bisphosphate. Alternatively, these cards can be printed single-sided, and the instructor can tape or glue the alternate structures onto the backs of the corresponding Fischer projections. In our provided template Supporting File S3, the instructor will find structures depicting cofactors (NAD^+ and NADH) and substrates (ADP, ATP, water, and inorganic phosphate). Certain structures (namely, NAD^+, NADH, ADP, and ATP) are included multiple times within Supporting File S3 to enable use in several reactions in a later stage of the activity (Phase 3, Table 1 and Figure 1). The cofactor and substrate cards should be printed single-sided.

The instructor should cut individual cards from the sheets of printed glycolytic intermediate cards and cofactor/substrate cards by following the horizontal and vertical grid lines separating each structure; scissors or a paper cutter can accomplish this task. There should be one molecular structure on each card. Cards can be laminated to extend their longevity. The instructor should note that the structures displayed on the colored cards are smaller than those on the glycolytic intermediate cards; this size difference will help ensure students have sufficient room to arrange all cards within the confines of their workspace. Once all cards are cut, the instructor should shuffle each set of cards (taking care to keep each set of white cards separate from each set of colored cards) and can use a paper clip or binder clip to keep each set of cards together. The instructor may wish to store sets of cards in Ziploc® bags to minimize chances of any cards becoming lost prior to the start of the activity.

Each group of students will need a large, flat working surface to spread out and move cards around to satisfying sequences. Tables used for group activities are ideal. Alternatively, large cardboard-backed sheets of flip chart paper (roughly 20 in x 23 in; we used Post-it® Super Sticky Tabletop Easel Pads, 3M) can be balanced among several movable chairs which have the added effect of keeping student groups close and engaged in the activity. We suggest one pad of flip chart paper per group; however, if tables in the classroom are present, each group of students can instead use a single sheet of flip chart paper (as opposed to an entire pad), as tables would provide the necessary flat surface for the activity.

The instructor will also need to provide masking or painter's tape and markers. To keep cards movable, yet held in place, small pieces of tape can be attached to each card between phases of the card sorting activity. The instructor may choose to give each group strips of tape or their own roll depending on resources. After the cards are placed in their desired positions, students will use markers to draw reaction arrows and balance equations. We recommend providing each group with at least one marker but having extras on hand to supply to groups as needed.

Finally, the instructor should print out one copy of the Energy for Life POGIL®-inspired worksheet (Supporting File S4) for each student.

Progressing Through the Activity: Class Session I

1. Introduction and Distribution of Materials for the Card Sorting Activity

The activity begins with the instructor introducing the card sorting activity as a chemical problem that students must decipher (Supporting File S1, slides 1–3). It is paramount for the instructor to take care not to reveal the activity is linked to glycolysis or metabolism, as students are to use their knowledge and chemical intuition to collaboratively deduce logical arrangements for the cards they receive. If students are told the cards relate to glycolysis, they might rely on their memory of the glycolytic pathway or look it up in a textbook or online, which compromises the goals of this activity. For this reason, we recommend the instructor prohibits the use of cell phones and any access to the Internet—along with any access to textbooks or class notes—during the activity.

As the instructor distributes supplies for the activity, students form small groups (we recommend four to five students per group), clear their desks, and assign group roles. We recommend each group selects one group member to serve as the "scribe" to manage the markers, and another member to serve as the "assembler" to manage the tape.

Each group of students should receive the following materials: one set of shuffled white glycolytic intermediate cards, at least one marker, tape, and one pad (or sheet) of large flip chart paper.

2. Phase 1 of the Card Sorting Activity

Once students have received all supplies and have assigned group roles, the instructor introduces Phase 1 of the card sorting activity (Supporting File S1, slide 4). Here, students are presented with the task of arranging the white cards in a chemically reasonable way such that there is a succession of similarly structured molecules. Before students begin, the instructor should tell the class to check the backs of each card for alternate representations of select compounds.

We recommend students spread out their cards and move them around atop their sheet of flip chart paper; using this sheet as their work surface will help students transition smoothly to the next phase of this activity. As students examine structures on their cards and work together to arrange the cards into a logical sequence, the instructor circulates between groups to identify any potential conceptual difficulties that may arise, as well as to monitor student participation and progress. Some students may begin to assemble the pathway in reverse (namely, by starting with three-carbon molecules and progressing in the direction of gluconeogenesis); if this scenario arises, the instructor may choose to give a hint regarding the number of carbons in the first and/or last structure in the pathway. Students will also likely need help transitioning from the six-carbon sugars to the three-carbon sugars, so the instructor may wish to issue a hint to each group that one of the compounds is involved in three reactions. However, as each group will be working at its own pace, we recommend that the instructor delivers these hints to each group at the appropriate time, as opposed to revealing them to the entire class at the same time.

Once each group has agreed on an order, students ask their instructor to check their work.

Suggested answers for each phase of the card sorting activity are provided for the instructor's consideration (Supporting File S6). After the instructor approves of the group's layout, the group can begin preparing for Phase 2 of the card sorting activity. Alternatively, the instructor may elect to hold a whole-class check-in at the end of each phase of the activity if they wish to keep groups in sync.

3. Preparing for Phase 2 of the Card Sorting Activity

As students begin to complete Phase 1 of the activity, the instructor displays instructions to prepare each group for Phase 2 (Supporting File S1, slide 5). Students ensure there is sufficient space surrounding each card (we recommend at least one card's length of space from all sides). They then roll pieces of tape into loops (sticky side out) to affix one side of each card (arranged in order) to their large sheet of flip chart paper. The instructor should encourage students to apply tape gently so they can readjust the location and space between their cards for subsequent tasks of the activity. Finally, students use markers to draw reaction arrows between cards to connect adjacent compounds. Students may struggle with drawing reaction arrows to connect the six-carbon sugars to the three-carbon sugars, so we recommend the instructor encourages students to ask for assistance if needed.

