LEGO Biochemistry: Fatty Acid Beta-Oxidation Made Visual

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Abstract

Fatty Acid Beta-Oxidation (FABO) is often taught as part of the lipid metabolism chapter in many undergraduate biochemistry syllabi. As a spiral metabolic pathway, its number of cycles depend on the length and types of fatty acid being metabolized. For simplification purposes, textbooks usually omit certain steps involved in the metabolism of fatty acid, showing only about 6 steps for a 32 steps reaction. This greatly reduces students' cognitive clarity towards the process and its subsequent ATP calculations. To overcome such issues, an approach to teach FABO using LEGO bricks as hands-on visualization tools has been developed and implemented. Students could now follow the full progression of FABO by interacting with the palm-sized bricks, regardless of the length and types of fatty acid being studied. The approach is able to help students learn FABO and its corresponding ATP calculations with ease and further encourages the use of LEGO bricks in the visualization of other metabolic pathways in biochemistry.

Keywords

Undergraduates, Biochemistry, Visualization Tools, Hands-On Learning, Fatty Acids, Metabolism, Reactions, LEGO

Introduction

Biochemistry is an important curriculum for many life science undergraduate programs. It encompasses the study of chemical processes within living organisms that helped maintain the healthy operation of cells. Metabolism is often the core focus within a biochemistry syllabus, with key topics centered around glucose metabolism (glycolysis, gluconeogenesis, tricarboxylic acid cycle (TCA), pentose phosphate pathway), fatty acid metabolism (beta-oxidation, biosynthesis), to name but a few. The complex and overlapping nature of metabolic pathways hinder students' overall view of the processes, making it challenging for educators to teach and for students to learn metabolism effectively without the need to memorize.[1-3] Various efforts to improve the situation have been reported over the years ranging from the implementation of special teaching arrangements,[1] role-play,[4] The Polygonal Model,[5] team-based learning,[6] animation,[2] virtual reality (VR),[7] augmented reality (AR)[8] and LEGO brick modeling.[9] Together, these efforts were targeting metabolism as a whole, with selected few putting emphasis on glucose metabolism.

Challenges Involved in Teaching Fatty Acid Beta-Oxidation (FABO)

Biochemistry Textbooks

FABO is an important catabolic process nested under the lipid metabolism chapter in many biochemistry textbooks. In FABO, the number of cycles involved vary with the length and types of fatty acid being metabolized. For instance, an 18-carbon long saturated fatty acid will have to go through 8 complete Oxidation-Hydrolysis-Oxidation-Thiolysis (OHOT) cycles (32 steps in total) to be broken down into 9 Acetyl-CoA, 8 NADH and 8 $FADH_2$ to be further processed into ATP. As a result, textbooks would opt to display their fatty acids as condensed structures and have many of the steps omitted (Figure 1) for simplification and space-saving purposes. This aggravates the situation, leaving students unable to fully comprehend the connection between the missing steps and the subsequent ATP calculations.

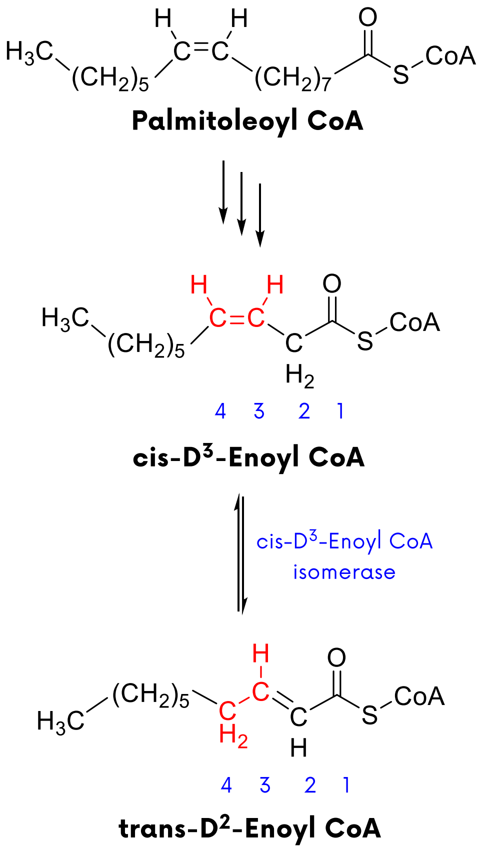


Figure 1. An example of how FABO is usually shown in textbooks.

