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METODOLOGIAS ATIVAS PARA O ENSINO DE ESTRUTURA PROTEICA E ENZIMOLOGIA NO ENSINO SUPERIOR

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS

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METODOLOGIAS ATIVAS PARA O ENSINO DE ESTRUTURA PROTEICA E ENZIMOLOGIA NO ENSINO SUPERIOR

por

Cláudia Sirlene de Oliveira

Dissertação apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Ciências Biológicas: Bioquímica Toxicológica.**

Orientadora: Profª. Drª. Ana Lúcia Anversa Segatto

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METODOLOGIAS ATIVAS PARA O ENSINO DE ESTRUTURA PROTEICA E ENZIMOLOGIA NO ENSINO SUPERIOR

Elaborada por **Cláudia Sirlene de Oliveira** como requisito parcial para a obtenção do grau de **Mestre em Ciências Biológicas: Bioquímica Toxicológica**

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3. RESULTADOS

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

Artigo: Cockroaches: an alternative model to teach enzymatic inhibition to undergraduate students

LISTA DE REDUÇÕES (ABREVIATURAS, SIGLAS E SÍMBOLOS)

3D: Tridimensional

3Rs: do inglês: *Replacement, Reduction and Refinement*

AChE: acetilcolinesterase

ASCh: acetiltiocolina

BSCh: butiriltiocolinesterase

CNE: Conselho Nacional de Educação

CPF: clorpirifós

DNA: Ácido desoxirribonucleico

Fab Labs: do inglês *fabrication laboratory*

grupo R: Cadeia lateral dos aminoácidos

mRNA: Ácido ribonucleico mensageiro

RNA: Ácido ribonucleico

α: Alfa

β: Beta

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APRESENTAÇÃO

No item **INTRODUÇÃO** está descrito uma revisão sucinta sobre os temas trabalhados nesta dissertação. No final deste item estão apresentados os objetivos geral e específicos.

Os **RESULTADOS** estão dispostos na forma de artigo científico e manuscrito submetido à publicação. As seções Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no artigo e nos manuscritos e representam a integra deste estudo.

No item **CONCLUSÕES** são apresentadas as conclusões gerais do presente trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** apresentadas no final da dissertação referem-se somente as citações que aparecem no item **INTRODUÇÃO**.

RESUMO

Dissertação de Mestrado
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Universidade Federal de Santa Maria, RS, Brasil

METODOLOGIAS ATIVAS PARA O ENSINO DE ESTRUTURA PROTEICA E ENZIMOLOGIA NO ENSINO SUPERIOR

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A Bioquímica é uma disciplina com enorme potencial de contextualização e interdisciplinaridade, porém, é vista como uma das disciplinas mais difíceis e com altos índices de reprovação entre os estudantes. Por conseguinte, é importante adaptar as estratégias de ensino de modo a tornar as aulas de Bioquímica atrativas, a fim de auxiliar o estudante na construção do conhecimento. Essa dissertação está dividida em dois estudos independentes. No primeiro estudo, aulas práticas investigativas foram realizadas com alunos do curso de Ciências Biológicas da Universidade Federal de Santa Maria. Os estudantes simularam a síntese do hormônio glucagon, desde a transcrição até a construção do modelo 3D. Os alunos ficaram motivados, afirmando que a construção do modelo proteico contribuiu para a assimilação do conhecimento sobre química de proteínas. No segundo estudo, foram realizadas aulas com estudantes dos cursos de graduação em Ciências Biológicas e Medicina Veterinária da mesma universidade, sobre os mecanismos de inibição enzimática. Foi utilizada a enzima acetilcolinesterase de cabeça de baratas da espécie *Nauphoeta cinerea*. Os alunos testaram diferentes fatores que alteram a atividade enzimática *in vitro* e *in vivo*. Os alunos, em sua maioria, demonstraram entusiasmo em trabalhar com animais nas aulas práticas, assim como apresentaram uma melhora nos conhecimentos sobre enzimologia. Dessa forma, nessa dissertação, foi possível demonstrar que a utilização de aulas práticas investigativas é importante para que os estudantes construam um conhecimento sólido sobre conteúdos da disciplina de Bioquímica.

Palavras-chave: Bioquímica, enzimas, baratas, modelo 3D

ABSTRACT

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METODOLOGIAS ATIVAS PARA O ENSINO DE ESTRUTURA PROTEICA E ENZIMOLOGIA NO ENSINO SUPERIOR

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Biochemistry is a discipline with enormous potential for contextualization and interdisciplinarity, however, it is seen as one of the most difficult disciplines and with high failure rates among students. Therefore, it is important to adapt teaching strategies to make Biochemistry classes more interesting, to assist the student in the construction of knowledge. This dissertation is divided into two independent studies. In the first study, investigative practical classes were carried out with students from the Biological Sciences undergraduate course at the Federal University of Santa Maria. Students simulated the synthesis of the hormone glucagon, from transcription to construction of the 3D model. The students were motivated, stating that the construction of the protein model contributed to the construction of knowledge about protein chemistry. In the second study, classes were held with students from undergraduate courses in Biological Sciences and Veterinary Medicine at the same university, on the mechanisms of enzyme inhibition. The enzyme acetylcholinesterase from the head of cockroaches of the species *Nauphoeta cinerea* was used. Students tested different factors that alter enzyme activity *in vitro* and *in vivo*. Most of the students showed enthusiasm in working with animals in practical classes, as well as an improvement in their knowledge of enzymology. Thus, in this dissertation, it was possible to demonstrate that the use of investigative practical classes is important for students to build solid knowledge about the contents of the Biochemistry discipline.

Keywords: Biochemistry, enzymes, cockroaches, 3D model

1 INTRODUÇÃO

A disciplina de Bioquímica é extremamente importante para o entendimento da composição e funcionamento das células e consequentemente dos tecidos, órgãos e organismos. Os cursos de graduação das áreas da saúde e das ciências naturais apresentam uma ou mais disciplinas de Bioquímica na grade curricular. Embora essencial, essa disciplina tem grande evasão e reprovação dos estudantes matriculados, uma vez que é complexa e requer dedicação e muita leitura (Andrade et al. 2015; De Faria et al. 2020; Souza et al. 2020). Recentemente, Nogara et al. (2018) demonstrou que a maioria dos estudantes de uma Universidade Federal acreditam que a disciplina de Bioquímica é essencial, porém, têm dificuldade em compreender o conteúdo ou relacioná-lo com as demais disciplinas do curso em que estão matriculados.

Dentre os conteúdos explorados no decorrer da disciplina, destacam-se, a química das proteínas, que abrange o estudo detalhado das interações químicas envolvidas na formação das estruturas primárias, secundárias, terciárias e quaternárias; e a enzimologia, que explora os mecanismos de inibição enzimática, dentre outros (Nelson e Cox 2008). As proteínas são responsáveis por uma variedade de funções bioquímicas, por exemplo, transporte, catálise, estrutural, hormonal, dentre outras. A síntese proteica ocorre através dos processos de transcrição (DNA → RNA) e tradução (RNA → Proteína) (Nelson e Cox 2008; Alberts 2017). As proteínas são construídas a partir de monômeros chamados aminoácidos unidos através da ligação peptídica. Os aminoácidos têm uma característica estrutural básica que inclui o carbono α ligado a um átomo de hidrogênio, um grupo carboxila, um grupo amina e a uma cadeia lateral (grupo R). A natureza química do grupo R confere propriedades físico-químicas específicas aos aminoácido, permitindo que eles sejam agrupados da seguinte forma (1) apolar; (2) polar sem carga; (3) polar com carga negativa (4) polar com carga positiva (Nelson e Cox 2008) (**Figura 1**).

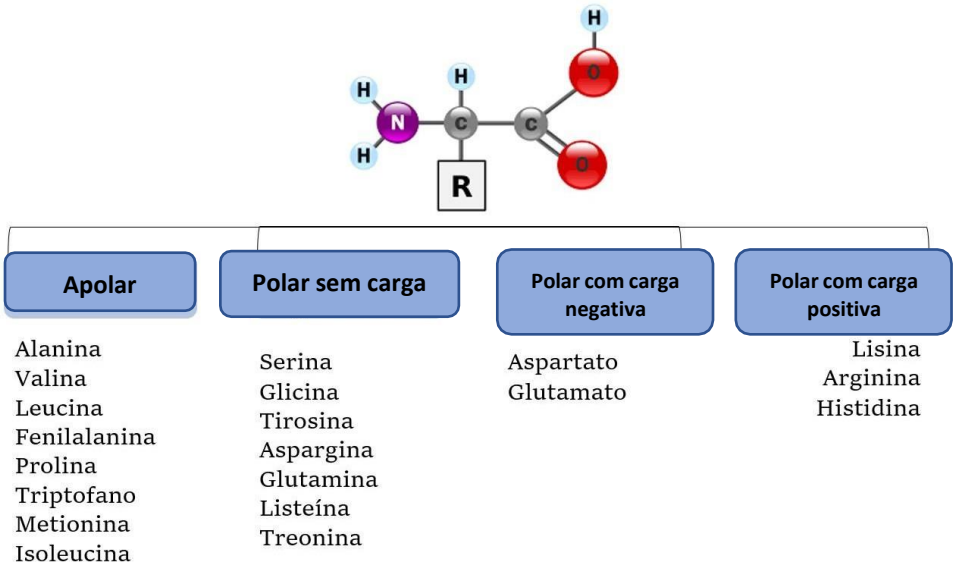

Figura 1: Característica estrutural dos aminoácidos e descrição dos 20 aminoácidos de acordo com as características físico-químicas da cadeia lateral. Fonte: Marzzoco e Torres (2015)

Em vista da grande importância das proteínas na manutenção da homeostasia celular e do funcionamento do organismo, é de fundamental importância que os alunos construam um conhecimento sólido e compreendam as proteínas e sua conformação espacial, bem como, entendam os mecanismos de inibição enzimática. Esses dois conteúdos são extensos e necessitam de horas de leitura, o que muitas vezes se torna exaustivo para os estudantes, visto que em um semestre os alunos têm em sua grade curricular de seis a oito disciplinas; ainda, muitos realizam estágio de iniciação científica. Além disso, é difícil compreender a estrutura tridimensional das proteínas, bem como, os mecanismos de inibição enzimática, apenas assistindo aulas teórico-expositivas. Por conseguinte, é importante adaptar as estratégias de ensino de modo a torná-las mais eficazes e motivacionais para os estudantes. Neste contexto, Spadaro (1993) observou um aumento na frequência e nas notas dos estudantes após a inclusão de atividades práticas em suas aulas teóricas.