4. Phase 2 of the Card Sorting Activity

Phase 2 of the card sorting activity begins with the instructor orienting students' thinking towards the individual reactions within the sequence of cards they have arranged (Supporting File S1, slide 6). At this point, it is appropriate for the instructor to reveal that these individual reactions are catalyzed by enzymes, as groups of students will have likely recognized the glycolytic pathway by this stage. Within their groups, students examine each pair of compounds in the sequence, identify the chemical differences between them, and write in the atoms and/or molecules gained or lost in each reaction to make each equation balanced. Students also number each pair of enzyme reactions (starting from the first reaction and working towards the last) as #1–11 and circle the reactions that are chemically similar.

Finally, if time allows, students can discuss how to best classify each reaction type (*e.g.,* hydrolysis, redox, isomerization, phosphorylation, or retro-aldol cleavage). We recommend the instructor determines whether students have been exposed to all of the aforementioned reaction types in their organic chemistry classes to date. If not, the instructor could preface this phase of the activity with a brief overview of these reaction types, or to provide brief definitions of each class of reaction for students to match to each of their 11 reactions. Students should not spend much time on this particular task. It is more important to the goals of the activity that students can see that among the 11 reactions, there are similarities, which allows them to exercise their skills in analysis. Classifying the reactions, in contrast, is at a lower level on Bloom's taxonomy (13).

5. Phase 3 of the Card Sorting Activity

For the final phase of the card sorting activity, the instructor distributes one set of shuffled colored cofactor and substrate cards to each group and reveals the prompts for Phase 3 (Supporting File S1, slide 7). Here, students are tasked with placing the colored cards above or below the reaction arrows on their sheets to indicate where they could be used to balance the original reactions. The colored cards feature the full molecular structures of the cofactors and substrates associated with this glycolytic pathway (ATP, ADP, NAD^+, NADH, water, and inorganic phosphate). Again, we highly encourage the instructor to not yet reveal that these cards are associated with glycolysis, in the event that all student groups have not yet arrived at this conclusion.

Before students launch into this phase of the activity, the instructor tells students where to place the inorganic phosphate card so students know which of the several phosphorylation reactions does not involve ATP as the source of the phosphoryl group. Alternatively, if time allows, the instructor can engage the class in a short whole class discussion to give students the opportunity to first think about where they would place the phosphate themselves.

As students work together to distribute and appropriately place the remaining cards in the pathway and determine their chemical names, the instructor circulates among groups to supply additional tape and markers (as needed) and to identify any additional conceptual difficulties that may arise. Once groups have agreed on the placement of all colored cards, students can again tear off pieces of tape into loops to attach the cards to their sheet of flip chart paper. Students can also use their markers to draw curved arrows through the main reaction arrow to indicate the flow of these cofactors and substrates into and out of the pathway.

6. Reflection and Feedback After the Card Sorting Activity

The instructor concludes Phase 3 of the card sorting activity by regrouping the class for a whole class discussion. The instructor begins by asking the class an open-ended question which results in students identifying the pathway. Potential questions include: "What aspects of the activity were difficult, and why? What aspects of the activity did you enjoy, and why?" (Supporting File S1, slide 8). By this stage, most groups will have likely deduced this activity focuses on the glycolytic pathway, but such clarification will be helpful to ensure all students make this connection before proceeding to the follow-up POGIL®-inspired activity (Supporting File S4).

When all students in the class are aware that this activity was centered around glycolysis, the instructor can ask students what each component of the activity represented (namely, the white cards represent the glycolytic intermediates, the colored cards represent the cofactors and substrates, and each arrow connecting adjacent compounds represents an enzyme reaction). Such knowledge will help students further solidify concepts from the card sorting activity and will be useful for their follow-up POGIL®-inspired worksheet (Supporting File S4). Finally, the instructor can ask: "If you were familiar with glycolysis before participating in this activity, how did this introduction to glycolysis differ from your previous experience?" (Supporting File S1, slide 8).

Such formative feedback will be useful for the instructor to assess student understanding and, if desired, to make iterations to the activity for future classes.

Post-Activity Follow-Up

At the end of the class, the instructor distributes one Energy for Life worksheet modeled after the POGIL® approach to each student as homework for students to complete outside of class time, either individually or in collaboration with their peers (Supporting File S4). Alternatively, the instructor can distribute the Energy for Life worksheet during the following class period, where groups of students could complete the activity in class, if time allows. Suggested answers to the POGIL®-inspired worksheet are provided for the instructor's consideration (Supporting File S7).

Finally, the instructor administers additional feedback questions on the card sorting activity for students to complete individually after class (Supporting File S5). We also encourage the instructor to make digital or printed versions of the glycolytic intermediate and cofactor/substrate cards available for students to access outside of class (Supporting Files S2, S3). If the instructor identifies any conceptual difficulties remaining after the activity, the instructor should revisit these concepts in class before any subsequent assessments. We also encourage the instructor to include questions inspired by the card sorting activity and follow-up POGIL®-inspired worksheet on future homework or exams. Four possible assessment questions and answers are provided (Supporting File S8).

While the above procedure follows the preparation and set-up that was used in this study, additional modifications or adaptations can be implemented to suit the needs and goals of the instructor and students. Here we provide some alternative ways in which the activity can be administered:

- The "bookends" of the pathway (*i.e.,* first and last structures) could be given as hints to students either before or during the initial sorting phase.
- The instructor could choose to use large white boards (roughly 3′ x 2′) across desks as an alternative surface for groups of students to assemble their glycolytic pathways. Students can use white board markers to annotate reaction types between the cards.
- Mounting putty (*e.g.,* Loctite® Fun-Tak® Low Strength Synthetic Rubber Mounting Putty, 2 oz, Henkel) could be used in place of tape for securing physical cards.
- The additional structures for acetaldehyde, thiamin pyrophosphate, and ethanol could be incorporated to accommodate glycolytic fermentations (provided in Supporting File S9).
- The names of cofactors and substrates could be provided rather than the full molecular structures (or, a double-sided option with structures on one side and names on the other could be employed), depending on students' backgrounds and class learning objectives. An example for the instructor's consideration is provided in Supporting File S10.
- An additional set of double-sided cards could be prepared with enzyme names on the front and reaction types on the back, so students can think about the connections between names (dehydrogenase, isomerase, etc.) and the reactions they catalyze. An example for the instructor's consideration is provided in Supporting File S10.
- Alternative pathways beyond glycolysis could be explored, such as the citric acid cycle, the pentose phosphate pathway, and various amino acid biosynthetic and degradative pathways. This could also serve as a way to introduce additional coenzymes.
- A gallery walk (38) could be administered either between phases of the card sorting activity or at the end, where groups of students could visit other groups' stations to observe how their peers solved the same problem, share ideas, and/or provide additional feedback to the other groups.
- A virtual version of the card sorting activity could be employed instead. Two such examples are provided in Supporting Files S11 and S12.