Students’ Background

Xiamen University Malaysia offers CME319 Biochemistry as a core course for their second-year undergraduates enrolled in the Bachelor of Chemical Engineering with Honors Program. Starting from April 2023 semester, however, a subset of the Biochemistry chapters has been relocated to part of a shared course of CME112 Introduction to Biochemical Engineering due to curriculum review. Chemical engineering students who are non-life science majors in this case often do not see a direct relevance of metabolism to their degree program, let alone FABO, making it even more challenging to teach.

LEGO Bricks as Visualization Tools

LEGO bricks are interlocking toy building blocks commonly played during childhood. As each brick can be of a variation of color, shape and size, and the bricks could easily be connected and disconnected during the assembly of a larger structure, it can represent anything ranging from an atom, a molecule all the way to polymeric structures.[10] In view of its diverse array of possibilities, it has been widely utilized as teaching aids to illustrate basic chemical concepts (e.g. the periodic table of elements[11] and its trends,[12] density,[13] Octet rule,[14] Lewis structures[15]), chemical structures of larger polymers (e.g. polypropylene with different tacticity,[16] polydimethylsiloxane[17]), chemical reaction (catalysis,[16] hydrogenation of alkenes[18]) and even in the constructions of handmade instruments (e.g. spectrophotometer,[19] calorimeters,[20] polarimeter[21]) to visualize their working mechanisms. In biochemistry, however, LEGO bricks are far less used in comparison (e.g. showcase basic genetic concept and gel electrophoresis,[22] in the building of biological structures like ATP synthase involved in metabolism,[9] and in the construction of student-built fluorescence spectrometer[23]).

Although a combination of the aforementioned challenges made learning of FABO non-intuitive, the incorporation of LEGO bricks will enable clearer visualization of the steps involved through hands-on learning by the dynamic assembly/disassembly of real or virtual LEGO bricks. This in turn will counter the issue of a lack of interactivity as previously reported in the learning of chemical reactions and reaction mechanisms,[24] thereby helping students to learn better in the process. In this article, an approach to teach FABO using LEGO bricks as visualization tools will be discussed.

Integrating the Use of LEGO in Teaching FABO

When teaching FABO in CME319 and CME112 lectures, students were firstly taught the theory of FABO without the use of LEGO bricks:

Catabolism of Fatty Acid

As shown in Figure 2, a fatty acid is combined with a CoA-SH molecule to yield a high-energy Fatty Acyl CoA in its activation step. In order to transport a fatty acid from the cytosol to the mitochondrial matrix where FABO occurs, the Fatty Acyl CoA will need to be transformed to Fatty Acyl Carnitine via the use of Carnitine Acyltransferase I. When it is shuttled through the inner mitochondrial membrane, Carnitine Acyltransferase II in the matrix catalyzes the reverse reaction transforming the Fatty Acyl Carnitine back to Fatty Acyl CoA. Once in the matrix, FABO can then take place. Each cycle of FABO produces a 2-carbon unit Acetyl CoA and a fatty acid that is shorter by 2 carbons. The cycle will repeat itself until the original fatty acid is completely metabolized to units of Acetyl CoA to be further processed in TCA.