Nos últimos anos, vários trabalhos foram publicados com alternativas pedagógicas para melhorar a aprendizagem dos conteúdos ensinados na disciplina de

Bioquímica, por exemplo, jogos, mapas conceituais, vídeos/filmes, modelos moleculares, dentre outros (Hageman 2010; Rostejnská e Klímová 2011; Oliveira e Dias 2012; Surapaneni e Tekian 2013; Ghosh et al. 2016; Oliveira et al. 2017; Cavalho et al. 2018; Barnes 2020; Gonçalves 2022; Schmitz et al. 2022). De forma geral, a utilização de atividades práticas é bem aceita pelos estudantes e aumenta a compreensão de conteúdos densos e abstratos, como os ensinados na disciplina de Bioquímica.

1.1 Importância de aulas práticas no ensino de Bioquímica

O mundo em que vivemos, no que diz respeito a ciência e tecnologia, avança a uma velocidade nunca vista. Os educadores têm o desafio de preparar profissionais que estejam prontos para viver e produzir em um mundo em constante mudança, com mercados que não existem hoje. Nesse sentido, muitos estudos tentam responder quais as metodologias de ensino-aprendizagem são mais eficientes para preparar esses cidadãos (Filatro e Cavalcanti 2018). A formação voltada apenas para o aspecto cognitivo vem dando lugar a uma formação que busca desenvolver a capacidade dos estudantes de construir seu próprio conhecimento (Masetto 2003). Esta perspectiva é fomentada pelo Conselho Nacional de Educação (CNE), ao evidenciar a formação cidadã como um dos objetivos principais do ensino, vinculando o conhecimento científico e tecnológico e as questões sociais e ambientais.

Neste sentido, estudantes e profissionais deixam de ser receptores de informações, no que chamamos de educação tradicional, para assumir o protagonismo da sua própria aprendizagem. Assim, surge um método de ensino que chamamos de Metodologias Ativas. Essas metodologias são vistas frequentemente como um contraponto do ensino

tradicional transmissivo, no entanto não é a estratégia de ensino que caracteriza uma metodologia ativa (Sanders et al. 2017). O que caracteriza uma metodologia ativa é a presença de dois aspectos fundamentais: ação e reflexão. Ou seja, o estudante precisa envolver-se com seu aprendizado e refletir sobre aquilo que está fazendo (Bonwell e Eison 1991).

A Bioquímica é a ciência que estuda os processos químicos e físicos que ocorrem nos organismos vivos. Trata da estrutura e função metabólica de componentes celulares como proteínas, carboidratos, lipídios, ácidos nucleicos e outras biomoléculas (Lehninger et al. 2014). A relevância do aprendizado de Bioquímica nunca foi tão grande, com o avanço da Biotecnologia, Medicina, Agricultura, e muitos outros campos. Apesar de ser uma disciplina com enorme potencial de contextualização e interdisciplinaridade é vista como uma das disciplinas mais difíceis e com altos índices de reprovação nas Universidades. É considerada também pelos professores uma disciplina difícil de ser ministrada pela complexidade de seus conteúdos que muitas vezes não são abstraídos e compreendidos pelos estudantes (Torres et al. 2004).

As aulas práticas aparecem como um método ativo de ensino, que favorece a aprendizagem da ciência e de seus conteúdos tornando-os mais palpáveis e relacionados com a realidade do estudante (Millar 2004). Além disso, práticas têm o potencial de aumentar o interesse e participação dos estudantes nas aulas de ciências (Bertusso et al. 2020). Cabe ao professor direcionar a construção do conhecimento para que o estudante reflita sobre o que aprendeu, correlacionando a prática com os conteúdos teóricos, o curso, o trabalho e a vida em sociedade. Nessa dissertação definimos atividade prática como qualquer atividade de ensino e aprendizagem em que se observa ou manipula os objetos e matérias que estão sendo estudados (Millar, 2004).

1.2 Aulas práticas investigativas

Os experimentos investigativos, ou atividades práticas investigativas diferem de outras atividades por envolverem a discussão de ideias, elaboração de hipóteses e explicações para os experimentos realizados, exigindo grande participação do aluno (Campos e Nigro 1999). Na literatura, são encontradas diferentes denominações para o ensino por investigação como: ensino por descoberta, aprendizagem por projetos, questionamentos, resolução de problemas, aprendizagem baseada em problemas, entre outros (Zompero e Laburú 2011).

Costuma-se associar o ensino por investigação de forma direta com as aulas experimentais no laboratório, no entanto, muitas atividades experimentais não apresentam características essenciais da investigação, assim como atividades que não são práticas podem ter grande potencial investigativo (Munford e Lima 2007). O professor tem o papel de mediação, fazendo com que a atividade tenha caráter investigativo, sendo importante que os alunos não saibam de antemão a solução para as questões (DeBoer 2006, Binatto et al. 2015). As práticas investigativas têm por finalidade a valorização de raciocínio de maneira que o estudante aproveite todo o procedimento para observar, levantar questões, tirar conclusões e entender por que elas estão corretas ou não (Rodrigues et al. 2021).

Nas aulas de laboratório reconhecidas como tradicionais, geralmente, um roteiro fechado é entregue ao estudante, os passos devem ser seguidos à risca e o estudante realiza as atividades acerca de fenômenos previamente determinados pelo professor (Tamir 1991). Nas práticas investigativas os grupos podem interagir e discutir a atividade proposta, inclusive dividindo responsabilidades e decidindo o que devem e como devem fazer diante da prática proposta, o que enriquece o aprendizado (Borges 2002) (**Figura 2**).

Aspectos	**Laboratório Tradicional**	**Atividades Investigativas**
Quanto ao grau de abertura	Roteiro pré-definido ⟵ Restrito grau de abertura	Variado grau de abertura ⟶ Liberdade total no planejamento
Objetivo da aula	Comprovar leis	Explorar fenômenos
Atitude do estudante	Compromisso com o resultado	Responsabilidade na investigação

Figura 2: Diferenças entre o laboratório tradicional e atividades investigativas. Fonte: Borges, 2002.

A abordagem investigativa deve apresentar pelo menos três etapas: 1) os estudantes elaboram hipóteses; 2) observam, medem e manipulam variáveis; 3) analisam e interpretam os resultados. A avaliação e interpretação dos resultados não é necessariamente a última etapa, novas questões podem ser formuladas e o plano revisto (Wellington, 2000).

1.3 Química de Proteínas e construção de modelos 3D

O estudo da química das proteínas envolve a compreensão de muitos termos e conceitos complexos e abstratos, bem como, é importante ter um conhecimento prévio de Química Orgânica. O primeiro passo é apresentar aos estudantes os monômeros que compõem as proteínas, os aminoácidos (**Figura 1**), e suas particularidades físico-químicas. Os aminoácidos são ligados entre si através de ligações peptídicas, conceituada como a ligação entre o carbono do grupo carboxila de um aminoácido e o nitrogênio do grupo amino de um aminoácido vizinho (**Figura 3**). Essa ligação não ocorre de forma espontânea, e requer um aparato complexo, o qual apresentamos aos alunos como síntese

proteica, ou seja, dos processos de transcrição (DNA → RNA) e tradução (RNA → Proteína). Esse processo envolve diferentes moléculas como ácidos nucleicos e enzimas e a organela ribossomo (Nelson e Cox 2008; Alberts 2017). Muitas vezes, os professores não fazem essa transição entre o conteúdo de síntese proteica, geralmente explorado nas disciplinas de Biologia Celular e Biologia Molecular, e o conteúdo de química de proteínas. Logo após a apresentação da ligação peptídica aos estudantes, inicia-se a explicação das estruturas proteicas, primária (sequência de aminoácidos), secundária (e os exemplos clássicos, α-hélice e β-folheada), terciária (conformação final ou funcional da proteína) e, em alguns casos, quaternária (união de duas ou mais estruturas terciárias) (**Figura 4**), bem como, das interações químicas que estabilizam as estruturas proteicas.

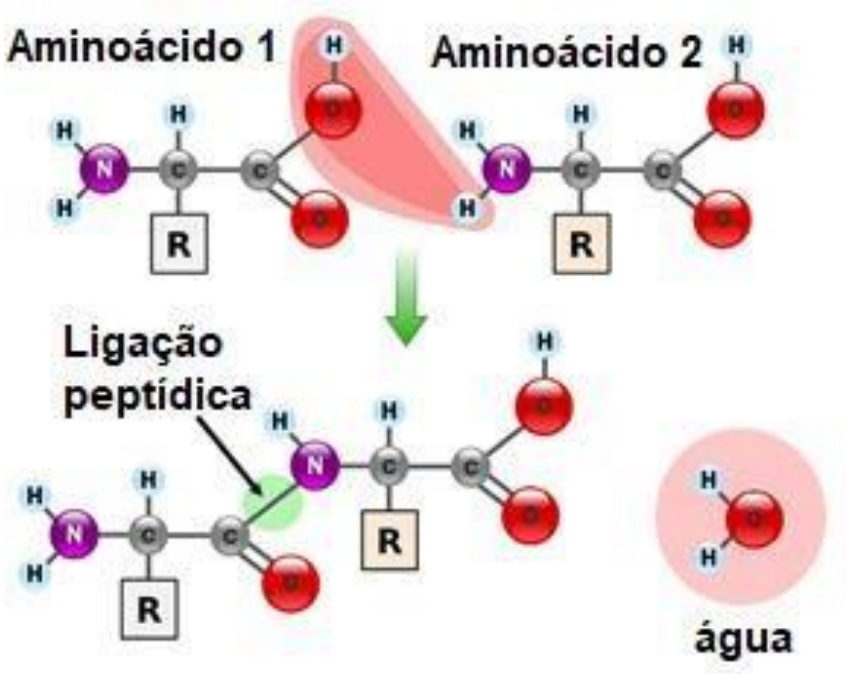

Figura 3: Ligação peptídica. Fonte: Banco de imagens do Google, com adaptações.