TEACHING DISCUSSION

Activity Management

While the initial card sorting activity and a few subsequent manipulations can be completed in a single class period, the time required to finish this activity will vary for different groups and classes and depend on the periodic guidance through hints that the instructor wishes to give. Student groups will likely not be able to complete the entire sequence of prompts for the card sorting activity and the follow-up POGIL®-inspired worksheet in a single class period. However, the card sorting activity can be connected to a POGIL®-inspired homework assignment that picks up wherever the students are at the end of class, which students can complete individually or with classmates outside of class before the next class meeting, when it would be discussed (Supporting File S4). Students with prior knowledge of glycolysis may interfere with group progress because they will see pyruvate rather than lactate as the final structure. They need to be reminded that this first pass is purely a chemical exercise that should not be influenced by their expectations. Alternatively, the instructor can ask such students the assumptions they are making by claiming that pyruvate is the final structure; under what conditions such an assumption might be true; and under what conditions such an assumption might be false or incomplete.

Virtually all groups have difficulty making the transition from six-carbon sugars to three-carbon sugars. At this point, hints can be provided such as: "one intermediate is involved in three reactions," to steer students towards placing glyceraldehyde 3-phosphate at the intersection of the aldolase, triose phosphate isomerase, and glyceraldehyde 3-phosphate dehydrogenase reactions, or "is anyone familiar with an aldol reaction from organic chemistry?" It may also be useful for the instructor to remind students in general that the role of an enzyme is to accomplish only one specific task. Such a reminder may help students rearrange adjacent cards in which more than one chemical reaction would have been needed to transform one intermediate into the next.

The chemical structures of sugars can be represented in multiple ways. For this activity, we prefer the more basic Fischer projections for several reasons. First, all atoms can be explicitly shown in the Fischer projections, making it easier for students to balance atoms in each reaction equation. Use of Fischer projections also allows for the representations of chemical structures to be consistent throughout the sequence, which may additionally aid students in arranging the structures in order and determining which atoms are lost or gained in each reaction. Furthermore, with Fischer projections the activity does not require chemical knowledge. It requires logical association of similar structures (objects). Students with an organic chemistry background will recognize the more familiar pyranose and furanose representations of hexoses (which we have provided), but that may cause some students to recognize the hexoses as glycolytic pathway intermediates seen in earlier courses and revert to a memorization mode rather than engage in a systematic analysis. In addition, the transition from pyranose to furanose structures and the retro-aldol cleavage are difficult to deduce logically compared to Fischer projection representations. Another complication of the structurally more accurate cyclic structures is that they have implications for the enzyme specificity for particular anomers. The product of one reaction may require nonenzymatic anomerization to be the substrate of the next reaction. These are important issues (39, 40), but better left for future discussion or advanced courses.

While students may not finish the activity during class time and may want to complete things they have not finished, they need to be reminded that they are being asked to do in a class period what took biochemists four decades to work out—a fact that may ameliorate any frustration. It can serve as a place to mention the challenges of isolating pure metabolites from cells in sufficient quantities to determine their structures using wet chemistry techniques at a time when modern chromatographic and spectroscopic methods did not exist. Deducing the glycolytic pathway proceeded slowly as additional structures were determined. Often researchers had to guess the structures of missing intermediates. To reinforce the historical aspect of the discovery of the glycolytic pathway, it may also be helpful for the instructor to point out enzymes whose names reflect the activity that was originally identified, rather than being named after the directionality of the pathway with respect to glycolysis; pyruvate kinase—whose enzymatic name and correlating activity run counter to its role in glycolysis—is a good example of this. Through this activity, students can get a glimpse of the intellectual challenges involved and appreciate the multiple Nobel Prizes awarded along the way.

Activity Adaptations for Diverse Classroom Settings

The glycolysis card sorting activity was first presented by H.B.W. at the University of Delaware in January 2019, at an NSF grant-sponsored Biomolecular Visualization Workshop for faculty. Among the 14 participants, which included 12 life sciences faculty members from colleges and universities nationwide, three faculty members (at Providence College, Saint Leo University, and the University of Texas at Austin) adopted and modified the activity in their biochemistry courses (Table 2). Two additional faculty members who were not present at the workshop also introduced modified forms of the activity in their biochemistry courses (at Albright College and Saint Mary's College of California). Here we present how the activity was administered within each of these five instructors' classrooms beyond the University of Delaware; such accounts speak to the malleable nature of this activity to fit the diverse goals, backgrounds, and needs of instructors and students.

Common Features of the Adaptations

Student Demographics: The student populations were typically life science or chemistry majors in their junior or senior year. Most courses had a mixture of juniors and seniors who had taken at least one semester of organic chemistry. K.P.'s courses also served sophomores that had met prerequisites. Notably, one of B.B.'s courses targeted non-biochemistry majors; this particular implementation is described in detail in the *Variations of the Adaptations* section below.