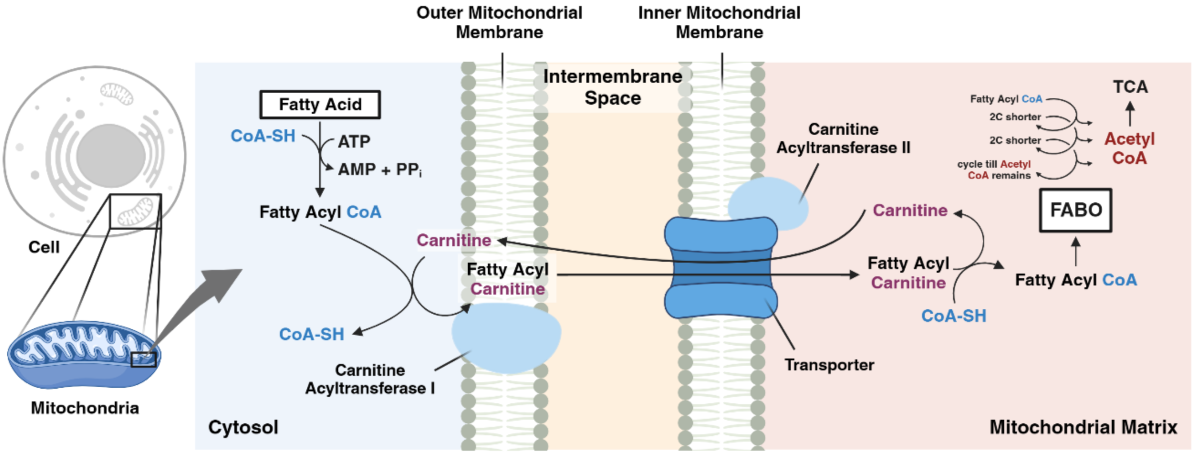


Figure 2. Overview of FABO with emphasis on the activation step (Fatty Acid to Fatty Acyl CoA) and the transfer of a Fatty Acyl CoA from the cytosol to the mitochondrial matrix.

FABO Cycle

Each cycle of FABO consist of 4 major steps: Oxidation-Hydration-Oxidation-Thiolysis (OHOT) as shown in Figure 3. In Step 1, a trans C=C bond is formed between the α and β carbons. In the process, $FADH_2$ is produced. In Step 2, a hydroxyl group is attached to the β carbon of the fatty acid via the addition of a water molecule. In Step 3, the secondary hydroxyl group on the β carbon is oxidized to a ketone, producing NADH. In Step 4, the $C_\alpha – C_\beta$ is cleaved to yield Acetyl CoA and a Fatty Acyl CoA that is 2 carbons shorter. The remaining Fatty Acyl CoA will then undergo several additional cycles of OHOT until it is fully metabolized to Acetyl CoA.

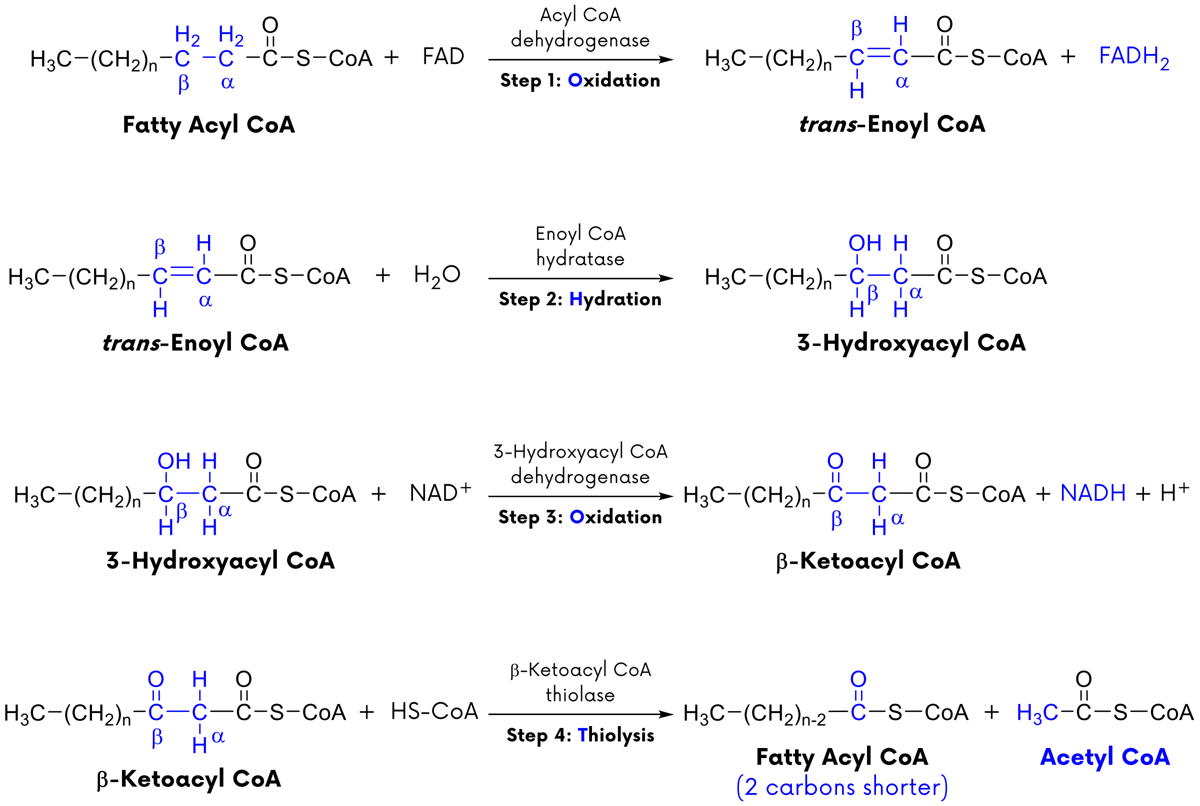


Figure 3. Oxidation-Hydration-Oxidation-Thiolysis (OHOT) Cycles in FABO.