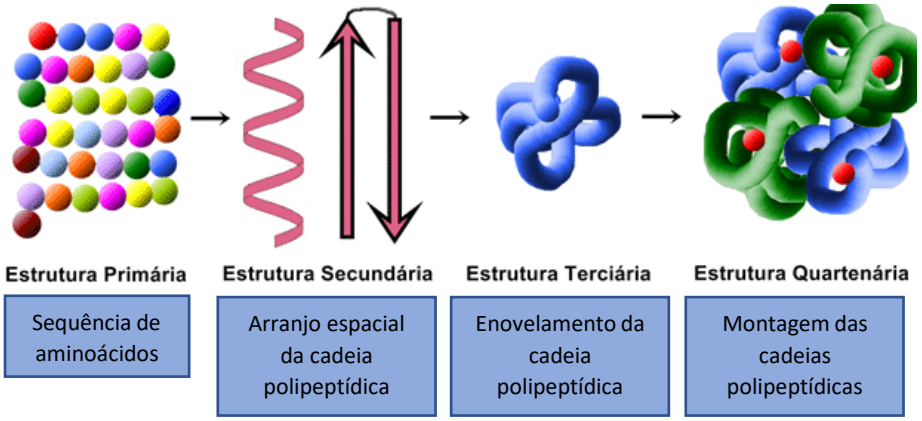

Figura 4: Organização estrutural das proteínas. Fonte: Banco de imagens do Google, com adaptações.

Nesse tópico, descrevemos de uma forma suscinta o que um aluno matriculado na disciplina de Bioquímica Básica irá encontrar ao frequentar uma aula teórico-expositiva sobre química de proteínas. A intenção aqui, não é reproduzir um livro texto sobre esse conteúdo, mas demonstrar a complexidade do tema, ressaltando a utilização de aulas interativas para estimular a participação dos alunos. Embora os livros de Bioquímica contenham figuras coloridas das moléculas e das rotas metabólicas que elas participam, faz falta uma visualização dimensional do que está sendo representado. Os modelos didáticos têm se comprovado eficientes em facilitar a aprendizagem e o entendimento de conceitos abstratos, permitindo que os conteúdos teóricos sejam revistos em aulas mais dinâmicas e interativas (Ceccantini 2006; Justina e Ferla 2006; Freitas et al., 2008; Orlando et al., 2009; Zierer 2017).

A construção de modelos didáticos pelos estudantes se encaixa no movimento *Maker*, que enfatiza a relevância do aprender fazendo a projeção e a construção de artefatos e a fabricação digital (Blikstein 2013). A forma mais comum de se adotar o movimento Maker na educação é a partir da criação e uso de Fab Labs (do inglês *fabrication laboratory*). Um Fab Lab é um espaço para prototipagem de objetos físicos conta com equipamentos específicos (de relativo baixo custo), como máquina de corte a

laser, impressoras 3D e máquina de corte de vinil, entre outros. No entanto, no âmbito escolar a na maioria das classes universitárias os professores adotam os princípios do movimento *Maker* usando materiais recicláveis ou de baixo custo, implementam a aprendizagem experiencial em sala de aula (Filatro e Cavalcanti 2018).

Recentemente, nosso grupo de pesquisa propôs a estudantes de Química bacharelado e licenciatura a construção da molécula de insulina utilizando para isso fio de cobre revestido e bolinhas de isopor (Oliveira et al. 2017). De uma forma geral, o trabalho foi bem aceito pelos estudantes; porém, como pontos negativos os alunos apontaram o tempo gasto na construção da molécula, visto que os estudantes construíram todos os 51 aminoácidos que fazem parte da molécula de insulina bem como fizeram as ligações peptídicas e pontes de hidrogênio. Outro ponto questionado pelos estudantes é o fato de como sabíamos a sequência dos aminoácidos, ou seja, os alunos desconheciam como ocorre o processo de síntese proteica.

A construção de modelo proteico 3D é aceita pelos estudantes. Porém, a escolha da proteína deve ser cuidadosa, prevendo o tempo que será gasto em todas as etapas envolvidas na construção do modelo. Ainda, a utilização de atividades lúdicas sobre o processo de síntese proteica, como jogos (Cavalho et al. 2018) ou encenações (Schmitz et al. 2022), ou o simples ato de o aluno transcrever o RNA "como se fosse um ribossomo" são fundamentais, e devem ser atividades realizadas previamente a construção do modelo proteico 3D.

1.4. Fatores que alteram a atividade enzimática e utilização de modelos animais em aulas práticas

Logo após o conteúdo de Química de proteínas, os estudantes da disciplina de Bioquímica Básica aprenderão sobre tipos de proteínas, dentre eles, as enzimas. Assim

como pontuado para o tópico de Química de proteínas, o estudo das enzimas, enzimologia, requer dos estudantes atenção e tempo de estudo, bem como, conhecimento prévio de alguns conceitos químicos e uma boa base de Química de proteínas.

As enzimas são catalisadores biológicos com alto poder de especificidade, bem como, são fundamentais para a manutenção da vida (Nelson e Cox 2008; Alberts 2017). É fundamental que o estudante compreenda o conteúdo de enzimologia, e não apenas decore, pois ao decorrer do curso escolhido (principalmente os cursos da área da saúde e das ciências agrárias) os alunos irão se deparar com diversas disciplinas que retomarão o tema enzimas, por exemplo, diversas doenças, independente da espécie estudada, podem ser causadas por déficit enzimático, diversos fármacos atuam inibindo a atividade de enzimas, dentre outras situações.

Como salientado no tópico 1.3, a ideia desse tópico não é reproduzir um livro texto sobre o conteúdo de enzimologia, mas sim, demonstrar a importância e complexidade desse conteúdo, pontuando para a necessidade de aulas práticas investigativas para que o aluno assimile o conteúdo ministrado nas aulas teórico-expositivas. Alguns autores têm implementado o uso de aulas práticas investigativas no ensino de enzimologia, e têm observados alunos mais motivados e comprometidos com o processo de aprendizagem (Pope et al. 1998; Kratasyuk e Kudinova 1999; Boyce et al. 2004; de Almeida et al. 2014; Gonçalves 2022).

Até pouco tempo animais vertebrados eram utilizados em aulas práticas de ciências, como por exemplo, em aulas para demonstrar o efeito de xenobióticos, como inibidores enzimáticos. Atualmente, as Universidades adequaram suas práticas ao princípio dos 3Rs (do inglês: *Replacement, Reduction and Refinement*), criado por Russell and Burch em 1959 como premissa para a pesquisa. Métodos que evitam ou substituem o uso de animais protegidos de acordo com *Scientific Procedures* Act 1986,

alterado 2012 (ASPA), em um experimento onde eles seriam utilizados, vêm sendo propostos. São considerados animais protegidos: todos os vertebrados vivos, incluindo algumas formas imaturas, e cefalópodes (por exemplo, polvo e lulas). As alternativas propostas incluem a substituição, como a utilização de tecidos e células, modelos matemáticos e animais invertebrados (como moscas e baratas).

Nesse contexto, surge a barata *Nauphoeta cinerae* (Blaberidae) (**Figure 5**) como uma alternativa ao uso de animais vertebrados em aulas práticas. Essa espécie de barata de origem africana, vive cerca de um ano, apresenta um tamanho semelhante a barata comum, mas um comportamento mais calmo e não voa. Em adição, essa espécie vem sendo utilizada como animal experimental em alguns trabalhos científicos, principalmente na área da toxicologia (Rodrigues et al., 2013; Adedara et al., 2015, 2016; Olagoke et al., 2021). São poucos os estudos que medem a percepção dos estudantes quanto à utilização de animais não vertebrados em aulas práticas. De maneira geral percebe-se que os estudantes consideram a utilização de animais em aulas importante para seu aprendizado, mesmo que alguns se sintam desconfortáveis em utilizá-los (Rochelle et al., 2016).

Figura 5: *Nauphoeta cinerae*; ninfas e adultos. Fonte: banco de imagens do Google.

2. OBJETIVOS

Avaliar a utilização de metodologias ativas no estudo de estruturas protéicas e enzimologia e na aprendizagem dos estudantes.

2.1 Os objetivos específicos foram:

- avaliar a aprendizagem dos estudantes de graduação antes e depois das aulas práticas;
- verificar a opinião dos estudantes sobre modelos proteicos;
- avaliar se a construção de um modelo proteico 3D melhora a compreensão dos estudantes sobre química de proteínas;
- avaliar se estudantes de graduação aceitam trabalhar com modelo de animais alternativos nas aulas práticas.

RESULTADOS

Os nossos resultados que fazem parte desta dissertação estão apresentados sob a forma de manuscrito submetido à publicação e artigo científico. Os itens Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se nos respectivos manuscrito e artigo.

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

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

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Abstract

Proteins are important macromolecules because they perform several roles in living organisms. Proteins' function is related to their three-dimensional (3D) structure and its synthesis occurs via a complex mechanism. Aiming to highlight this knowledge for students enrolled in a Biological Science undergraduate course, we performed practical activities in which students perform the steps of human glucagon synthesis and assembled a three-dimensional model of this protein. The activities involved 3 classes of 2 hours each. Fifteen students simulated the steps of human glucagon synthesis, from the gene transcription to the 3D structure assemblage, in an active way. In the end, we asked the students to answer an opinion survey and applied again after one year. The results indicate students were motivated and curious and the classes contributed to their learning in protein synthesis, although they lack chemistry skills related to intermolecular interactions and amino acids' later.

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

Introduction

Among the macromolecules, proteins are the main component of living organisms. Besides the structural function, proteins are responsible for most of the body's chemical reactions (Nelson and Cox 2008). Protein amino acid sequence is determined by the genetic code, being the sequence similarity among species proportional to the evolutionary distance in most cases (Nei and Kumar 2000).

The synthesis of one protein is determined by many environmental and internal signals that start and regulate its transcription and translation (Alberts 2017). The copy of the nucleotides sequence coded in the DNA (deoxyribonucleic acid) to mRNA (messenger ribonucleic acid) is the first step in protein synthesis, it depends on many transcriptional factors (regulatory proteins and RNAs) and RNA-polymerase. Once the mRNA is produced, in eukaryotes, it should be processed before leaving the nucleus. The translation occurs in the cytoplasm and depends on many translation factors (regulatory proteins and RNAs), ribosomes, and transport RNA. In the translation, the ribosome decodes the mRNA nucleotide triplets to ensure that the correct amino acids brought by the transport RNA will be added to the polypeptide chain and attached by a peptide bond (Alberts 2017). Many proteins suffer post-translational processing that includes the covalent addition of functional groups, proteolytic cleavage, or degradation of entire proteins.