Face-to-Face Classroom Settings: Several of the face-to-face adaptations (K.C. and K.P.) utilized active learning classrooms. In the A.S.G adaptations, student groups arranged their cards on 2′x2′ or 2′x3′ melamine white boards (crafted by A.S.G.), allowing groups to dynamically and reversibly add comments and reaction arrows with dry-erase markers. While no tape was used in this class setting (to prevent the tendency for students to "lock in" unfinished answers), instructors who wish to continue the activity during a second class period may find it helpful to provide magnets or tape so students can affix their cards at the end of the first class period, thus enabling students to save their progress. One of K.P.'s classrooms contained whiteboard tables, which also enabled students to draw and erase and maintain a dynamic working surface, but only during the class period.

Variations of the Adaptations

Alternative Card Sets

In the K.C., A.S., A.S.G., and B.B. modifications of the activity, cards featuring the names of cofactors and substrates rather than their molecular structures were used, along with cards featuring double-sided enzyme names and reaction types (Supporting File S10, slides 1–8, printed double-sided). The enzyme cards allow students to connect the names of enzymes to the reactions they catalyze (*e.g.,* phosphofructokinase with phosphorylation), as well as to associate enzyme names with the names of glycolytic intermediate chemical structures (*e.g.,* phosphofructokinase with fructose structures—or, if students have learned specific intermediate names, with fructose-6-phosphate and fructose-1,6-bisphosphate). Notably, instead of distributing the correct number of cofactor/substrate cards to each group, B.B. provided stacks of each card (Supporting File S10, slides 5–8, printed double-sided) on a central table, where student groups were given the additional task of determining the number of each cofactor and substrate they would need. This approach encourages students to think critically about the chemical role of each species.

B.B. additionally incorporated cards with the names of the pathway intermediates (Supporting File S15) for students to tape below each intermediate's corresponding structure. The inclusion of lactate was especially beneficial, because it prevented the students from relying solely on rote memorization of glycolysis when placing the cards.

Online and Hybrid Implementations

At the onset of the COVID-19 pandemic in 2020, A.S. modified the sorting activity to be delivered in an online format: namely, via Zoom and Google Jamboard, a collaborative digital whiteboard tool. In this virtual learning environment, students worked collaboratively in Zoom breakout rooms to sort the cards on their Jamboards; the entire class was periodically brought back together for group discussions. At the end of the activity, students shared major take home messages and identified strengths and weaknesses of sorting activities. As A.S. notes, "*The Jamboards worked great. The students were engaged while working collaboratively, from remote areas. I was able to easily transition between the boards – I used audio cues as well as left hints when they were struggling or notes of encouragement when they were on the right track. This also allowed me to confirm progress through the different phases of the activity.*" A.S. also commented on the advantage of using a digital tool that can save students' progress: "*The activity was offered over two 50-minute lectures. Another reason the Jamboards were great, the students didn't have to reassemble their progress [at the start of the second class period].*"

An implementation of the card sorting activity at the University of Texas at Austin was carried out by K.P. while classes were still held online due to the COVID-19 pandemic in 2021. Google Jamboard was again used for students to sort the cards virtually in a 50-minute discussion section held via Zoom. The shorter time period allowed for only the sorting part of the activity to be implemented, which was followed by placement of the structures on energy diagrams and discussion of the activity in the next class period (Supporting File S12). Importantly, this modularity of the online activity lent itself to ready implementation the following two semesters, when hybrid discussions were offered and students were frequently absent due to illness.

Tailoring the Activity to Emphasize Enzymatic and Metabolic Logic

The B.B. implementations at Saint Mary's College of California present an interesting strategy, because here, the instructor pre-sorted the intermediate cards (Supporting File S10, slides 1–2, printed double-sided) and taped them to large sheets of paper (roughly 20 in x 23 in; oriented in "landscape" configuration) with the Fischer projection side visible. From here, students still focused on reasoning involving the glycolytic pathway, but the focus was on enzymatic and metabolic logic instead of the chemical logic of the intermediate ordering. Descriptions of each implementation are presented below.

Targeting Enzymatic Logic: The first implementation at Saint Mary's College of California was carried out in the first semester of a two-semester biochemistry course for biochemistry and chemistry majors. Rather than use the activity to teach glycolysis, it was used as part of a unit on enzymes to give students practice recognizing enzymatic reaction types and identifying when and which cofactors will be needed. During the first class period, groups of students worked at the board to compare each intermediate to the prior one to identify what had changed chemically and predict what type of enzyme would be needed to catalyze that change. They were instructed to write their predictions on their sheets, which enabled the instructor to quickly assess their progress while walking around amongst the groups and asking them to explain the rationale for their answers. During the second class period, students first added enzyme cards to their sheets (Supporting File S10, slides 3–4, printed double-sided), followed by substrate/cofactor cards (Supporting File S10, slides 5–8, printed double-sided). Placing the cards via this staged approach provided students with insight into the logic of enzymatic nomenclature and biochemical conventions for representing cofactor inclusion with curved arrows.

Targeting the Logic of Metabolic Pathways: The second implementation at Saint Mary's College of California was carried out in a one-semester biochemistry course for biology and other non-biochemistry majors, as an introduction to glycolysis and gluconeogenesis. During the first class period, students were tasked with placing the cofactor/substrate cards on their sheets (Supporting File S10, slides 5–8, printed double-sided). In this class, rather than providing each group with the exact number of substrate/cofactor cards required, stacks of each of the cards were placed on a central table, and groups of students had to figure out how many they would need of each. This encouraged students to think critically about the chemical role of each species. Students participated in this activity immediately after a lesson on oxidation-reduction reactions; as such, groups were encouraged to calculate oxidation numbers whenever disagreement arose regarding whether or not oxidation or reduction was occurring. Moreover, discussions on redox further reinforced where NADH or NAD^+ cards were needed within the map and allowed students to discover the stoichiometric equivalence of these cofactors by acquiring and placing the NADH and NAD^+ cards in a 1:1 ratio. The inclusion of the pyruvate to lactate step led to

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a discussion about the role of fermentation to regenerate NAD^+. A key realization for the students was that the glyceraldehyde 3-phosphate dehydrogenase step involves oxidation and phosphorylation, whereas the other phosphorylation steps do not involve oxidation. This led to discussions of the distinction between phosphate groups and phosphoryl groups and the difference between kinases and phosphatases. Further details are provided for the instructor in the notes section of Supporting File S10 (see slide 9).