Types of Fatty Acids

Students were then introduced to the types of fatty acids covered in their course syllabus:

- Saturated Fatty Acid (FABO with calculation)
- Odd Carbon Saturated Fatty Acid (FABO without calculation)
- Odd-numbered Double Bonds Unsaturated Fatty Acid (FABO with calculation)
- Even-numbered Double Bonds Unsaturated Fatty Acid (FABO with calculation)
- Mix-numbered (Odd and Even) Double Bonds Unsaturated Fatty Acid (FABO with calculation)

They were reminded that each of these fatty acids will undergo FABO in a slightly different way with the exception of saturated fatty acid being the most straightforward. Students were then guided through a complete FABO of steric acid (18-carbon saturated fatty acid) from the first cycle to its eighth cycle to further consolidate their understanding of OHOT cycles. Right after, they were introduced to the use of LEGO bricks as a hands-on visualization tool.

LEGO Bricks Version of FABO

Using an 8-carbon saturated fatty acid as an example (Figure 4), students were informed of the legend – a 1 x 2 blue translucent brick represents a trans C=C double bond, a yellow circular translucent cap represents a hydroxyl (–OH) group, two yellow circular translucent cap stacked upon each other represent a carbonyl (=O) group and a 1 x 2 white brick with a pink flat cap transparent brick represents a molecule of Acetyl CoA. The students were then guided through each cycle's OHOT steps brick-by-brick to ensure they were skilled enough to explore FABO on their own when given a saturated fatty acid of different length. Emphasis was given to step 1 and 3 of each cycle as 1 molecule of $FADH_2$ and 1 molecule of NADH will be produced, respectively. Altogether, an 8-carbon saturated fatty acid will yield 4 Acetyl CoA through 3 OHOT cycles alongside 3 $FADH_2$ and 3 NADH.

By utilizing the number of $FADH_2$, NADH and Acetyl CoA from FABO, coupled with preliminary knowledge of TCA and Oxidative Phosphorylation, students were then taught how to use Table 1 to calculate the total number of ATP generated. Students were provided with another saturated fatty acid to attempt on their own before proceeding with other types of fatty acids.