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, also known as the R group. The general structure without R groups is called the protein backbone. The interaction by hydrogen bonds and other molecular forces among the protein backbone determine the secondary structures, which are α-helix (helicoidal structure) and

β-sheet (beta strands connected laterally forming a flatted and rigid structure). 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.

The glucose homeostasis *in vivo* in both animals and humans is regulated by two proteins (hormones) insulin and glucagon (Jiang and Zhang 2003). Insulin secretion from pancreatic beta-cells is stimulated largely by elevated glucose concentrations, reducing circulating glucose levels via inhibition of glycogenolysis and gluconeogenesis, accompanied by stimulation of glycogen synthesis in the liver (Nelson and Cox 2008; Jiang and Zhang 2003). Hyperglycemic actions of glucagon are mediated through the promotion of glycogenolysis and gluconeogenesis in the liver, whilst also 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 fashion. It is accepted that 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. The polypeptide hormone glucagon has 29 amino acids and is produced by PC2-mediated cleavage of proglucagon in pancreatic alpha cells (**Figure 1**). Glucagon is well-known even to people outside the academy which make it a good candidate to be used in the teaching of protein synthesis, structure, and function.

Protein synthesis, structure, and function in undergraduate courses are taught mainly in Biochemistry and Molecular Biology classes and are contents considered difficult and abstract by the students. The construction of three-dimensional (3D) models demonstrated effectiveness to improve 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 proposed the construction of a 3D model of glucagon in an undergraduate course in Biological Science at a Brazilian public University. We choose glucagon because it is well-known in the general sense, is translated as proglucagon, is processed in different manners 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.

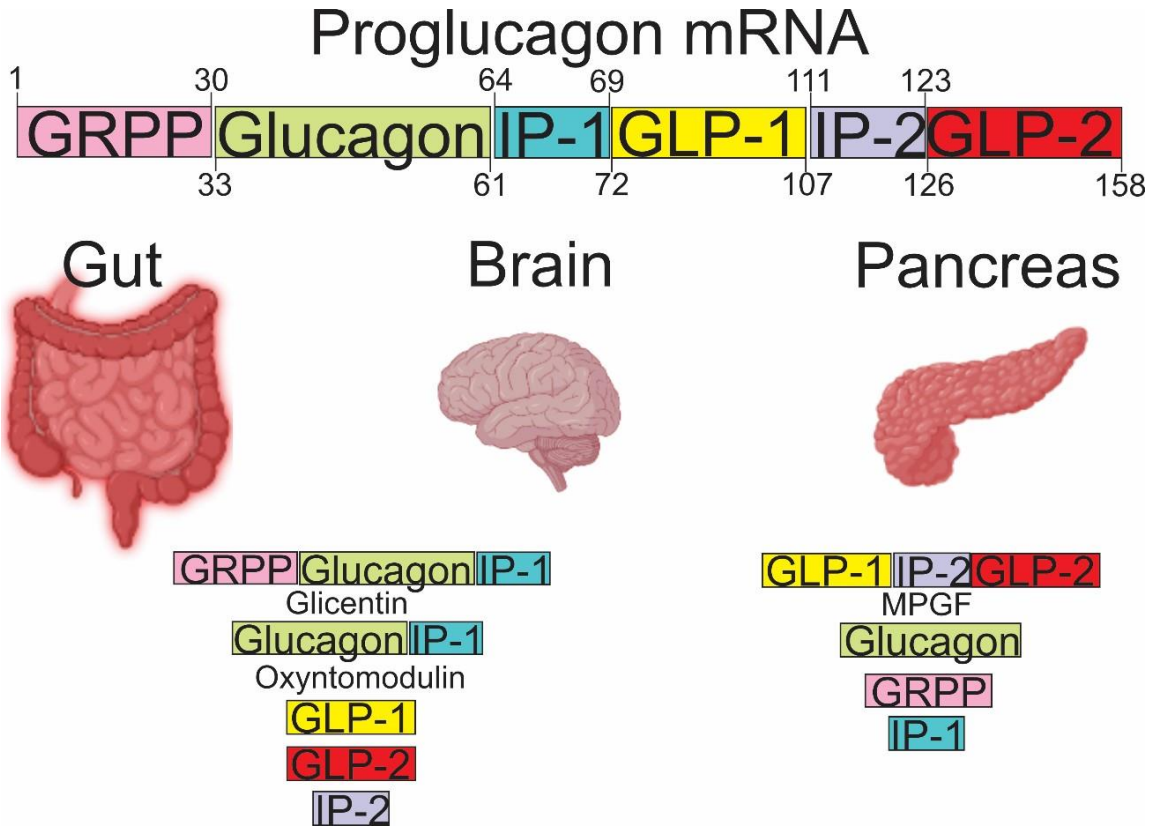

Figure 1: A schematic overview of tissue-specific proglucagon processing. In the gut and in the brain, proglucagon is processed by convertase 1/3 (PC1/3) to generate glicentin, oxyntomodulin, glucagon-like peptides-1 and -2 (GLP-1, GLP-2), and intervening

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.

Materials and Methods

This study was applied to Biological Science undergraduate students who were enrolled in the Practical Biochemistry course at a Brazilian Public University. The study was conducted with 15 students and the experimental activities were divided into three classes of two hours each. During the classes, the students were continuously asked/challenged about the protein structure, properties, and function.

In the first class, the protein synthesis topic was briefly reviewed. The students received a printed copy of the 20 amino acids' structural formula, the genetic code, and the human pancreatic proglucagon mRNA (NM_002054.5:100; O'Leary et al. 2016) (**Figure 2**). The students translated the proglucagon mRNA and after that, the Professors discussed with the students the post-translational modifications of proglucagon (158 amino acids) to glucagon (29 amino acids) (**Figure 3**).

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 the amino acids using beads of different colors (according to the choice of each group) to represent the atoms of each element, besides pliers, wire (1-4 mm) or nylon cords, and hot-melt adhesive were used to connect the beads (atoms). Each group was responsible to determine the number of beads and amino acids necessary to do the activity. Considering the bond distances in the amino acids, the students chose freely the scale used to do the protein model.

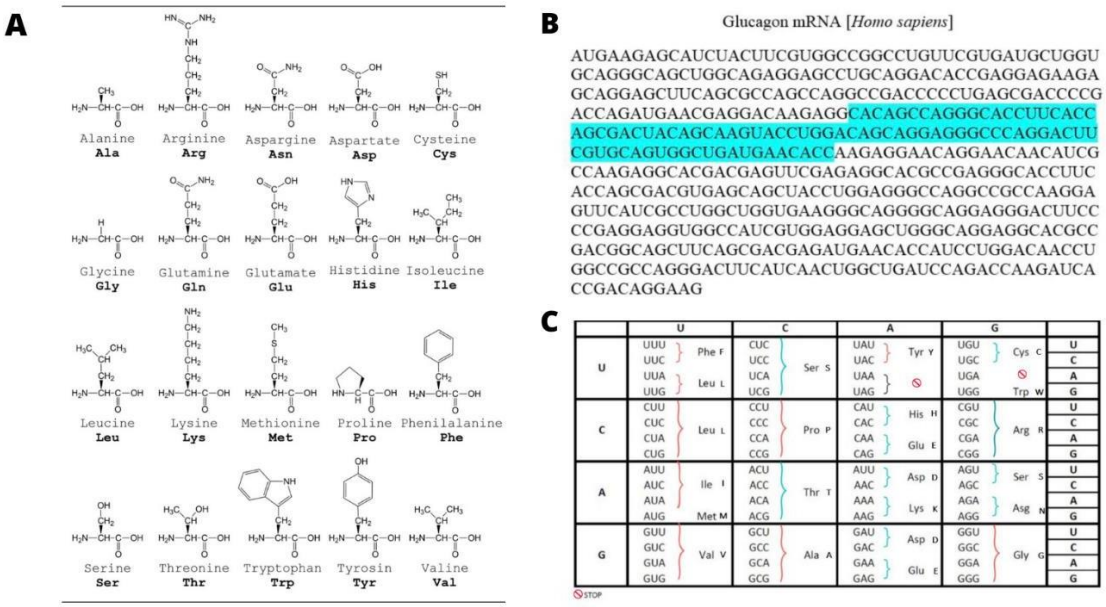

Figure 2: Material distributed to students. (A) amino acids structural formula; (B) proglucagon mRNA. The glucagon mRNA is highlighted in blue; (C) Genetic code.

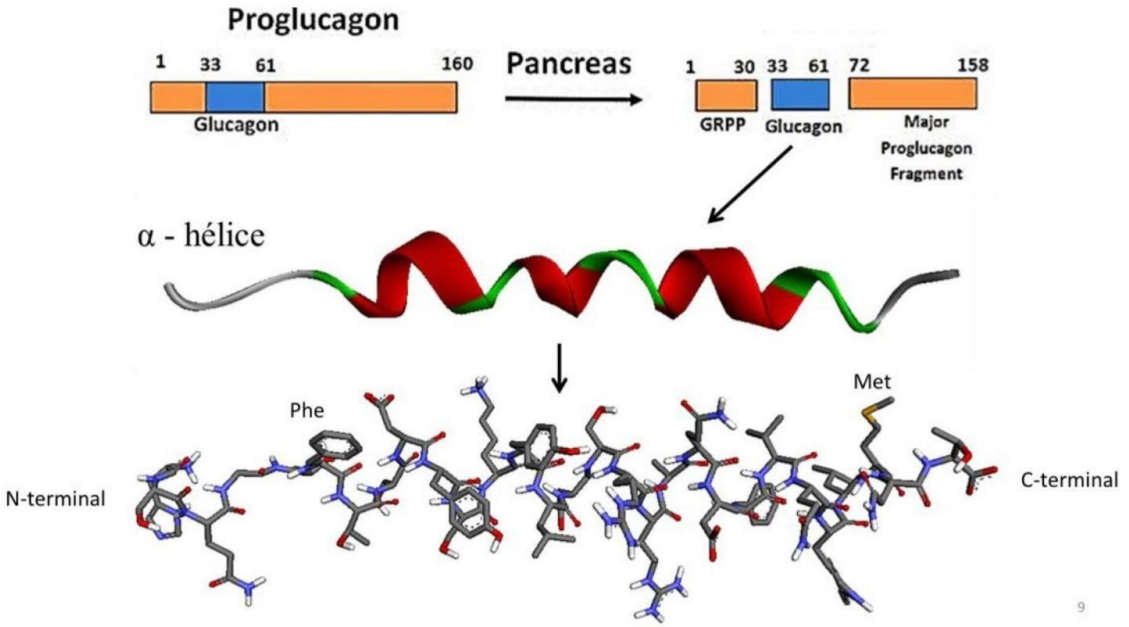

Figure 3: Material distributed to students. Proglucagon translational modifications and glucagon tertiary structure.