Assessment and Feedback

Two face-to-face implementations utilized pre- and post-tests to measure student understanding before and immediately following the card sorting activity. Both involved administering identical questions to the students at the beginning and end of the single class period in which the activity was featured.

At the University of Texas at Austin, pre- and post-test were administered online via the Canvas Learning Management System, which each student completed individually. While the pre- and post-tests consisted of the same questions, the order in which the questions were presented varied between assessments. Pre/post-test questions can be referenced in Supporting File S13, and student results from the Fall 2023 semester are presented in Figure 2.

One of the concepts K.P. had specifically emphasized during the card sorting activity (upon bringing the class together once students had completed their initial sort) was the identification of the redox reaction in glycolysis, where K.P. directed students to discuss the structural changes within the substrate after classifying that reaction. Accordingly, Q1 was a multiple choice question that asked students to select the redox reaction from three distractor reactions: alkene addition, elimination, and phosphorylation (see Supporting File S13). Analogous to the first phase of the card sorting activity, only the major organic reactant and product were presented to evaluate whether students were able to recognize the structural changes caused by the gain or

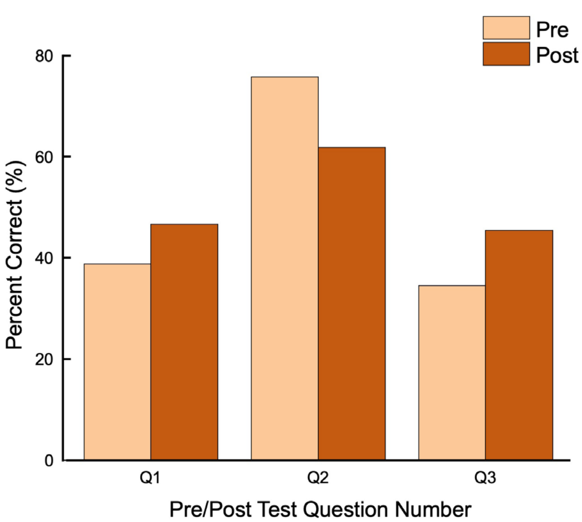

Figure 2. Percent of students answering correctly before and after the card sorting activity on pre/post-test questions at the University of Texas at Austin. Light orange bars represent each pre-test question, while dark orange bars represent each post-test question (*n* = 165). Pre/post-test questions are presented in Supporting File S13.

loss of electrons without the additional hint of the coenzyme presence. Satisfyingly, an increase in percent correct was observed on the post-test (pre = 38.8%, post = 45.5%, *n* = 165).

Mixed results were observed with Q2 and Q3, which were ordering questions designed to assess students' reasoning regarding the progression of metabolic pathways. Two structures were presented at the top, as starting and ending points for the metabolic steps students were asked to arrange. Below, three structures were presented in a random order by the online learning management system, and students dragged the remaining structures into the correct order. Some students taking the pre/post-tests on mobile devices and/or tablets verbally reported they had difficulties that caused them to drag their metabolites into an undesired order. Therefore, we recommend the instructor tests the questions on mobile devices and tablets when crafting questions of this type, and potentially encourages the use of computers for the pre/post-tests if technical difficulties are encountered.

The pathway for Q2 was derived from steps of the citric acid cycle and gluconeogenesis, beginning with oxaloacetate and ending with phosphoenolpyruvate. Thus, this pathway shares one intermediate with the glycolytic pathway. Q3 followed glycerol metabolism though dihydroxyacetone phosphate, and then ended with its conversion to 1,3-bisphosphoglycerate, sharing the final two steps with the glycolysis pathway. For Q2, the percent correct decreased from pre- to post-test (pre = 75.6%, post = 61.8%, *n* = 165), while we observed an improvement in performance for Q3 (pre = 34.5%, post = 45.5%, *n* = 165, Supporting File S13). We surmise that the number of overlapping steps with glycolysis could have contributed to the improvement observed for Q3 immediately following the card sort activity. Students may have more easily determined the penultimate intermediate given that two metabolites are shared with glycolysis. It is not completely clear why Q2 showed a decrease in performance pre to post; however, this pathway includes the FADH-promoted oxidation to form an alkene, and an analogous reaction is not found in glycolysis.

At Providence College, assessment of student learning was measured by administering a five-item multiple choice test to the students using the online assessment platform [Top Hat] immediately before the start of the activity, and then administering the identical test to the students at the end of the class period. Each student individually completed the pre- and post-tests. Pre/post-test questions can be referenced in Supporting File S14, and student results from the Fall 2023 semester are presented in Figure 3.

While student improvement was observed on all five questions, Questions 2 and 5 show the greatest increases in student performance. Q2 (pre = 27%, post = 100%, *n* = 22) asks students to identify the enzyme that catalyzes the cleavage of fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Many students tended to struggle with arranging these glycolytic intermediates in the correct order during the initial card sort; therefore, we hypothesize that this reaction may have stood out for students as a dramatic change while completing the activity, making it more memorable from the whole class discussion that followed. Q5 (pre = 9%, post = 70%, *n* = 22) asks students to identify the only redox reaction in

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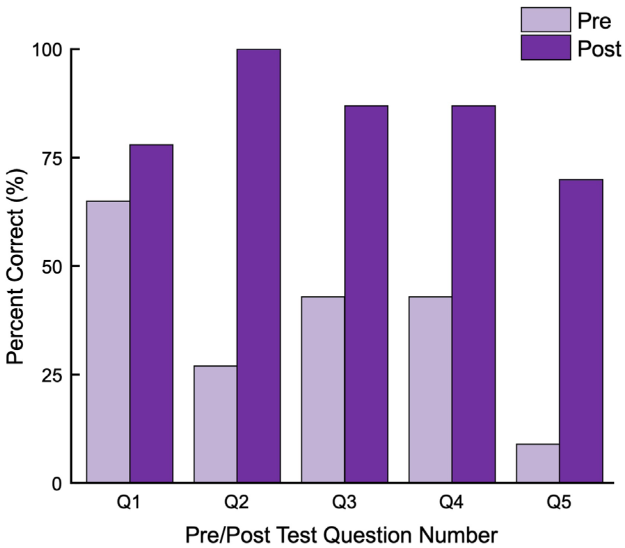

Figure 3. Percent of students answering correctly before and after the card sorting activity on pre/post-test questions at Providence College. Light purple bars represent each pre-test question, while dark purple bars represent each post-test question (*n* = 22). Pre/post-test questions are presented in Supporting File S14.

glycolysis. As this particular reaction was emphasized heavily in K.C.'s post-activity whole class discussion, we surmise that this connection may have also been memorable to the students.