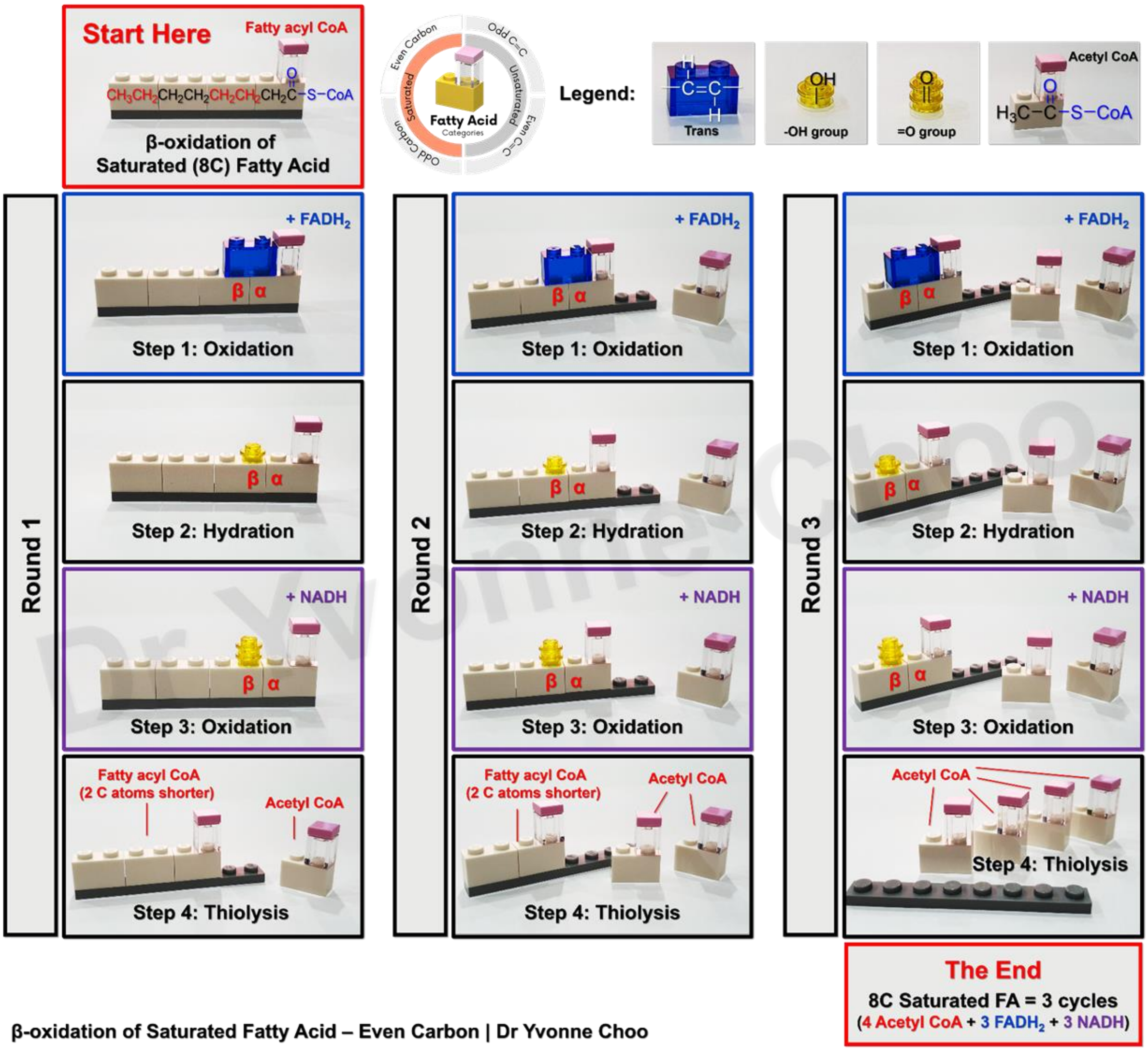


Figure 4. Complete FABO of saturated fatty acid demonstrated with LEGO bricks.

Table 1. Example of ATP calculation for an 8-carbon saturated fatty acid.

Fatty Acid: (Delta Nomenclature, 8:0)	**ATP**	**NADH**	**$FADH_2$**	**ATP** (after Oxidative Phosphorylation)[a]
β-oxidation (no. of cycles = $\frac{8}{2} - 1 = 3$)[b]	0	3	3	3(2.5) + 3(1.5) = 12
TCA (no. of Acetyl CoA $= \frac{8}{2} = 4$)[c]	4	12	4	4 + 12(2.5) + 4(1.5) = 40
Energy Used for Activation[d]	– 2	0	0	– 2
Total ATP Generated				12 + 40 – 2 = 50

[a] NADH = 2.5 ATP; $FADH_2$ = 1.5 ATP. [b] Number of cycles = Number of Carbons on Fatty Acid divided by 2, subtract by 1; [c] Number of Acetyl CoA = Number of Carbons on Fatty Acid divided by 2; Each Acetyl CoA that enters TCA will generate 1 ATP, 3 NADH, 1 $FADH_2$; [d] Energy Used for Activation is from the conversion of Fatty Acid to Fatty Acyl CoA, – 2 ATP by default.

For the case of unsaturated fatty acid, the location of the double bonds present will lead to variations in the OHOT cycle. Hence, in order to facilitate additive learning, students were taught the odd-numbered double bonds unsaturated fatty acid first before the even-numbered and mix-numbered ones. Similar to the saturated fatty acid, students were briefed about the legend used. Noticed how the bricks are now yellow in color to provide visual distinction between the different types of fatty acids.

Counting from the carboxyl (or Delta) end of the fatty acid molecule, if no C=C double bond is present on the first 4 carbons as shown in Figure 5 (Round 1 and 3), then a typical OHOT cycle will take place, resulting in the removal of an Acetyl CoA. Moving on, if a C=C double bond is occupying the third carbon as depicted by the 1 x 2 yellow translucent brick in Step 4 of Round 1, then an isomerization process will take place – converting a *cis*-Δ-3 isomer to a *trans*-Δ-2 isomer, followed by HOT. The isomerization step resembles the first oxidation step in OHOT but does not generate $FADH_2$. This means that for every odd-numbered double bond present in the unsaturated fatty acid, FABO will yield 1 less $FADH_2$. Students were given time to visualize the process and try out the ATP calculations themselves as shown in Table 2 before an in-class discussion take place.