Finally, in the third class, the students constructed the protein backbone using the same pattern of beads and joined the amino acids by placing them in the correct sequence while assembling the α-helix and making the respective hydrogen bonds. In this step, the

students used the glucagon crystallographic structure (PDB ID 1GCN) as a comparison, 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 (**Table 1, part A**). With the goal to test the learning, one year later another questionnaire was applied to the same students (**Table 1, part B**).

Table 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 ( ) strongly agree ( )
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 ( )

Results and Discussion

Protein chemistry is an abstract subject to teach and learn. It is pivotal that undergraduate students dominate this topic to understand numerous cellular mechanisms, disease development, and drug action, among other cellular processes. The use of didactic tools to facilitate the students' understanding of proteins 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.

Considering that our sample already was enrolled in theoretical Biochemistry classes and had concluded the Cell Biology theoretical topic, at the first moment we briefly reviewed the main topics involved in protein synthesis. The Professors explained the steps of protein synthesis according to the central dogma of molecular biology: the DNA is transcribed into mRNA that, in turn, mRNA is translated into the protein (Alberts 2017). A good alternative to review the protein synthesis subject is the use of playful activities, such as board games (Cavalho et al. 2018) and the representation (students acting in a play) of the protein synthesis (Schmitz et al. 2022). Unfortunately, we could not apply these activities in our classes due to the discipline schedule. In fact, thinking about the discipline schedule and aiming to optimize the time, we choose the glucagon hormone for the practical classes, because it is a relatively small protein, with 29 amino acids.

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, performed the translation of 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 students and Professors discussed the post-translational protein's modifications and how the proglucagon is modified to its active form, glucagon. 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**). The colors were also chosen by students, so we had different colors among the groups.

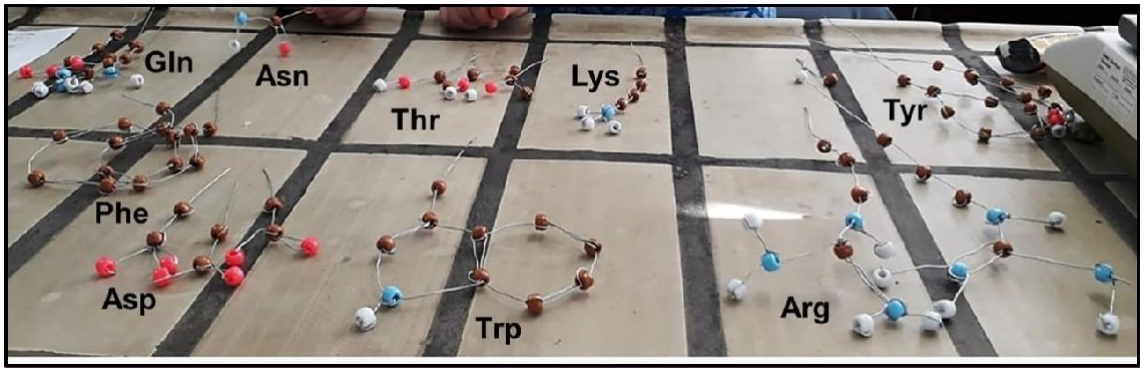

Figure 4: Examples of lateral chains assembled by the students.

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, and after the lateral chains were added to the protein.

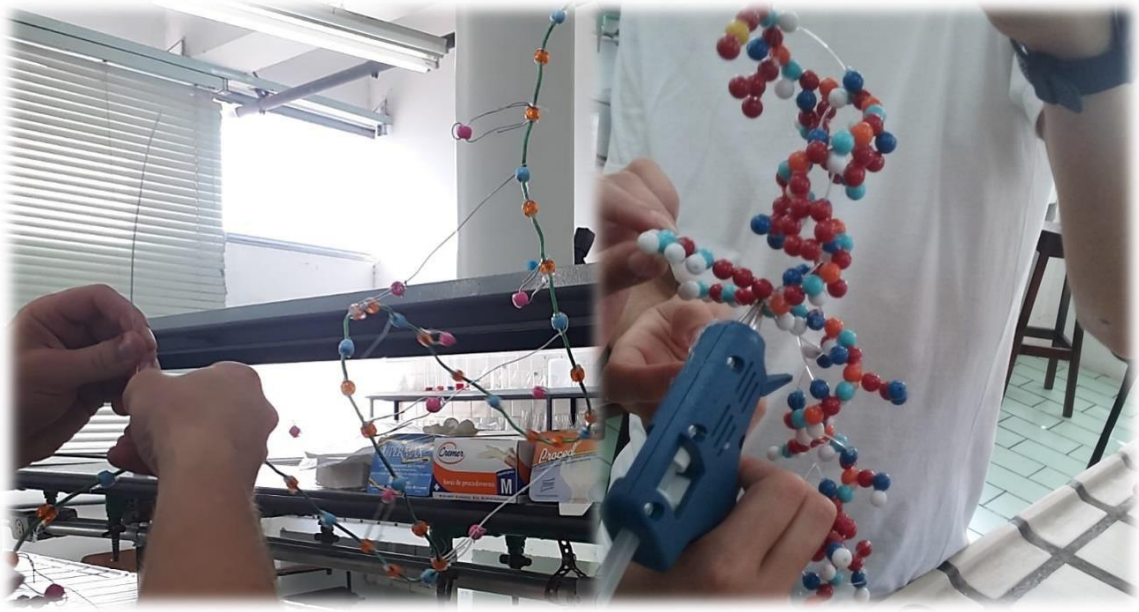

Figure 5: Glucagon being assembled by the students.

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. In this way, Professors pointed out that the sequence of amino acids determines the tertiary structure, and in consequence, the protein function. It is important to remember that this sample of students already had theoretical biochemistry classes, so, we were performing a review of those contents.

Figure 6: Different glucagon molecules assembled by the students.

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 proteins' 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 agree with this. In addition, ~70% of students strongly agree 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 problems comprehending chemistry subjects, such as intramolecular interactions, which probably have the origin in middle school. In fact, Schmitz et al (2022) demonstrated that 9th-grade students had difficulty in the comprehension of the atom concept and or molecular interactions. Nogara et al. (2018) demonstrated that Brazilian undergraduate students considered their chemistry skills from high school incomplete. Interestingly, Alves et al. (2013) demonstrated that ~50% of the Biological Sciences undergraduate students did not conclude the General Chemistry program at a Brazilian University.

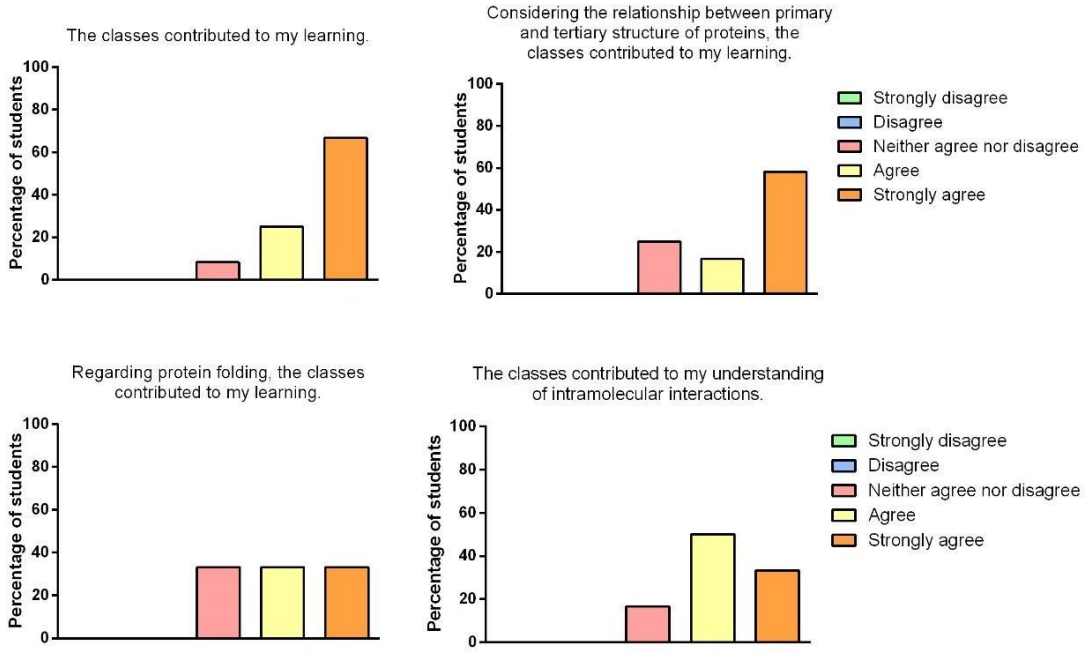

Figure 7: Students opinion about the classes' contribution to their knowledge improvement.

We also asked the students which were the classes' positive and negative points. **Table 2** shows the categories that emerged from the data analysis. The data analysis was performed using Content Analysis (Bardin, 2011). In relation to the positive aspects, students called attention to the way the professors conducted the class, 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. 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). 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. In fact, 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). 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. Several studies have been demonstrating that practical classes to explain an abstract topic are well accepted by students and, in a general way, students that participate in this kind of classes had a better performance in the topic's evaluation (White et al. 2002; Silva et al. 2016; Oliveira et al. 2017; Schmitz et al. 2020). Neto and Oliveira (2015) demonstrated that majority of Biological Sciences degree students considered practical classes as an instrument to explain a given theory.

Table 2: Practical classes' positive and negative aspects pointed by the students.

Category	Occurrence
Positive points	
Differentiated class	5
Articulation between theory and practice	1
Development of students' autonomy	3
3D structure visualization	3
Knowledgeable and earing teachers	6
Did not answer	1
Negative points	
Long class	1
Did not explain the distance between amino acids	1
Did not answer	10

Regarding the practical classes' negative aspects, most of the students did not answer this question, indicating they did not recognize any negative aspects. Although, one student pointed out that the class demands a long period of time, 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, mostly, and teachers 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 student's protagonist in their own 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. The students' answers are described in **Table 3** and **Figure 8**. A great number of students (~70%) remembered the practical, and the other 30% vaguely remembered it. More than 90% of the students remembered which protein was assembled in the classes, glucagon. 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. In fact, 100% of the students answered correctly the three first questions about protein's primary, secondary, and tertiary structures. But only 36% of the students correctly answered the question "Which region of amino acids is responsible for protein polarity and molecular stabilization?", emphasizing, again, the student's lack of chemical skills.