We did not collect any open-ended feedback to accompany the pre- and post-test data, so we can only hypothesize about the student thought process that led to changes in performance on the pre/post-tests. An interesting follow up would be a think-aloud to further probe the thought process of the students as they approach these problems to see if they describe any changes in their reasoning after having performed the card sort.

Additional Feedback

Student Responses from the University of Delaware

- "I like that it forced us to really think through the pathway rather than just relying on memorization."
- "I liked the physicality of it and having cards in your hand and sorting them rather than writing it on paper/ looking at it on a PowerPoint. It felt like I was doing a matching game or puzzle more than an assignment."
- "I did really like how interactive it was. Everyone in the group was able to add to someone's previous thought so it just kept growing."
- "Figuring out the pathway on our own. I enjoyed this because it allowed us to think and troubleshoot which we don't get to do when we are told the answer."
- "I really enjoyed being able to think out loud and talk the process out with a team, while also being able to see it all in front of me."
- "I feel like the hints were helpful without giving too much away so we could still do the assignment ourselves."

Instructor Responses Across Institutions

- "These have been the best glycolysis lectures ever. This approach resulted in many smiles and the best collaborative engagement within the groups; the students truly functioned as cohesive teams on this. It was a beautiful thing." (A.S.)
- "I felt that quite a few students were so involved that they wanted to keep working on the problem beyond the end of the class period." (H.B.W.)
- "The scores on the multiple choice test at the conclusion of the activity were remarkably higher than the scores on the same test given at the beginning of class, even though most of my students had seen the glycolytic pathway before in their previous biology courses." (K.C.)
- "The glycolysis card sorting exercise works seamlessly with other active-learning methods and POGIL® in biochemistry classes, and can be easily modified. After the success of the exercise in the first year, I incorporated an amino acid card sorting exercise at the beginning of the semester. Card sorting is now in my go-to repertoire of active-learning activities." (A.S.G.)
- "Too often, students, especially non-majors, see molecules as words to memorize rather than chemical entities with distinct properties. This activity helped change these students' viewpoints on glycolysis and enabled them to think critically about the chemical logic behind the pathway." (B.B.)
- "One of my student groups perfectly reconstructed the pathway in reverse, starting with the three-carbon compounds. They felt like they had done something wrong, so I reassured them they were perfectly following metabolic logic, and this was an alternative solution only they had picked up on. I also told them they were extra prepared for gluconeogenesis, which we'd be covering next. I was also pleased to see how well this worked in my class of 105. After introducing the activity, many students shifted so that some were standing around the continuous surface desks while two remained seated. The groups collaborated well, working independently for the most part, so even with about 25 groups, three undergraduate assistants and I were able to effectively answer questions and check over card order." (K.P., Figure 4)

Figure 4. Implementation of the card sorting activity in a large lecture hall with continuous surfaces at the University of Texas at Austin.

Responses from Additional Biomolecular Visualization Workshop Participants

- "I did include more open ended group activities in part because [H.B.W.]'s demonstration helped me to understand that they can be very simple in design. These are working excellently in my grad class, which also appreciates them." (Rebecca Roston, University of Nebraska, Lincoln)
- "Since that workshop, I have changed how I present glycolysis based on those cards but I would not say that I do a formal activity. I don't use the cards per se, but I do show the structures in random order from each phase of glycolysis and spend a few minutes discussing their order." (Kelly Keenan, Stockton University)

Conclusion

In this novel approach to teaching glycolysis, students deduce the glycolytic pathway by applying chemical logic to visual objects through a card sorting activity. Visual literacy is essential in biochemical education (41), and this activity encourages students to operate at higher Bloom's levels to differentiate, discriminate, organize, and integrate visual information (42). The original implementation of this exercise involves arranging the intermediates of a metabolic pathway and deducing needed cofactors and substrates, followed by an accompanying POGIL®-inspired worksheet; however, adaptations can be tailored to meet the specific needs of the class, for example, to emphasize enzymatic reaction classification rather than ordering (B.B. adaptation). To underscore its modularity, we have demonstrated that this activity is transferable by highlighting its adoption across six institutions, modification for online delivery, and implementation in both small and large classes ranging from ~12 to over 100 students.

Our activity is relatively simple in design, as noted by an instructor who attended the Biomolecular Visualization Workshop in which this card sorting exercise was first introduced. It requires minimal preparation time for instructors, and students are able to approach the problem given only physical (or virtual) decks of cards with chemical structures alongside brief instructions. Despite its simplicity, the activity is engaging and effective, evidenced by positive student feedback and improved performance on post-test questions. We hope that the straightforward nature of this exercise lowers the barrier to entry for instructors wishing to introduce active learning in their classrooms.

As emphasized by Boud and Feletti, learning is initiated with a problem (43), and card sorting is adaptable to various biochemistry-related problems. We envision ready application of this approach to the deduction of other metabolic pathways. Moreover, A.S.G. describes a card sorting approach to amino acid classification, illustrating its utility in additional biochemical contexts. Therefore, we welcome instructors to use our glycolysis card sorting activity as-is, but encourage them to consider adaptations both within and outside of the context of metabolism. We are limited only by our own imaginations when encouraging creative problem solving in our students.