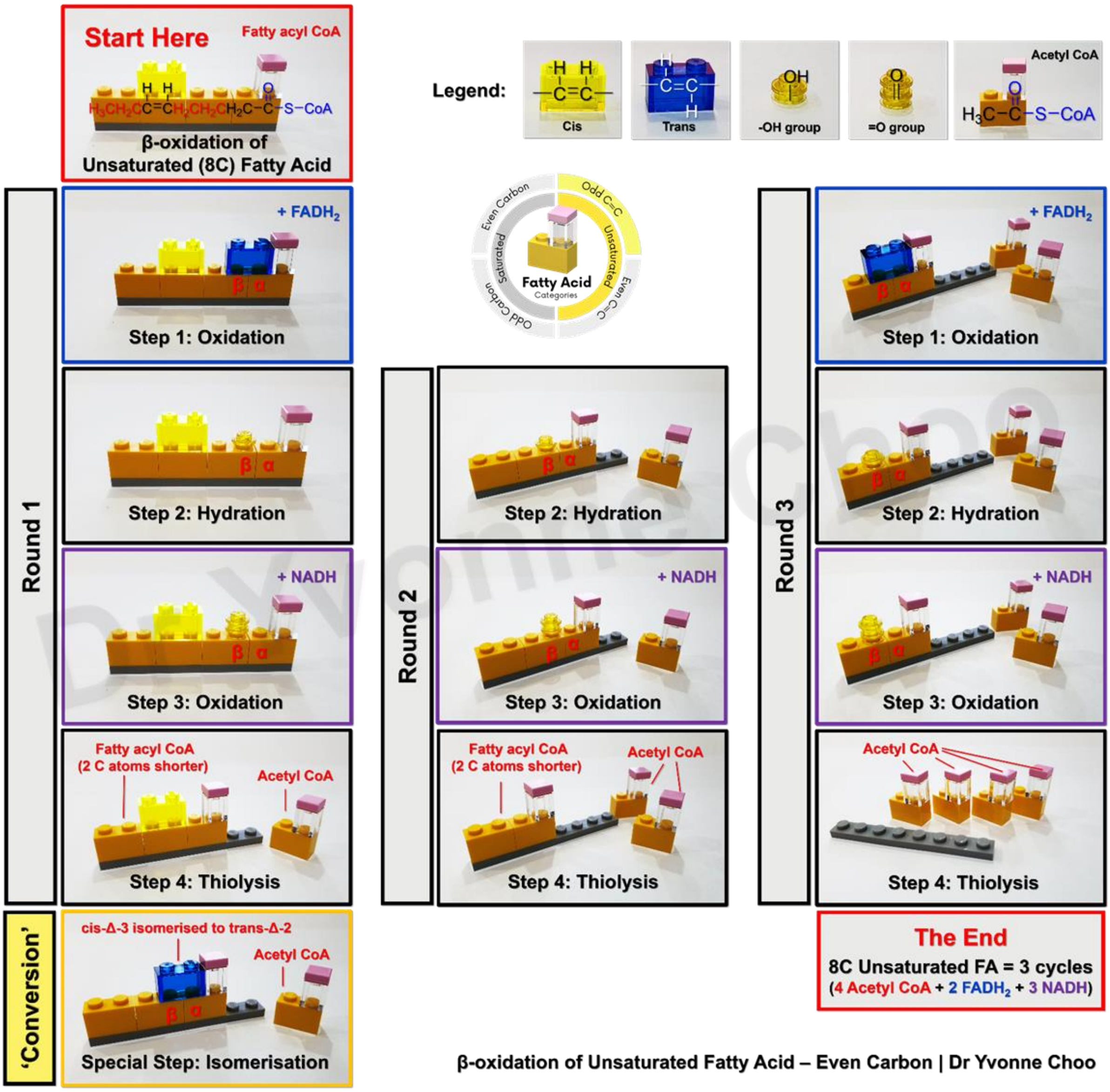


Figure 5. Complete FABO of odd-numbered double bonds unsaturated fatty acid demonstrated with LEGO bricks.

