Construction of a three-dimensional glucagon model as a didactic tool in an undergraduate course

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Abstract: Proteins, essential macromolecules, play diverse and critical roles in organisms, encompassing structural support, catalyzing chemical reactions, providing defense mechanisms, and acting as hormones. The synthesis of proteins is a complex process that ultimately determines their specific functions, which are intimately linked to their three-dimensional (3D) structure. To facilitate students' understanding of this topic, we designed a set of educational activities for an undergraduate Biological Sciences course. During these activities, the students simulated the steps of human glucagon synthesis and assembled a three-dimensional model of this protein. The activities involved three classes of two hours each. Fifteen students simulated the steps of human glucagon synthesis, from the gene transcription to the 3D structure assemblage, in an active way. Immediately after the end of the third class and one year after the classes, we asked the students to answer an opinion survey. The activities pedagogical outcomes provided the basis for a research analysis. Our findings indicate the effectiveness of practical, hands-on activities in enhancing students' understanding of protein synthesis. While the approach fostered motivation and interest, the observed deficiency in chemistry-related skills indicates an opportunity for targeted intervention in future classes.

Keywords: Glucagon; 3D model; biology course; practical activities; proteins synthesis.

Introduction

Among the macromolecules, proteins are the main component of cells. Besides the structural function, proteins are responsible for most of the body's chemical reactions (Nelson and Cox, 2008). The amino acid sequence of a given protein is determined by the genetic code, with sequence similarity among species generally being proportional to their evolutionary distance (Nei and Kumar, 2000).

The linear sequence of amino acids is the primary structure of one protein. Amino acids have a general structure composed of an α-carbon bound with a carboxylic group (-COOH), an amino group ($-NH_2$), a

hydrogen atom, and a variable side chain, known as the R group. The general structure without the R group is called the protein backbone. The interaction by hydrogen bonds and other molecular forces among the protein backbone determines the secondary structure, for instance, α-helix or β-sheet. The next level of protein organization is due to the hydrogen bonds and other molecular forces among amino acid radicals and determines a tertiary structure. If more than one polypeptide is used in the final protein structure, it is known as the quaternary structure (Alberts, 2017; Wilson *et al.* 2018). Protein function is directly related to its final conformation.

Two proteins, insulin and glucagon, play a central role in glucose homeostasis (Jiang and Zhang, 2003). Insulin, secreted by pancreatic beta-cells, responds predominantly to elevated glucose concentrations and acts to reduce circulating glucose levels. It does so by inhibiting glycogenolysis and gluconeogenesis while promoting glycogen synthesis in the liver (Nelson and Cox, 2008; Jiang and Zhang, 2003). In contrast, glucagon exerts hyperglycemic actions by stimulating glycogenolysis and gluconeogenesis in the liver while inhibiting glycolysis and glycogenesis (Jiang and Zhang, 2003). Proglucagon is expressed in various tissues (e.g., brain, pancreas, and intestine) and suffers pos-translational processing by enzymes termed prohormone convertases (PCs) into multiple peptide hormones (proglucagon-derived peptides - PGDP's) in a tissue-specific manner. Pancreatic alpha-cells mainly possess PC2, which cleaves dibasic Lys-Arg sites within proglucagon to generate glicentin-related pancreatic peptide (GRPP), glucagon, intervening peptide-1 (IP-1), and major proglucagon fragment (MPGF) (Lafferty *et al.* 2021). The proglucagon has 158 amino acid residues, and the polypeptide hormone glucagon has 29 amino acids and is produced by PC2-mediated cleavage of proglucagon in pancreatic alpha cells (Figure 1). Glucagon action is well-known even to people outside the academy which makes it a good candidate to be used in the teaching of protein synthesis, structure, and function.

Protein synthesis, structure, and function are primarily taught in undergraduate Biochemistry and Molecular Biology courses. This topic is considered difficult and abstract by students, resulting in diminished interest in the classes (Fisher 1985). Mechanisms such as protein synthesis, which defy representation through two-dimensional figures and remain imperceptible to the naked eye or traditional microscopes, are frequently subject to misunderstanding in the classroom setting. Traditional teaching methods, characterized by direct narration and the presentation of images and text, often fall short in facilitating sufficient conceptual learning in these cases (Concannon and Buzzetta, 2010). Alternatively, educational approaches incorporating three-dimensional objects, such as animations and models, along with activities involving active student participation, prove to be more effective. Nevertheless, understanding 3D interactive figures in a computer can pose a challenge for students lacking foundational knowledge, particularly those with limited spatial ability (Wu and Shan, 2004; Herman *et al.* 2006).