SUPPORTING MATERIALS

- S1. Rediscovering Glycolysis – Activity Slides with Instructor Notes
- S2. Rediscovering Glycolysis – Glycolytic Intermediate Cards
- S3. Rediscovering Glycolysis – Cofactor and Substrate Cards
- S4. Rediscovering Glycolysis – Energy for Life POGIL®-inspired Worksheet
- S5. Rediscovering Glycolysis – Card Sorting Activity Student Feedback Questions
- S6. Rediscovering Glycolysis – Card Sorting Activity Answer Key
- S7. Rediscovering Glycolysis – Energy for Life POGIL®-inspired Worksheet Answer Key
- S8. Rediscovering Glycolysis – Additional Assessment Questions and Answer Key
- S9. Rediscovering Glycolysis – Optional Glycolytic Fermentation Cards
- S10. Rediscovering Glycolysis – Alternative Cards and Answer Key
- S11. Rediscovering Glycolysis – Virtual Cards for Card Sorting Activity
- S12. Rediscovering Glycolysis – Metabolism Jamboard Activity
- S13. Rediscovering Glycolysis – UT Austin Pre/Post-Test Questions
- S14. Rediscovering Glycolysis – PC Pre/Post-Test Questions
- S15. Rediscovering Glycolysis – Glycolytic Intermediate Name Cards
- S16. Rediscovering Glycolysis – Metabolic Logic Guided Questions
- S17. Rediscovering Glycolysis – Metabolic Logic Answer Key

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- The students for engaging in the activity and answering the assessment and feedback questions.

Table 1. Rediscovering Glycolysis: Teaching timeline. Progression through the activity with approximate timestamps.

Activity	Description	Estimated Time	Notes
Preparation for Class			
Instructor preparation	1. Review activity slides and learning goals and objectives. 2. Print out (double-sided) one copy of the glycolytic intermediate cards for each group of students on white cardstock or paper; laminate if desired. 3. Print out one copy of the cofactor and substrate cards for each group of students on colored cardstock or paper; laminate if desired. 4. Print out one copy of the Energy for Life POGIL®-inspired worksheet for each student. 5. Cut, shuffle, and assemble the printed cards (one set of glycolytic intermediate cards and one set of cofactor/substrate cards per group). 6. Gather masking or painter's tape, markers (at least one marker per group), and large pads of flip chart paper (one pad per group). 7. Rearrange the classroom for the activity to ensure students have sufficient space (ideally large flat working surfaces for students to spread out cards and move them around).	60–120 min	• Activity slides are provided in Supporting File S1. • Glycolytic intermediate cards are provided in Supporting File S2. • Cofactor and substrate cards are provided in Supporting File S3. • The Energy for Life POGIL®-inspired worksheet is provided in Supporting File S4. • We recommend printing the glycolytic intermediate cards double-sided so that the alternate representations appear onto the backs of the corresponding cards. Alternatively, these cards can be printed single-sided, and the instructor can tape or glue the alternate structures onto the back. • The glycolytic intermediate cards and cofactor/substrate cards can be laminated to extend their longevity. • After cutting the cards, keep each set of cards together with a paper clip or binder clip. The instructor may wish to store sets of cards in Ziploc® bags to minimize chances of any cards becoming lost prior to the start of the activity. • Tables present in the classroom would be ideal for this activity; alternatively, a large sheet of cardboard-backed flip chart paper (*e.g.,* Post-it® Super Sticky Tabletop Easel Pads, 20″ x 23″) can be balanced among several movable desks which have the added effect of keeping student groups close and engaged in the activity.
Class Session I (75 minutes)			
1. Introduction and distribution of materials for the card sorting activity	1. Instruct students to sit with their groups or to form small groups of 4–5 students (slide 1). 2. Introduce the card sorting activity by framing the activity as a chemical problem that students must figure out (slides 2–3). 3. Students clear their desks, put away all electronic devices, and assign group roles as the instructor distributes supplies for the activity. 4. Each student group should receive: one set of the white (glycolytic intermediate) cards, at least one marker, one roll (or sufficiently long strip) of masking or painter's tape, and one pad of flip chart paper.	5 min	• Slides 1–3 are provided in Supporting File S1. • It is paramount for the instructor to not reveal the cards are linked to glycolysis or metabolism, as this could compromise the goal of the activity (namely, for groups of students to rely on chemical structures to deduce a logical order of the intermediates, as opposed to relying on their memory of the glycolytic pathway). • Suggested group roles include one scribe (to manage the markers) and one assembler (to manage the tape), but additional group roles can be assigned. • The instructor may choose to give each group strips of tape or their own roll depending on resources. • If tables in the classroom are present, each group of students can instead use a single sheet of flip chart paper (as opposed to an entire pad), as tables would provide the necessary flat surface for the activity.