Table 2. Example of ATP calculation for an 8-carbon, odd-numbered double bond unsaturated fatty acid.

Fatty Acid: (8:1 Δ-5)	**ATP**	**NADH**	**$FADH_2$**	**ATP** (after Oxidative Phosphorylation)[a]
β-oxidation (no. of cycles = $\frac{8}{2} - 1 = 3$)[b]	0	3	2	3(2.5) + 2(1.5) = 10.5
TCA (no. of Acetyl CoA = $\frac{8}{2} = 4$)[c]	4	12	4	4 + 12(2.5) + 4(1.5) = 40
Energy Used for Activation[d]	– 2	0	0	– 2
			Total ATP Generated	10.5 + 40 – 2 = 48.5

[a] NADH = 2.5 ATP; $FADH_2$ = 1.5 ATP. [b] Number of cycles = Number of Carbons on Fatty Acid divided by 2, subtract by 1; [c] Number of Acetyl CoA = Number of Carbons on Fatty Acid divided by 2; Each Acetyl CoA that enters TCA will generate 1 ATP, 3 NADH, 1 $FADH_2$; [d] Energy Used for Activation is from the conversion of Fatty Acid to Fatty Acyl CoA, – 2 ATP by default.

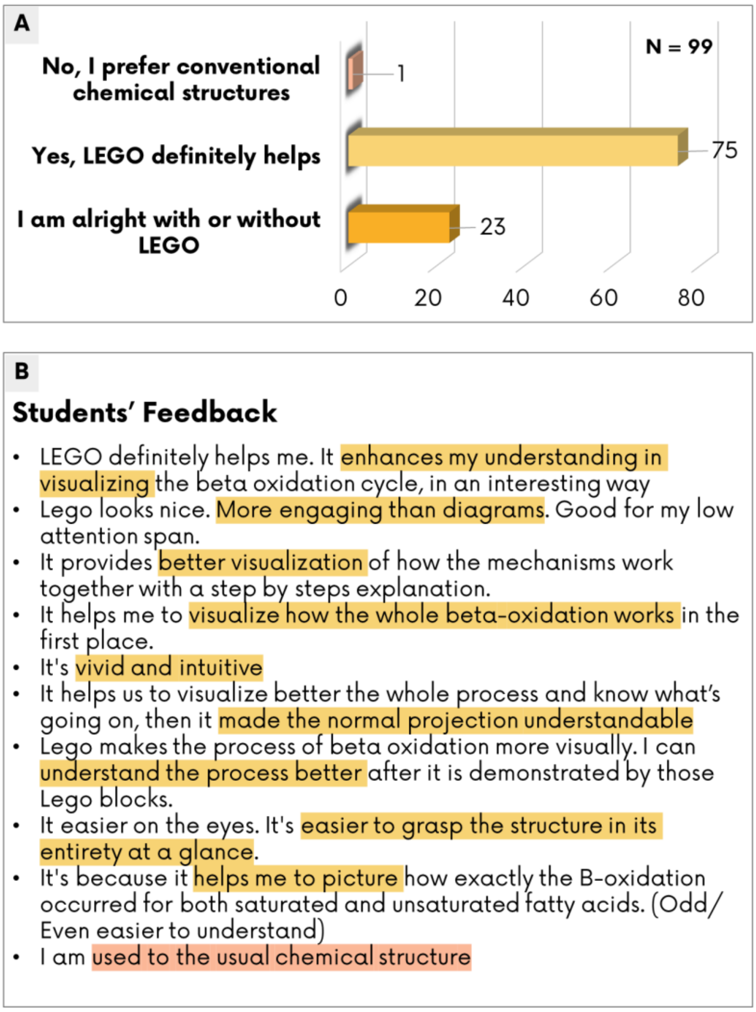
Students who have shown mastery in the first two types of fatty acid were then taught the even-numbered and mix-numbered double bonds unsaturated fatty acids (see Supporting Information). Students were encouraged to analyze the different types of unsaturated fatty acids for trends – e.g. for every even-numbered double bond present, 1 NADPH molecule is used which resulted in a reduction of 3.5 ATP from the total number of ATP generated; for every odd-numbered double bond present, 1 less $FADH_2$ is generated, which resulted in a reduction of 1.5 ATP from the total number of ATP generated.