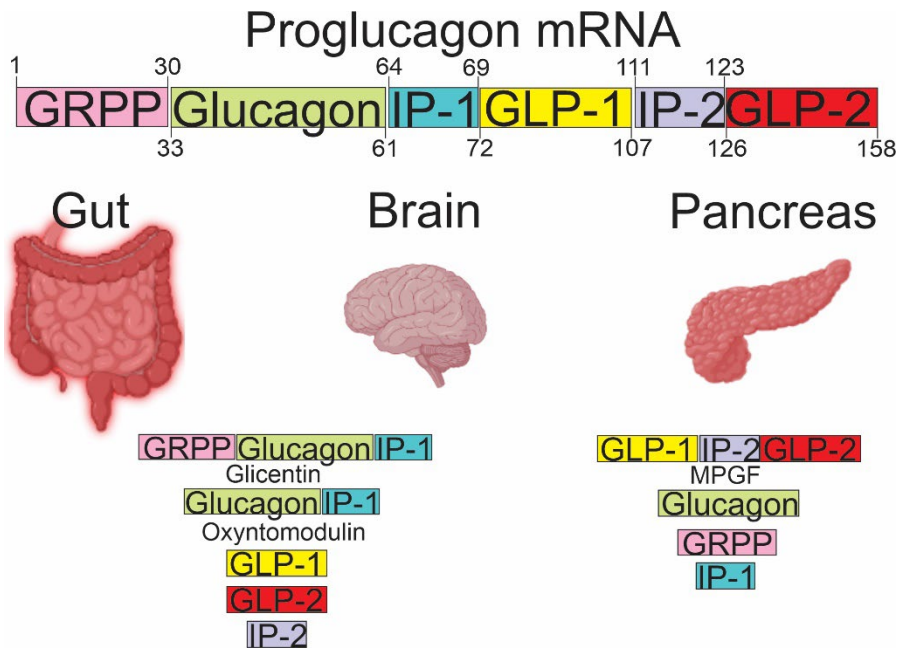

Figure 1 - A schematic overview of tissue-specific proglucagon processing. In the gut and the brain, proglucagon is processed by convertase 1/3 (PC1/3) to generate glicentin, oxyntomodulin, glucagon-like peptides-1 and -2 (GLP-1, GLP-2), and intervening peptide-2 (IP-2). In pancreatic alpha-cells, convertase 2 (PC2) is responsible for the generation of the major proglucagon fragment (MPGF), glucagon, glicentin-related pancreatic polypeptide (GRPP), and intervening peptide-1 (IP-1). The numbers in the proglucagon mRNA indicate the amino acid positions. Source: the authors.

The construction of three-dimensional (3D) models demonstrated effectiveness in improving the teaching of protein structures to high school students (Schmitz *et al.* 2022) and undergraduate students (Oliveira *et al.* 2017; White *et al.* 2002; Azer and Azer, 2016). In this work, we propose the construction of a 3D model of glucagon for a Biological Sciences undergraduate course at a Brazilian public university. We choose glucagon because it is well-known, is translated as proglucagon, is processed differently depending on the tissue, and is a small protein (29 amino acids). The students were introduced to protein synthesis and processing, amino acid characteristics, and protein function related to its 3D conformation. By employing this practical approach, we aimed to enhance students' comprehension and appreciation of these intricate topics in the field of protein biology.

Materials and methods

This study is derived from an educational activity applied to Biological Sciences undergraduate students enrolled in the Practical Biochemistry course at a Brazilian public university. The study was conducted with 15 students that were already enrolled in theoretical Biochemistry classes and had concluded the Cell Biology theoretical topic. The activities were divided into three classes of two hours each. During the classes, the students were

consistently engaged in discussions and challenges related to protein structure, properties, and function.

In the first class, the topic of protein synthesis was briefly reviewed. The Professors explained the steps of protein synthesis according to the central dogma of molecular biology (Alberts, 2017). The students received printed materials containing the structural formulas of the 20 amino acids, the genetic code, and the human pancreatic proglucagon mRNA (NM_002054.5:100; O'Leary et al. 2016) (Figure 2). They were tasked with translating the proglucagon mRNA, and subsequently, the instructors discussed with the students the post-translational modifications that convert proglucagon (158 amino acids) into glucagon (29 amino acids) and its folding into a short alpha helix (Figure 3).

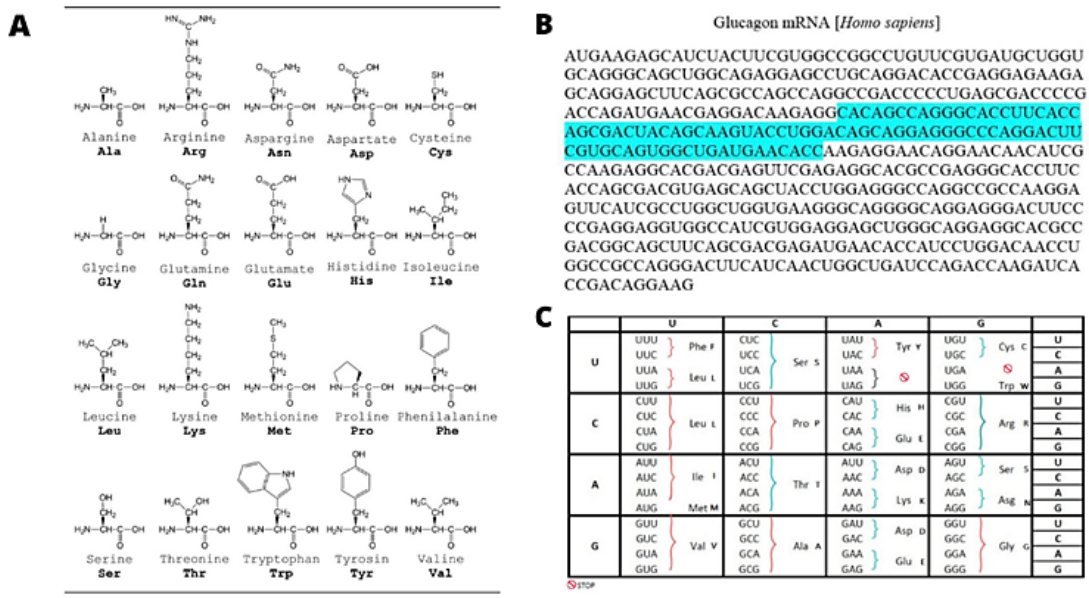

Figure 2 - Material distributed to students. (A) amino acids structural formula; (B) proglucagon mRNA. The glucagon mRNA is highlighted in blue; (C) Genetic code. Source: the authors.