Activity	Description	Estimated Time	Notes
2. Phase 1 of the card sorting activity	1. Reveal the first task for the card sorting activity (slide 4). 2. Instruct students to examine the white cards and to check the backs of each card for alternate representations. 3. Students work in small groups to place the glycolytic intermediate cards in a logical order by examining their chemical structures. 4. Students ask their instructor to check their work upon completion of this task.	20–25 min	• Slide 4 is provided in Supporting File S1. • The white cards feature the chemical structures of the glycolytic intermediates; however, the instructor should refrain from revealing that the cards are associated with glycolysis or metabolism. • The backs of some cards feature chair conformations and Haworth projections which are chemically identical but more complicated than the corresponding Fischer projection on the front of that card; some students may prefer one representation over the other. • Some students may begin to assemble the pathway in reverse (namely, by starting with three-carbon molecules and progressing in the direction of gluconeogenesis); if this scenario arises, the instructor may choose to give a hint regarding the number of carbons in the first and/or last structure in the pathway. Students may also struggle with making the transition from six-carbon sugars to three-carbon sugars, so providing them with the hint that one of the compounds is involved in three reactions may be helpful. These hints would be needed at different times for different groups. Discretion would be needed in revealing the hints to the entire class at the same time. • Students should arrange their cards atop their sheet of flip chart paper; this will help them transition smoothly to Phase 2.
3. Preparing for Phase 2 of the card sorting activity	1. Wrap up Phase 1 of the activity by displaying instructions to prepare students for Phase 2 (slide 5). 2. Students use tape to affix one side of each card (arranged in order) to their large sheet of flip chart paper. 3. Students use a marker to draw reaction arrows between cards to connect adjacent compounds.	5 min	• Slide 5 is provided in Supporting File S1. • Pieces of tape should be torn off and rolled into loops (sticky side out) to attach cards to the sheet. • Students should apply tape gently so they can readjust the location and space between cards for subsequent tasks of the card sorting activity. • Students may struggle with drawing reaction arrows to connect the six-carbon sugars to the three-carbon sugars, so encouraging them to ask for assistance if needed may be helpful.
4. Phase 2 of the card sorting activity	1. Orient student thinking towards the individual reactions within the sequence by presenting the prompts for Phase 2 of the activity (slide 6). 2. Students examine chemical differences between pairs of compounds, write in the atoms and/or molecules gained or lost in each reaction to balance each equation. 3. Students number each pair of enzyme reactions, circle the reactions that are chemically similar, and classify each reaction type.	20–25 min	• Slide 6 is provided in Supporting File S1. • Students may wish to address the provided prompts in any order they would like. • The final bullet point on slide 6 may need to be modified if students have not yet encountered all of the provided classes of reactions (*e.g.*, students may not have been exposed to aldol cleavages in their organic chemistry classes to date). Alternatively, the instructor could provide brief definitions of each class of reaction for students to match to each of their 11 reactions.

Activity	Description	Estimated Time	Notes
5. Phase 3 of the card sorting activity	1. Introduce students to Phase 3 of the activity (slide 7). 2. Distribute one set of colored (cofactor and substrate) cards to each group. Distribute additional tape and markers if needed. 3. Instruct students where to place the inorganic phosphate card. 4. Students name the structures of the remaining colored cards and distribute and tape them on their sheets where they could be used to balance the original reactions. They can also use their markers to draw curved arrows through the main reaction arrow to indicate the flow of these cofactors and substrates into and out of the pathway.	10–15 min	• Slide 7 is provided in Supporting File S1. • The colored cards feature the chemical structures of the cofactors and substrates associated with this glycolytic pathway (NAD^+, $NADH+H^+$, ADP, ATP, water, and inorganic phosphate). Again, we highly encourage the instructor to not yet reveal that these cards are associated with glycolysis. • Pieces of tape should be torn off and rolled into loops (sticky side out) to attach cards to the sheet.
6. Reflection and feedback after the card sorting activity	1. Regroup the class to have an open discussion about the activity (suggested prompts can be found on slide 8). 2. Briefly highlight the main points of the activity, as well as what each set of cards and arrows represent within the glycolytic pathway. 3. If time allows, introduce the follow-up Energy for Life POGIL®-inspired worksheet (slide 9).	10 min	• Slides 8 and 9 are provided in Supporting File S1. • Once all students in the class are aware that this activity focuses on the glycolytic pathway, the instructor should emphasize the white cards represent the glycolytic intermediates, the colored cards represent the cofactors and substrates, and each arrow connecting adjacent compounds represents an enzyme reaction. Such knowledge may help students connect concepts from the card sorting activity to their follow-up POGIL®-inspired worksheet.
Follow-Up			
Instructor completion	1. Administer feedback questions on card sorting activity after Class Session I. Review feedback. 2. Distribute one Energy for Life POGIL®-inspired worksheet to each student. 3. Assign the POGIL®-inspired worksheet as homework after Class Session I for students to complete outside of class time.	10–20 min	• The card sorting activity feedback questions are provided in Supporting File S5. • The Energy for Life POGIL®-inspired worksheet is provided in Supporting File S4. • Alternatively, the instructor can wait to distribute the Energy for Life POGIL®-inspired worksheet during the following class period, where groups of students could complete the activity in class, if time allows.
Student completion	1. Complete feedback questions on card sorting activity. 2. Complete the Energy for Life POGIL®-inspired worksheet.	45–60 min	• Students may complete the Energy of Life POGIL®-inspired worksheet individually or in collaboration with their peers.

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Table 2. Rediscovering Glycolysis: Implementation of the glycolysis card sort outside of the University of Delaware.

Instructor/Year	Institution	No. Students	Mode	Class Period	Notable Features of Adaptation
K.C. / 2019	Providence College	24	Face-to-face	50 minutes × 1 session	• Used alternative card set featuring names of enzymes, reaction types, and names of cofactors/substrates (Supporting File S10, slides 1–8)
A.S. / 2019	Saint Leo University	24	Face-to-face	50 minutes × 2 sessions	
A.S. / 2020	Saint Leo University	24–28 (2 sections)	Online	50 minutes × 2 sessions	• Delivered via collaborative whiteboard (Supporting File S12, pages 1–5)
K.P. / 2021	The University of Texas at Austin	46–75 (2 sections)	Online/ hybrid	50 minutes × 1 session	• Adapted collaborative whiteboard to include energy diagrams (Supporting File S12)
A.S.G. / 2020–22	Albright College	12–18	Face-to-face	90 minutes × 1 session	• Used alternative card set (Supporting File S10, slides 1–8)
K.P. / 2023	The University of Texas at Austin	72–105 (2 sections)	Face-to-face	90 minutes × 1 session	• Pre- and post-test administered (Supporting File S13)
K.C. / 2023	Providence College	20	Face-to-face	50 minutes × 1 session	• Pre- and post-test administered (Supporting File S14)
B.B. / 2023	Saint Mary's College of California	12–14 (2 sections)	Face-to-face	50 minutes × 2 sessions	• Used alternative card set (Supporting File S10, slides 1–8) • Used additional card set featuring intermediate names (Supporting File S15) • Used guided questions to incorporate principles of metabolic regulation, metabolic reversibility, and reactions of gluconeogenesis (Supporting Files S16, S17)

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