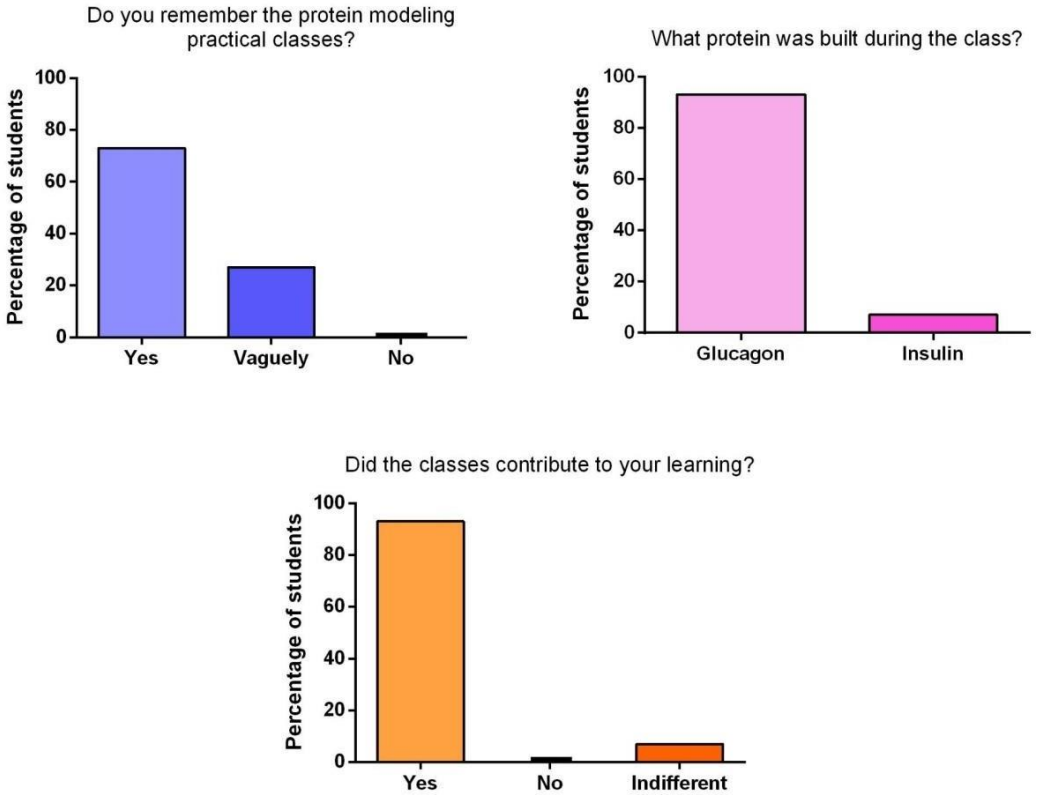

Figure 8: Answer of students to one year after the classes survey.

Table 3: Percentage of students correct answers about protein chemistry one year later the practical classes.

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%

Conclusion

In this study, the biological science degree students assembled a 3D model of a protein, namely, glucagon, in three practical classes of approximately 2 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. Unfortunately, it was possible to identify that these students had a lack of chemistry skills, which are necessary to understand intra- and intermolecular interactions, among other concepts. In future classes, the Professors must evaluate the student's chemistry skills, and prepare some activities to overcome this problem before starting the protein 3D model construction.

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3.2 Artigo – Cockroaches: an alternative model to teach enzymatic inhibition to undergraduate students

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Cockroaches: an alternative model to teach enzymatic inhibition to undergraduate students

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ABSTRACT

Enzymes are organic molecules known for their catalytic nature because they accelerate metabolic reactions inorganisms. The study of enzyme functions and modes of action is essential to understand Biochemistry. This study aimed to analyse the acetylcholinesterase enzymatic activity, as well as the enzymatic inhibition mechanism in practical classes. The enzyme was obtained from the cockroach (*Nauphoeta cinerea)* head, an alternative model to the use of vertebrates in practical classes. For this study, practical classes were carried out in two undergraduate courses: Biology and Veterinary Medicine. It was observed the knowledge consolidation through the analysis of the answers given to the questionnaire. The students' enthusiasm to work with animals was evident. This fact led to a better understanding about the subject and more attractive classes.

KEYWORDS

Enzymes; biochemistry teaching; practical classes; cockroaches; AChE; CPF

Introduction

Enzymology has an essential role in different areas, for instance, medical diagnostics, proteomics, and environmental and occupational toxicology (Robinson 2015; Nogara et al. 2018). The study of enzymes, which comprises their structures, functions, kinetics and factors that alter enzymatic activity (temperature, pH, substrate, cofactors and xenobiotics) is considered abstract. Such abstraction makes the enzymology course unattractive to students. A way to teach enzymology is with theoretical classes where concepts and procedures are exposed to students. Experimental classes are an efficient way to improve students' learning since the practice of the phenomenon promotes understanding, transforming the abstract concept into a concrete one (Galiazzi and Gonçalves 2004). This way, experiments are a great tool to verify a given phenomenon, the professor is a guide/helper, correcting mistakes, and misconceptions, helping the students reach a conclusion (Domin 1999; Oliveira 2010).

A common characteristic of experimental classes is the use of real world problems as starting points. This contributes even more to decrease the abstraction of certain topics. In this context, several questions regarding the safety of organophosphates to the environment and public health still need answers (Voorhees et al. 2017). The Brazilian economy relies on agriculture, where dozens of pesticides are used, including organophosphates. One common organophosphate is chlorpyrifos (CPF), which acts via the inhibition of acetylcholinesterase (AChE) (Bednarska et al. 2017). AChE

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catalyzes the cleavage of the acetylcholine in choline and acetate, stopping the interaction between the neurotransmitter and the cholinergic receptor. When the AChE activity is negatively affected, the cholinergic receptor will be continuously excited. This phenomenon can be seen in classic physical effects such as contortion, vibration, convulsion and even death (Amenta et al. 2006; Gorecki et al. 2016).

Considering that CPF is used as a pesticide in agriculture, we proposed the use of an insect model to teach its effects. The cockroach *Nauphoeta cinerea*, as an experimental model, has safe handling and maintenance, simple anatomy and a rapid proliferation cycle (Wilson-Sanders 2011; Rodrigues et al. 2013; Sharma, Khurana, and Muthuraman 2017; Silva et al. 2018). The ethical concerns for the use of vertebrates in research, classes and experiments need to be considered. The use of vertebrates has positive aspects; however, it needs to be rational. Invertebrates, including insects, are being used by researchers and professors as alternative models in toxicological studies (Ahmad 1995; Rodrigues et al. 2013; Silva et al. 2018) and experimental classes (Vital et al. 2004; Porntrai and Damrongphol 2008; Fala, Correia, and Pereira 2010; Bonachea 2019).

In this context, this work aimed to use for the first time the cockroach *Nauphoeta cinerea* as an experimental model in practical classes. This animal model helped to teach and make clear to students the factors that alter enzymatic activity, as well as demonstrating a real world problem generated by organophosphate intoxication.

Materials and methods

This study was carried out at the Federal University of Santa Maria, Brazil. Thirty-nine undergraduate students participated in the practical classes, 23 students from the undergraduate course in Veterinary Medicine and 16 from the undergraduate course in Biology. This work was divided into 3 practical classes of 2 hours each.

First class – factors that affect enzymatic activity

Firstly, a questionnaire was given to the students aiming to analyse their previous knowledge about enzymes (Table 1).

After answering the questionnaire, the students were divided into three groups and each group received a different protocol (Figure 1): a- different pH; b– different substrate; and c– different temperature. It was reinforced that the students must wear gloves, glasses, and a laboratory coat.

Before starting the tests, the professor lectured using the blackboard to explain the enzymatic assay (Ellman's method) used to measure AChE activity (Figure 2) (Ellman et al. 1961). AChE was obtained from the head of the cockroaches (*Nauphoeta cinerea*) as described by Silva et al (2018).

After the explanation, the students were instructed to start the experiments. Although in our class, the students only observed the reaction (qualitative experiment), the quantitative experiment would involve spectrophotometrically measuring the reaction at 412 nm.

When the experiments were finished, a group discussion was carried out. Each group presented their findings and, guided by the professor, explained the possible mechanism of enzymatic inhibition and inferred the best substrate, pH, and temperature to the AChE enzymatic assay.

Second class – cockroaches' behaviour

In this class, students evaluated the effects of CPF exposure on cockroach *N. cinerea* behaviour. CPF (100 times diluted in water) was injected in the cockroaches' head and body, as depicted in Figure 3. In the control group, water was injected in the cockroaches head and body. It was reinforced that the students must wear gloves and a laboratory coat.

Before beginning the experiments, the professor made a short lecture explaining the uses of CPF and its intoxication effects. The injections were performed in the fume hood.

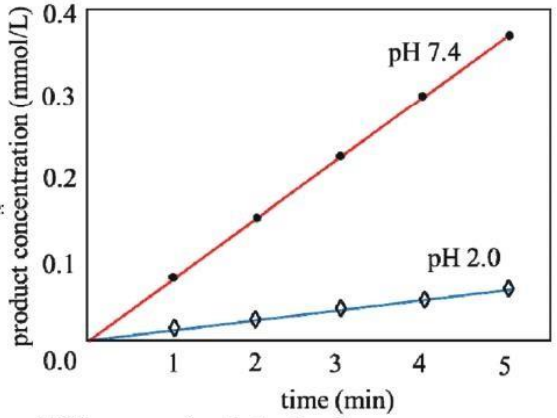
Table 1. Questionnaire used to evaluate the students' pre- and post-practical classes knowledge.