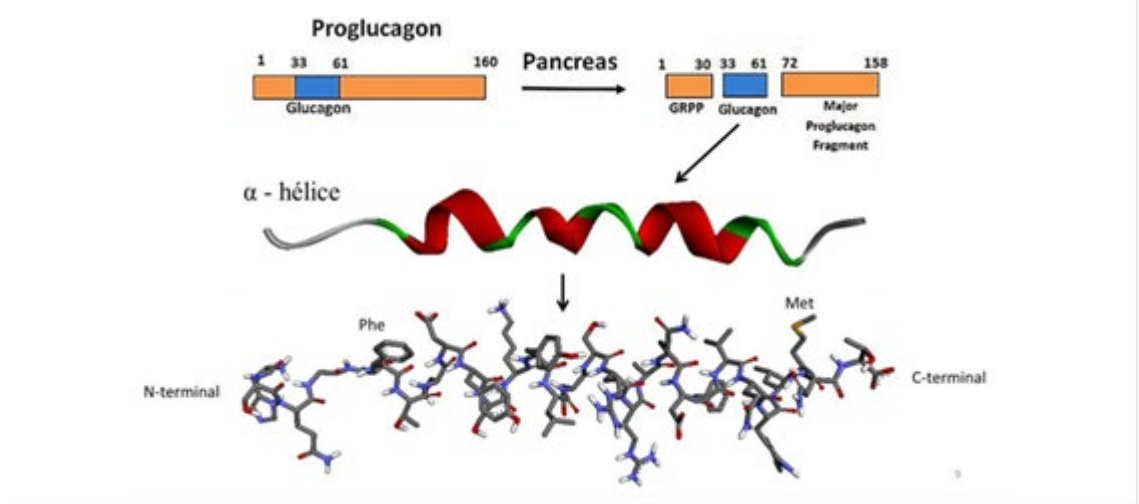

Figure 3 - Material distributed to students. Proglucagon translational modifications and glucagon tertiary structure. Source: the authors.

In the second class, the 3D model of the active form of glucagon started to be built. The students were divided into pairs to build the 3D structure of amino acids using beads of different colors (chosen by each group) to represent the atoms of each element. They also used pliers, wire (1–4 mm) or nylon cords, and hot-melt adhesive to connect the beads (atoms). Each group was responsible for determining the number of beads and amino acids necessary for the activity. Considering the bond distances in the amino acids, the students freely chose the scale to use for the protein model.

Finally, in the third class, the students constructed the protein backbone using the same bead pattern and joined the amino acids in the correct sequence while assembling the α-helix and forming the respective hydrogen bonds. In this step, the students used the glucagon crystallographic structure (PDB ID 1GCN) to compare, using the Discovery Studio Visualizer software (https://discover.3ds.com/discovery-studio-visualizer). At the end of the experimental activity, a group discussion was made, the glucagon models were compared, and a questionnaire was applied to the students (Appendix 1, part A). To assess learning retention, a follow-up questionnaire was applied to the same students one year later (Appendix 1, part B). All 15 original participants were located during another class and invited to complete the follow-up assessment (Table 1, part B).

Results and discussion

Teaching and learning protein chemistry can be challenging due to its abstract nature. However, understanding this subject is crucial for comprehending various cellular mechanisms, including disease development and drug action. The use of didactic tools to facilitate the students' understanding of protein synthesis, structure, and functions has been widely explored (Oliveira et al. 2017; Cavalho et al. 2018; Barnes, 2020; Gonçalves, 2022; Schmitz et al. 2020, 2022) and it is a simple and effective tool to help the students to construct and consolidate the knowledge about proteins. Alternatively, one could consider initially using interactive computer images and subsequently constructing physical models. However, the utilization of 3D construction has proven instrumental in elucidating the intricate interactions among amino acid radicals, rendering the molecular world more comprehensible for students. The subsequent integration of images and computer simulations becomes more accessible in educational settings following the prior introduction of concrete models. Presently, numerous free online resources are available for learning about protein structures and interactions, such as PDB-101 (Zardecki et al. 2022) and the Swiss Model (Waterhouse et al. 2018). After students have established a foundational understanding, these resources can be effectively incorporated into classes with a reduced risk of being misunderstood (Roberts et al. 2005; Herman et al. 2006). In that regard, we emphasize our approach as an effective method to enhance the learning of protein structures.

As we aimed to build the 3D structure of human glucagon, the human proglucagon mRNA was given to the students (Figure 2B). Once they had the mRNA, students, divided into groups, translated codons to the primary structure, i.e., amino acids sequence. This step was conducted by consulting the genetic code, in which the amino acids are related to their respective codon (Figure 2C). After translating the proglucagon, the post-translational modifications of proglucagon to its active form, glucagon, were discussed with the students. Also, the molecular formula of the 20 essential amino acids was provided; thus, the students could recognize it and build the lateral chains of those amino acids that compose the human glucagon.