Discussion

Students' Receptivity Towards the Approach

In order to informally gauge students' receptivity towards the use of LEGO bricks as visualization tools in the teaching and learning of FABO across the 4 semesters (April 2021, 2022 and 2023 and September 2021), anonymized post-implementation surveys (N = 99) were conducted via Microsoft Forms and the representative feedbacks were shown in Figure 6. When students were asked whether the use of

LEGO helped to visualize beta oxidation cycles better than conventional chemical structures, 75 out of 99 respondents (76%) found LEGO as a helpful tool, 1 out of 99 respondents (1%) preferred the use of conventional structures while the remaining 23 out of 99 respondents (23%) were alright with or without it. In the next open-ended question, students were given the opportunity to elaborate on how the approach helped them or if they preferred some other approaches. Out of 99 respondents, 76 students answered and gave their feedbacks but only 9 of them were chosen as representative responses to prioritize ones that are non-repetitive in nature.



Students' Feedback

- LEGO definitely helps me. It enhances my understanding in visualizing the beta oxidation cycle, in an interesting way
- Lego looks nice. More engaging than diagrams. Good for my low attention span.
- It provides better visualization of how the mechanisms work together with a step by steps explanation.
- It helps me to visualize how the whole beta-oxidation works in the first place.
- It's vivid and intuitive
- It helps us to visualize better the whole process and know what's going on, then it made the normal projection understandable
- Lego makes the process of beta oxidation more visually. I can understand the process better after it is demonstrated by those Lego blocks.
- It easier on the eyes. It's easier to grasp the structure in its entirety at a glance.
- It's because it helps me to picture how exactly the B-oxidation occurred for both saturated and unsaturated fatty acids. (Odd/ Even easier to understand)
- I am used to the usual chemical structure

Figure 6. Graphical depiction of students' responses to the anonymized post-implementation survey. (A) Answers to question regarding whether the use of LEGO helped to visualize beta oxidation cycles better than conventional chemical structures, and (B) Representative feedbacks to the open-ended question on how the approach helped them or if they preferred some other approaches.

Limitations

The ATP calculations for odd carbon saturated fatty acids are not covered as part of CME319 and CME112 syllabi because students were not taught the catabolism of Propionyl CoA through TCA. Hence, only the FABO portion was described (see Supporting Information). As shown in Table 1 and Table 2, the ATP calculations are not as effortless as the ATP calculator webtool[25] reported for odd and even carbon saturated fatty acids. However, our reported approach would address their limitations when it comes to ATP calculations for FABO of unsaturated fatty acids.

As not every student in physical, online or hybrid lectures have access to real LEGO bricks, the perceived knowledge gain from the visualization tool may differ from individual to individual. However, all students would at the very least have access to BrickLink Studio (https://www.bricklink.com/v3/studio/download.page), a free digital building software that allows them to access and interact with similar set of bricks in accordance to the teaching material. Since not all students are tech-savvy, there is a need to guide students through the download and software installation as well as the navigation of user interface before it is used as a visualization tool.

Unlike the ball and stick model, LEGO bricks do not have the ability to visualize the stereochemistry of each fatty acid molecule.[14] Hence, the *cis* and *trans* C=C double bonds found in unsaturated fatty acids could only be visualized with the use of 1 x 2 yellow translucent brick and 1 x 2 blue translucent brick, respectively.

Future Work

Similar approach can be applied to teach fatty acid biosynthesis as it is akin to FABO, where each cycle involves a growth of 2 carbons. Other potential developments include the use of LEGO bricks in the teaching of linear metabolic pathway such as glycolysis and cyclic metabolic pathway like TCA due to the linearity of the metabolites/intermediates. The widespread use of LEGO bricks in Biochemistry will give meaning to the term LEGO Biochemistry coined in the title of this article.

Conclusion

The utilization of LEGO bricks as hands-on visualization tools has allowed students to grasp the concept of FABO, enabling them to follow through all of the steps involved in the catabolism of fatty acid, regardless of its length and type. Based on the informal, anonymized post-implementation surveys conducted, the approach has been perceived by students as being more engaging and intuitive in nature as compared to conventional chemical structures. It is envisaged that such use of LEGO bricks can be adapted to cover other complex metabolic pathways to revolutionize the teaching and learning of biochemistry.

Supporting Information

Details about FABO for mix-numbered double bonds unsaturated fatty acids and odd carbon saturated fatty acids; An empty table for the calculation of ATP (PDF)

Snippet of an Online Pre-Recorded Lecture Video on FABO of Saturated Fatty Acid (link)

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