Name:

Please, indicate whether the statements below are true or false:

( ) Enzymes are biological catalysts.
( ) Enzymes are responsible for speeding up body's reactions.
( ) Enzymes are composed of fatty acids and are part of cholesterol metabolism.
( ) Enzymes are constructed by amino acids.
( ) All enzymes have at least one protein moiety.
( ) The non-protein component of an enzyme is called active site.
( ) The active site is where the substrate interacts with the enzyme.
( ) One of the enzymes functions is to decrease the activation energy of metabolic reactions.
( ) Enzymes act independently despite the type of substrate.
( ) There are enzymes that have their optimal activity with acidic pH.
( ) Enzymes activity can be altered due to changes in the pH medium
( ) Denaturation alters the primary enzyme structure.
( ) Xenobiotics can inhibit enzymes activity
( ) Different molecules of the substrate may interact with enzyme regions that are different from the active site.
( ) The medium temperature can change the enzyme activity in both ways, positively and negatively.
( ) The activity of an enzyme can be measured from the products of its reaction with the substrate.

The figure below shows the acetylcholinesterase (AChE) enzyme activity in media with different pH values. Please indicate whether the following statements are true or false:



( ) AChE is an enzyme found in the stomach
( ) AChE shows higher activity at pH 7.4
( ) In an acidic medium, AChE has higher catalytic activity
( ) At pH 2, AChE is denatured
( ) The product concentration decreases over time

During the class, students observed the cockroaches' behaviour for 30 min. After the behavioural observation, the insects were cryoanesthesied, their heads were cut off, homogenised and centrifuged for posterior biochemical analyses (Silva et al. 2018). The supernatant was frozen until the next class.

Third class – *Ex vivo* experiments

In this class, students analysed the AChE activity in the heads of the cockroaches injected with CPF or water (control) in the previous class.

Firstly, the professor reminded the students about the best pH, water temperature and substrate to perform the AChE enzymatic assay. In sequence, the students needed to check their notes from the first class. It was reinforced that the students must wear gloves, glasses, and a laboratory coat.

a

Experimental protocol – *in vitro* assay: different pH

In a 5 mL glass tube (room temperature), add the reagents as described below.

Glass tube	H_2O (mL)	pH 7.4 (mL)	pH 4.4 (mL)	pH 0.4 (mL)	Sample (mL)	ASCh (mL)	DTNB (mL)
A	1.5	2.0	0.0	0.0	0.5	0.5	0.5
B	1.5	0.0	2.0	0.0	0.5	0.5	0.5
C	1.5	0.0	0.0	2.0	0.5	0.5	0.5

Take notes and make some comparisons.

Additional information:

pH 7.4: 100 mM Potassium phosphate solution, ajusted to pH 7.4 with HCl

pH 4.4: 100 mM Potassium phosphate solution, ajusted to pH 4.4 with HCl

pH 0.4: 100 mM Potassium phosphate solution, ajusted to pH 0.4 with HCl

Sample: supernatant from homogenized cockroaches head

ASCh: 4 mM acetylthiocholine

DTNB: 25 mM 5,5-dithio-bis-(2-nitrobenzoic acid)

b

Experimental protocol – *in vitro* assay: different substrates

In a 5 mL glass tube (room temperature), add the reagents as described below.

Glass tube	H_2O (mL)	pH 7.4 (mL)	Sample (mL)	ASCh (mL)	BSCh (mL)	DTNB (mL)
A	1.5	2.0	0.5	0.5	0.0	0.5
B	1.5	2.0	0.5	0.0	0.5	0.5

Take notes and make some comparisons.

Additional information:

pH 7.4: 100 mM Potassium phosphate solution, ajusted to pH 7.4 with HCl

Sample: supernatant from homogenized cockroaches head

ASCh: 4 mM acetylthiocholine

BSCh: 4 mM butyrylthiocholine

DTNB: 25 mM 5,5-dithio-bis-(2-nitrobenzoic acid)

c

Experimental protocol – *in vitro* assay: different temperatures

In a 5 mL glass tube (room temperature), add the reagents as described below.

Glass tube	H_2O (mL)	pH 7.4 (mL)	Sample (mL)	Water temperature (°C)	ASCh (mL)	DTNB (mL)
A	1.5	2.0	0.5	0–5	0.5	0.5
B	1.5	2.0	0.5	25–27	0.5	0.5
C	1.5	2.0	0.5	90–100	0.5	0.5

Take notes and make some comparisons.

Additional information:

pH 7.4: 100 mM Potassium phosphate solution, ajusted to pH 7.4 with HCl

Sample: supernatant from homogenized cockroaches head

ASCh: 4 mM acetylthiocholine

DTNB: 25 mM 5,5-dithio-bis-(2-nitrobenzoic acid)

Figure 1. Experimental protocols (a, b, and c) used in the first practical class.

After a brief explanation about the class objectives, the students received the protocol (Figure 4) to start the enzymatic assay.

In the end of the class, a group discussion was organised aimed to examine the obtained results. Students were challenged to make a correlation between the behaviour observed in the previous

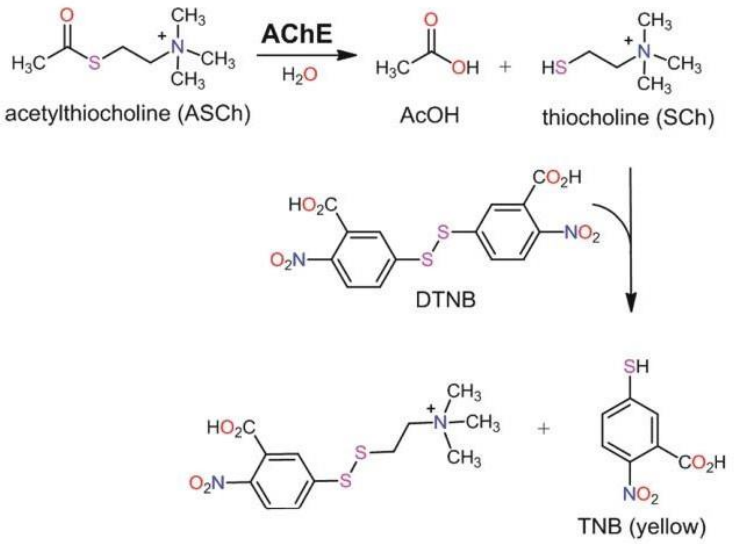


Figure 2. Ellman's method. The acetyltiocholinesterase (ASCh) is used as a substrate. After ASCh hydrolysis by AChE, thiocholine (SCh), it can react with DTNB leading to the yellow compound TNB. The yellow intensity can be measure at 412 nm.

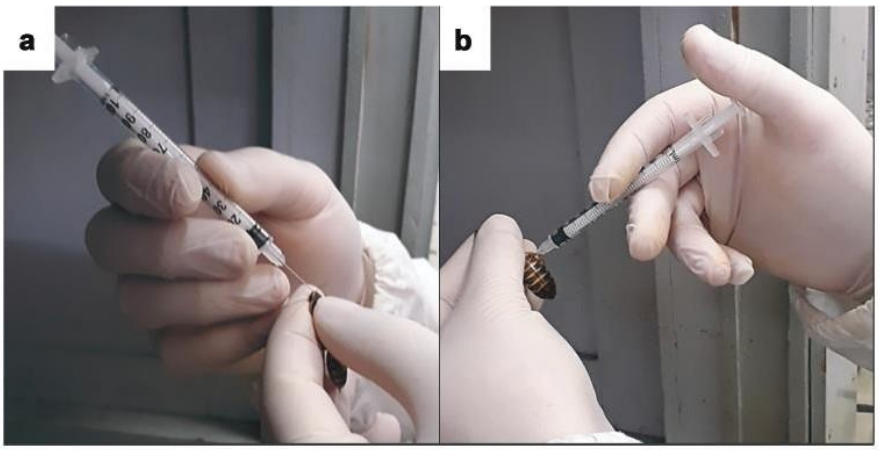


Figure 3. Injection sites. a- Injection in the head and b- in the body.

class and the AChE activity inhibition. In addition, students answered the questionnaire described in Table 1 again, so we could see the improvements in their learning.

Online questionnaire about students' opinion

Four weeks after finishing the practical classes, an online questionnaire was sent to the students to get their opinion about the classes. The questionnaire is available in Table 2.

Experimental protocol – *ex vivo* assay

In a 5 mL glass tube (room temperature), add the reagents as described below.

Glass tube	Injection	H_2O (mL)	pH 7.4 (mL)	Sample (mL)	ASCh (mL)	DTNB (mL)
A	CPF-head	1.5	2.0	0.5	0.5	0.5
B	CPF-body	1.5	2.0	0.5	0.5	0.5
C	Water-head	1.5	2.0	0.5	0.5	0.5
D	Water-body	1.5	2.0	0.5	0.5	0.5

Take notes and make some comparisons.

Additional information:

pH 7.4: 100 mM Potassium phosphate solution, ajusted to pH 7.4 with HCl

Sample: supernatant from homogenized cockroaches head

ASCh: 4 mM acetylthiocholine

DTNB: 25 mM 5,5-dithio-bis-(2-nitrobenzoic acid)

Figure 4. *Ex vivo* protocol.

Table 2. Online questionnaire answered by the students after the classes. Zero means completely disagree and five completely agree.

Online questionnaire						
Questions	0	1	2	3	4	5
A) How much do the classes contributed to your knowledge?						
B) Do you agree with the use of cockroaches in classes?						
C) Do the classes helped understand the factors that alter enzyme activity?						

Results

First class – factors that affect enzymatic activity

In the first class, the students were divided into three groups. Each group analysed one factor that may affect enzymatic activity: pH, temperature and substrate specificity.

The yellow colour presence in the test tubes was used as indicative of enzymatic (AChE) activity (Ellman et al. 1961). After the reaction, the students were challenged to make assumptions about what happened in each tube. The students observed that the assays carried out in the acidic medium, as well as in the high- and low-temperature medium did not have an intense yellow colour which is characteristic of enzyme activity. The assays performed with BSCh as a substrate

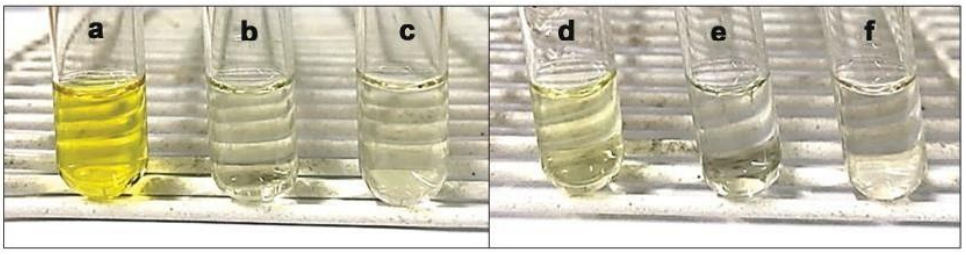


Figure 5. Test tubes for the AChE activity, using different conditions. a) ASCh + pH 7.4; b) ASCh + pH 4.4; c) ASCh + pH 0.4; d) BSCh + pH 7.4; e) BSCh + pH 4.4; f) BSCh + pH 0.4. In these examples, assays were performed at room temperature.

showed low yellow intensity when compared with the assay performed with ASCh, indicating low enzymatic activity. In this way, the best conditions found to determine the AChE activity (from cockroaches) were using the ASCh substrate, pH 7.4 and room temperature (~25°C) once its parameters culminated in a fast high yellow intensity in tested mediums. Figure 5 represents the findings. These parameters were established as standards to the third class.