The second assignment was to build the amino acids necessary for the glucagon 3D structure. Using the molecular formula of amino acids, students should create a scale for the bond length to the 3D model, which resulted in 3D models with different sizes. Afterward, the lateral chains were built using wire or nylon cords and beads of different colors representing the carbon, hydrogen, oxygen, nitrogen, and sulfur atoms (Figure 4). Students also chose the colors, so we had different colors among the groups.

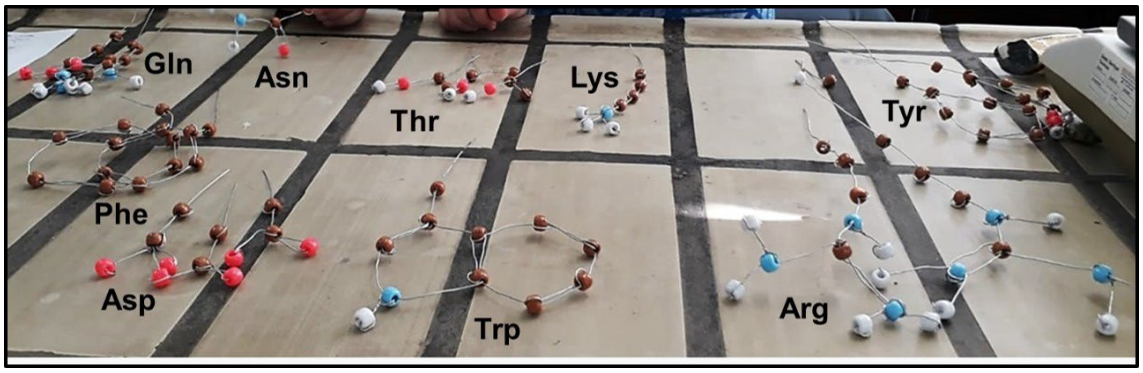

Figure 4 - Examples of lateral chains assembled by the students. Here, the hydrogen (H), carbon (C), nitrogen (N), and oxygen (O) atoms are represented by white, brown, blue, and pink colors of the beads. Source: the authors.

Once the lateral chains were assembled, students started to organize the protein backbone using a thicker wire or nylon cord and the same pattern of beads' colors (Figure 5). The protein backbone was assembled respecting the peptide bonds and α-carbon patterns after the lateral chains were added to the protein.

When the students had the glucagon primary structure done, they started to build the glucagon secondary structure, the α-helix. In Figure 6 we can observe examples of the final structure assembled, which consists of the human glucagon, with a 3D conformation, in different sizes, according to the students' standardized scale. To assemble the α-helix it was necessary to remember the interactions between the lateral chains of amino acids. In this way, students classified the amino acids as polar and non-polar and assembled the hydrogen bonds between the amino acid residues. At this point, the relationship between the primary and tertiary structures of proteins was highlighted. This step highlighted the relationship between the primary and tertiary structures of proteins, emphasizing that the sequence

of amino acids determines the tertiary structure and the protein function. It is important to remember that this sample of students already had theoretical Biochemistry classes, so, we were reviewing those contents.

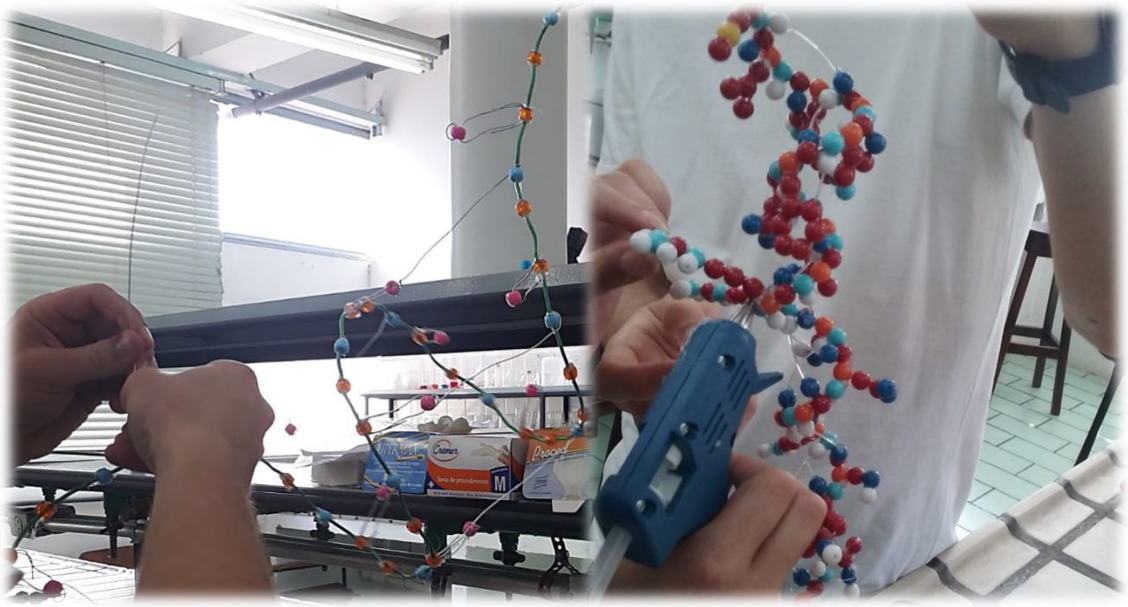

Figure 5 - Glucagon being assembled by the students. Source: the authors.

Figure 6 - Glucagon molecules assembled by the students. Source: the authors.

At the end of the activity, we asked the students to answer an opinion survey to understand how they feel about the contributions of those classes to their understanding of protein structures (Figure 7). The statements are answered via a five-point scale ranging from "strongly disagree" to "strongly agree". The first statement was "The classes contributed to my learning.", and 70% of the students strongly agreed with this. In addition, 70% of students strongly agreed with the second statement "Considering

the relationship between the primary and tertiary structure of proteins, the classes contributed to my learning." Otherwise when the statements were about complex subjects, i.e., "Regarding protein folding, the classes contributed to my learning." and "The classes contributed to my understanding of intramolecular interactions", only 30% of students strongly agree with the statements. Here, the students demonstrated some difficulties in comprehending chemistry subjects, such as intramolecular interactions, which likely originated in middle school. Schmitz et al (2022) demonstrated that 9th-grade students had difficulty comprehending the concept of the atom and molecular interactions. Nogara et al (2018) demonstrated that Brazilian undergraduate students considered their chemistry skills from high school incomplete. Interestingly, Paim et al (2011) demonstrated that 50% of the Biological Sciences undergraduate students did not conclude the General Chemistry program at a Brazilian University.

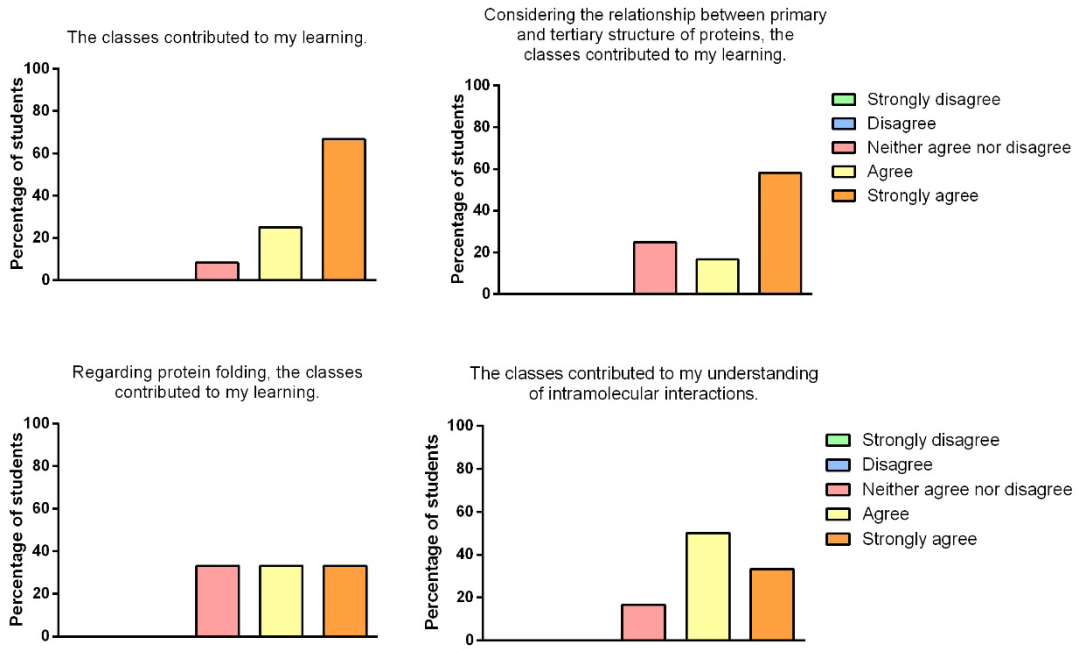

Figure 7 - Students' opinions about the classes' contribution to their knowledge improvement. Source: the authors.

We also asked the students what the classes' positive and negative points were. Table 1 shows the categories that emerged from the data analysis. The data analysis was performed using Content Analysis (Bardin, 2011). About the positive aspects, students called attention to the way the professors conducted the class: "The professors were very good and attentive", characterizing the Professors as knowledgeable and earnest. Also, highlighted the way the classes were developed, calling it a differentiated class, compared to the traditional expositive classes: "We learn more playfully". Other categories are related to the articulation between the theory (studied in theoretical Biochemistry classes) and practice (the organelles work to synthesize proteins), an important aspect

of Brazilian undergraduate courses (Schmitz, 2022): "the visualization of structures facilitates understanding the interaction among molecules on a microscopic scale". Also, students pointed out that the class helps to develop their autonomy, so they are protagonists of their learning, and the teacher is the moderator: "The activity encourages thinking about the topic". The idea that the Professor must transmit all the knowledge and give all the answers is being replaced by the idea of a professor that gives motivation and instigates the students to search the knowledge, helping the student to develop autonomy (Silva et al. 2019) as proposed by constructivism learning theory (Ausubel, 1982). Thus, in constructivism, the students' knowledge and understanding occurs through a "mental construction" based on personal experiences, social interactions and prior knowledge, which will creates the foundation for new concepts and understanding.

Category	**Occurrence**
Positive points	
Knowledgeable and earing teachers	6
Differentiated class	5
Development of students' autonomy	3
3D structure visualization	3
Articulation between theory and practice	1
Did not answer	1
Negative points	
Did not answer	13
Long class	1
Did not explain the distance between amino acids	1

Table 1 - Practical classes' positive and negative aspects pointed out by the students.

The students are the protagonists of their learning and participate actively. In this sense, practical pedagogical activities are of paramount importance. Several studies have demonstrated that practical classes to explain an abstract topic are well accepted by students and, in a general way, students who participate in this kind of classes had a better performance in the topic's evaluation (White et al. 2002; Oliveira et al. 2017; Schmitz et al. 2020). Neto and Oliveira (2015) demonstrated that the majority of Biological Sciences students considered practical classes to be an effective tool for explaining a given theory. Additionally, the class contributes to students' learning because it allows them to visualize the 3D structure of proteins, a content that could be abstract and difficult to understand. The great understanding of molecular science and the details

we currently know about its mechanisms increased the difficulties of communicating such processes. The tactile visualizations provide the recognition of proteins as concrete entities (Roberts et al. 2005; Bain et al. 2006; Herman et al. 2006; Oliveira et al. 2017). The use of physical models allows the easy manipulation, allowing students to explore the molecule from various perspectives. Active participation in the construction process not only facilitates comprehension but also heightens student interest in the subject (Roberts et al. 2005; Bain et al. 2006).

Regarding the practical classes' negative aspects, most of the students did not answer this question, indicating they did not recognize any negative aspects. However, one student pointed out that the class demands a long period, in the category of long class. Also, one student pointed out that teachers did not explain the distance they should use between the amino acids. This category demonstrates the fact that some students are not comfortable making decisions in class, as exemplified by the fact they should create the scale for the bond length. In general, students have difficulties performing an active role in the learning process (Felder and Brent, 2016). In this way, active methodologies are being proposed, as in our work, to stimulate the students' protagonist in their learning.

After 1 year we contacted the students asking them to answer another questionnaire, aiming to see if their opinions changed over time, as well as their knowledge about protein chemistry. A great number of students (70%) remembered the practical, and the other 30% vaguely remembered it (Figure 8). More than 90% of the students remembered which protein was assembled in the classes, glucagon (Figure 8). Corroborating the first survey, most of the students (90%) pointed out that the construction of a protein 3D model contributed to their knowledge construction and learning process (Figure 8). Indeed, 100% of the students answered correctly the three first questions about protein's primary, secondary, and tertiary structures (Table 2).

Question	**Correct Answer?**	
	Yes	**No**
What is the protein backbone?	100%	-
What changes in the protein's primary structure might alter?	100%	-
What are the two most common types of protein's secondary structure?	100%	-
What does the tertiary structure of proteins determine?	87%	13%
Which region of amino acids is responsible for protein polarity and molecular stabilization?	36%	64%

Table 2 - Percentage of students' correct answers about protein chemistry one year later the practical classes.

But only 36% of the students correctly answered the question "Which region of amino acids is responsible for protein polarity and molecular stabilization?" (Table 2). The incorrect answers represent a limitation in the understanding, further emphasizing the students lack of chemistry-related skills. It is important to note, however, that the students were already familiar with these concepts from previously attended theoretical biochemistry classes, and that the practical sessions described in this work reinforced these topics.

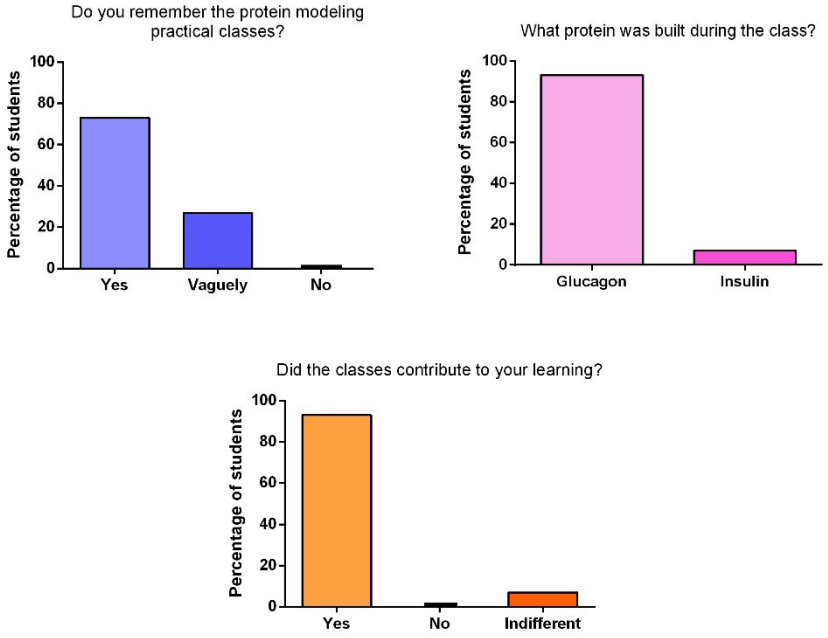

Figure 8 - Answers of students one year after the class survey. Source: the authors.

The final aspect to be addressed pertains to the recyclability and reusability of the materials employed. A similar work by Oliveira *et al* (2017) involved the utilization of styrofoam balls to depict atoms within the insulin protein. The researchers underscored the challenge of recycling styrofoam in numerous locations, as well as its inherent difficulty in being reused. It is imperative to acknowledge that a considerable portion of the materials employed in our study are recyclable and amenable to reuse, particularly when the structural components are disassembled.

Conclusion

In this study, the biological sciences students assembled a 3D model of glucagon, in three practical classes of approximately two hours each. The students showed motivation and curiosity during the classes, which are essential to knowledge construction. In addition, even one year after the practical classes, most of the students believe this kind of class contributes to their learning process, as well as they presented a good performance on the content test. However, it was evident that many students exhibited a lack of proficiency in chemistry skills, particularly concerning the

understanding of intra- and intermolecular interactions, among other related concepts. This finding reinforces the difficulty of understanding the abstract mechanism, and explains our results, as learning becomes meaningful when it is associated with already-known concepts. To address this challenge in future classes, the Professors need to evaluate the students' chemistry skills before commencing the protein 3D model construction. By addressing this issue proactively, the Professors can optimize the overall learning experience, ensuring that students are better equipped to tackle the intricate concepts of protein chemistry. Additionally, facilitating a solid understanding of chemistry concepts will likely improve the student's performance in subsequent practical classes and enhance their proficiency in the broader field of Biological Sciences.

References

Alberts, B. (2017). *Molecular Biology of the Cell* (6th ed.). New York: W.W. Norton & Company.

Ausubel, D. P. (1982). *A aprendizagem significativa: a teoria de David Ausubel*. São Paulo: Moraes.

Azer, S. A., Azer, S. (2016). 3D anatomy models and impact on learning: A review of the quality of the literature. *Health Professions Education*, *2*(2), 80–98. https://doi.org/10.1016/j.hpe.2016.05.002.

Bain, G. A., Yi, J., Beikmohamadi, M., Herman, T. M., Patrick, M. A. (2006). Using physical models of biomolecular structures to teach concepts of biochemical structure and structure depiction in the introductory chemistry laboratory. *Journal of Chemical Education*, *83*(9), 1322. https://doi.org/10.1021/ed083p1322.

Bardin, L. (2011). *Análise de conteúdo*. São Paulo: Edições 70.

Barnes, R. L. (2020). A protein purification card game develops subject knowledge and transferable skills. *Journal of Biological Education*, *56*(4), 365–375. https://doi.org/10.1080/00219266.2020.1799844.

Cavalho, J. C. Q., Beltramini, L. M., Bossolan, N. R. S. (2018). Using a board game to teach protein synthesis to high school students. *Journal of Biological Education*, *53*(2), 205–216. https://doi.org/10.1080/00219266.2018.1469532.

Concannon, J., Buzzetta, M. (2010). Students conceptualizing transcription and translation from a cellular perspective. *Science Activities*, *47*(2), 83–88. https://doi.org/10.1080/00368121003753894.

Felder, R. M., Brent, R. (2016). *Teaching and learning STEM: A practical guide*. San Francisco: Jossey-Bass.

Fisher, K.M. (1985). A Misconception in Biology: Amino Acids and Translation. Journal of Research in Science Teaching, 22 (1), 53-62. https://doi.org/10.1002/tea.3660220105

Gonçalves, T. M. (2022). Action of the chymosin enzyme in milk: proposing a simple and low-cost experiment for the teaching of

Biochemistry in the Biology discipline. *Research, Society and Development*, *11*, e204111132494. http://dx.doi.org/10.33448/rsd-v11i11.324941.

Herman, T., Morris, J., Colton, S., Batiza, A., Patrick, M., Franzen, M., Goodsell, D. S. (2006). Tactile teaching: Exploring protein structure/function using physical models. *Biochemistry and Molecular Biology Education*, *34*(5), 247–254. https://doi.org/10.1002/bmb.2006.494034042649.

Jiang, G., Zhang, B. B. (2003). Glucagon and regulation of glucose metabolism. *American Journal of Physiology: Endocrinology and Metabolism*, *284*(4), E671–E678. https://doi.org/10.1152/ajpendo.00492.2002.

Lafferty, R. A., O'Harte, F. P. M., Irwin, N., Gault, V. A., Flatt, P. R. (2021). Proglucagon-derived peptides as therapeutics. *Frontiers in Endocrinology*, *12*, 689678. https://doi.org/10.3389/fendo.2021.689678.

Nei, M., Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

Nelson, D. L., Cox, M. M. (2008). *Lehninger Principles of Biochemistry* (5th ed.). New York: W.H. Freeman & Company.

Neto, L. S., Oliveira, M. L. A. (2015). Experimental classes in higher education: what the students of the biological sciences degree think about this practice. *Revista de Educação, Ciências e Matemática*, 5(1), 62-72.

Nogara, P. A., Schmitz, G. L., Eisenhardt, L. M., Moura, B. L. T., Rocha, J. B. T., Oliveira, C. S. (2018). Perception of undergraduate students of a Brazilian University about the Biochemistry course. *Journal of Biochemistry Education*, *16*(1), 5-24. https://doi.org/10.16923/reb.v16i1.753.

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, Y., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C. M., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V. S., Kodali, V. K., Li, W., Maglott, D., Masterson, P., McGarvey, K. M., Murphy, M. R., O'Neill, K., Pujar, S., Rangwala, S. H., Rausch, D., Riddick, L. D., Schoch, C., Shkeda, A., Storz, S. S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R. E., Vatsan, A. R., Wallin, C., Webb, D., Wu, W., Landrum, M. J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T. D., & Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*(D1), D733–D745. https://doi.org/10.1093/nar/gkv1189.

Oliveira, C. S., Nogara, P. A., Schmitz, G. L., Rocha, J. B. T. (2017). O hormônio insulina como um modelo para ensinar a estrutura tridimensional das proteínas. *Revista de Ensino de Bioquímica*, *15*(2), 114–130. https://doi.org/10.16923/reb.v15i2.741.

Paim, R. A., Santos, N. R. Z., Saidelles, A. P. F. (2011). *Análise do desempenho dos acadêmicos dos cursos de Ciências Biológicas,*

Biotecnologia e Gestão Ambiental na disciplina de Química Geral da Unipampa - campus São Gabriel. In: SIEPE, Uruguaiana: ANAIS SIEPE, v. 3.

Roberts, J. R., Hagedorn, E., Dillenburg, P., Patrick, M., Herman, T. (2005). Physical models enhance molecular 3D literacy in an introductory biochemistry course. *Biochemistry and Molecular Biology Education*, *33*(2), 105–110. https://doi.org/10.1002/bmb.2005.494033022426.

Schmitz, G. L. (2022). *A prática como componente curricular em cursos de licenciatura em ciências biológicas e em química* (Tese de doutorado). Universidade Federal de Santa Maria, Programa de Pós-graduação em Educação em Ciências: Química da Vida e Saúde, Santa Maria, Brasil.

Schmitz, G. L., Joras, L. E., Nogara, P. A., Galiciolli, M. E. A., Piccoli, B. C., Silva, F. D., Oliveira, C. S., Rocha, J. B. T. (2022). Representation of insulin synthesis and construction of a three-dimensional insulin model as didactic tools in basic education. *Journal of Biological Education*, *58*(2), 344–354. https://doi.org/10.1080/00219266.2022.2058983.

Schmitz, G. L., Nogara, P. A., Medina, N., Rocha, J. B. T., Barbosa, N. V., Segatto, A. L. A., Oliveira, C. S. (2020). Cockroaches: an alternative model to teach enzymatic inhibition to undergraduate students. *Journal of Biological Education*, *56*(4), 397–407. https://doi.org/10.1080/00219266.2020.1808512.

Silva, P., Felipe, E. H., Oliveira, R. C., Vicente, K. B. (2019). O perfil do professor no ensino superior no século XXI. *Multi Debate*, *3*(2), 124-136.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., de Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research, 46*(W1), W296-W303. https://doi.org/10.1093/nar/gky427.

White, B., Kim, S., Sherman, K., Weber, N. (2002). Evaluation of molecular visualization software for teaching protein structure: Differing outcomes from lecture and lab. *Biochemistry and Molecular Biology Education*, *30*(2), 130–136. https://doi.org/10.1002/bmb.2002.494030020026.

Wilson, K., Hofmann, A., Walker, J. M., Clokie, S. (2018). *Principles and Techniques of Biochemistry and Molecular Biology*. New York: Cambridge University Press.

Wu, H., Shah, P. (2004). Exploring visuospatial thinking in chemistry learning. *Science Education*, *88*(4), 465–492. https://doi.org/10.1002/sce.10126.

Zardecki, C., Dutta, S., Goodsell, D. S., Lowe, R., Voigt, M., Burley, S. K. (2022). PDB-101: Educational resources supporting molecular explorations through biology and medicine. *Protein Science*, *31*(1), 129-140. https://doi.org/10.1002/pro.4200.

Appendix 1 - Surveys applied to the students. A) At the end of the class. B) After one year.

A. At the end of the class

1. The classes contributed to my learning. ( ) strongly disagree
( ) disagree
( ) neither agree nor disagree
( ) agree
( ) strongly agree

2. Considering the relationship between primary and tertiary structure of proteins, the classes contributed to my learning. ( ) strongly disagree
( ) disagree
( ) neither agree nor disagree
( ) agree
( ) strongly agree

3. Regarding protein folding, the classes contributed to my learning. ( ) strongly disagree
( ) disagree
( ) neither agree nor disagree
( ) agree
( ) agree strongly

4. The classes contributed to my understanding of intramolecular interactions.( ) strongly disagree
( ) disagree
( ) neither agree nor disagree
( ) agree
( ) strongly agree

5. Which were the negative and positive points of the classes?

B. After one year

1. Do you remember the protein modeling practical classes? ( ) Yes
( ) Vaguely
( ) No

2. What protein was built during the class?( ) Glucagon
( ) Insulin

3. Did the classes contribute to your learning? ( ) Yes
( ) No
( ) Indifferent

4. What is the protein backbone? ( ) Set of nucleotides linked by peptide bonds
( ) Set of amino acids linked by peptide bonds
( ) Set of amino acids linked by glycosidic bonds
( ) Set of nucleotides linked by phosphodiester bonds

5. What changes in the protein's primary structure might alter? ( ) Ribosome
( ) Protein function
( ) DNA
( ) Cell composition

6. What are the two most common types of protein's secondary structure?
( ) Alpha helix and zinc fingers
( ) Beta sheet and alpha helix
( ) Beta sheet and zinc fingers
( ) Zinc fingers and TATA box

7. What does the tertiary structure of proteins determine? ( ) Protein function
( ) Order of amino acids
( ) Order of nucleotides

( ) Cell death

8. Which region of amino acids is responsible for protein polarity and molecular stabilization? ( ) Active site

( ) α-carbon

( ) Side chain

( ) Carboxylic group