In the end of the class, all students discussed the results. Through the discussion and with the guiding questions asked by the professor they concluded that in acidic medium and high temperature, the enzyme had low activity, probably because it is denatured. With low temperature, the enzyme activity is low due to the lower molecular motion (i.e., low reaction velocity). Regarding the substrates, it was explained that the BSCh has a bigger carbon chain than the ASCh and it cannot access the AChE active site with the same affinity as ASCh, probably because of the steric hindrance (Chatonnet and Lockridge 1989; Pezzementi et al. 2011; Rosenberry et al. 2017).

Second class – cockroach behavioural analysis

To demonstrate the *in vivo* effects of xenobiotic exposure, in the second class the students injected two substances in the cockroaches: CPF and water. The injections were made in the head and body of the insects to compare the cockroaches' behavioural and AChE activity (third class).

Table 3 summarises the observations in relation to the cockroaches' behaviour. In general, the cockroaches that were exposed to CPF presented agitation, loss of posture, abortion, convulsion and death, while the animals that were injected with water showed slow movements and did not convulse.

The students' analysis of the cockroach behavior in comparison to control (cockroaches that were injected with water) indicates that the symptoms observed may be related to nervous system damage. The symptoms reported by the students are similar to the literature description (Amenta et al. 2006; Gorecki et al. 2016), indicating the correct handling of the insects and injection.

Third class – AChE activity from cockroaches head

In the third class, the cockroaches' heads were used in the enzymatic assay (using the best parameters determined in the first class). The students tested the head AChE activity from the insects exposed to CPF or water.

The AChE assay demonstrated that the insects exposed to CPF (in the head or body) presented AChE inhibition (absence of yellow colour), while in the cockroaches exposed to water, the AChE activity was observed (presence of yellow colour) (Figure 6).

In a group discussion, the AChE inhibition was correlated with the cockroaches' behavioural alteration observed in the second class. AChE inhibition causes nervous and muscular dysfunctions; consequently, the cockroaches exposed to CPF exhibited contortion, vibration, convulsion and death (Amenta et al. 2006). CPF alters the contraction of the smooth muscles of the hollow organs in mammals (Çetinkaya and Baydan 2010; Darwiche et al. 2017), and probably similar phenomena could happen in insects, causing the observed abortion.

Table 3. Cockroach behaviour analysis after the injection of CPF or water.

		Behavioural analysis		
Substance	Injection	2 min	10 min	30 min
CPF	head	slow movements	abortion	convulsion/death
	body	slow movements	contortion	convulsion/death
Water	head	slow movements	slow movements	contortion
	body	slow movements	slow movements	slow movements

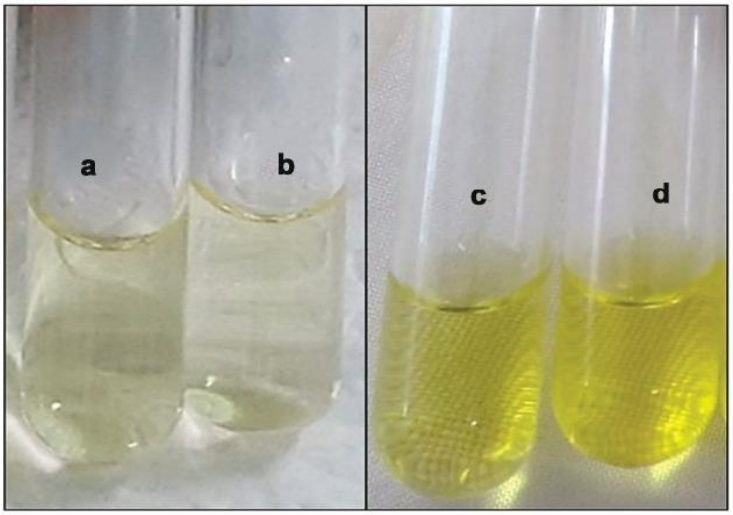


Figure 6. Cockroaches AChE activity after exposure to CPF (a: head and b: body) or water (c: head-d: body). The colourless indicates that the AChE is inhibited.

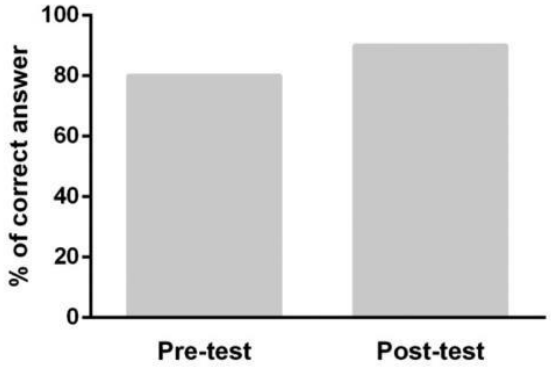


Figure 7. Percentage of pre- and post-test correct answers about enzymology knowledge (Table 1).

Students knowledge improvement

The percentage of pre- and post-class correct answers is depicted in Figure 7 as pre- and post-test, respectively. Concerning the questionnaire applied to evaluate the students' knowledge before and after the classes, we found that students already had an established knowledge about enzymology. Veterinary Medicine students already had the theoretical biochemistry course in an earlier semester and the Biology undergraduate students were taking the theoretical biochemistry class concomitantly. Therefore, we observed that our classes reinforced the concepts about enzymatic activity and increased knowledge.

Students evaluation of experimental classes

After the practical classes, an online questionnaire was sent to undergraduate students of both undergraduate courses. The students answered the questions using a 6-point scale, ranging from

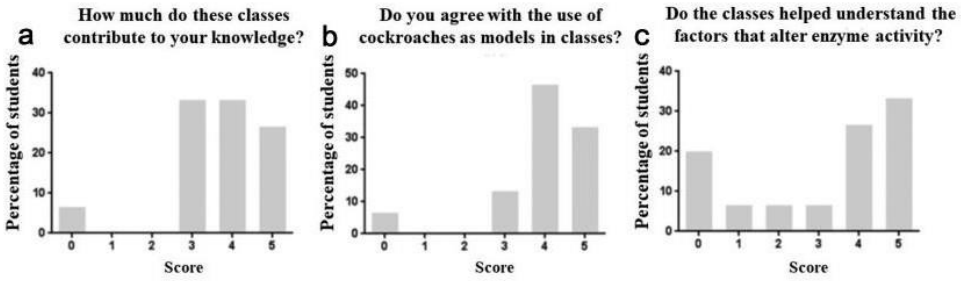


Figure 8. Percentage of answers for each question presented in Table 2.

zero to five, with zero being strong disagreement and five being strong agreement. Twenty students with a response rate of 51% answered the online questionnaire. The data showed that the majority of the students classified the classes as an excellent instrument to improve their learning about enzymes (Figure 8). These results showed that the protocols had a good design and they contributed to students' learning, reaching our objective.

Regarding the second question, 80% of the students agreed with the use of cockroaches as an alternative model for laboratory classes. In the third question, 70% of the students evaluated the topics discussed in the classes as necessary for learning about enzymatic activity and considered the classes a great contribution to their learning about the subject.

Discussion

In this work, we demonstrated for the first time the use of the cockroach *N. cinerea* as an experimental model to teach factors that alter enzymatic activity to undergraduate students.

Experimental classes may contribute to overcoming obstacles in learning scientific concepts, making use of their investigative nature and applying thescientific method. In our work, the colour change in the AChE enzymatic assay, induced by different factors, was visible in the first and third classes, as well as the different behaviour of cockroaches in the second class, which clarified factors that may interfere in enzymatic reactions and CPF effects in the nervous system. Visualisation has been the subject of research in science education, and its importance has been proven (Gilbert 2007). In this sense, the methodology used in our work followed the idea that visualisation brings the subject closer to the student's reality helping knowledge construction (Harris and Alexander 1998). Besides that, the results suggested that guided discussions were useful to help students reach their conclusions about the experiment.

It is becoming a consensus among education scientists that students will only learn something, i.e. build new knowledge, if they act and problematise their actions (Glaser 1991; Becker 1994; Mendes and da Silva 2018). Thus, it was possible, using an example of organophosphate insecticide, to improve the students' knowledge about factors that alter enzymatic activity and show the real world problem concerning the *in vivo* effects of pesticide exposure.

In our work, the majority of the students agreed with the animal experimental model. This observation is following previous studies of Fischer and Tamioso (2013), where students accept the animal use for educational purposes, including mammals. The use of alternative animals in classes is important and may be used as models to study the effects of toxicants, for example. In our case, we used cockroaches. We are aware of the ethical implications of using animals (even invertebrates), and again, we call the attention to the careful use of those models. On the other hand, a substantial percentage of students (20%) considered that the classes did not contribute to improve their knowledge about enzymatic activity. We believe these students are in disagreement with the use

of animals in experimental classes, mainly the Biology undergraduate students. In addition, a great number of Veterinary students considered biochemical experimental classes useless. These students reported that the classes could be theoretical, without using animals and they would contribute to learning in the same way.

In this study, it was possible to confirm through the enzymology questionnaire that practical classes were efficient. The majority of the students considered the classes important to understand the factors that alter enzymatic activity; they also considered interesting the use of cockroaches as an experimental organism. In general, our data from the undergraduate courses reinforced the importance of experimental activities to learn about enzymology, and it supports the viability and effectiveness of alternative models as a platform to reach this objective.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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3. CONCLUSÃO

A partir dos resultados encontrados nesta dissertação é possível concluir que:

- aulas práticas investigativas são bem aceitas pelos alunos da graduação em Ciências Biológicas e Medicina Veterinária.
- a construção do modelo proteico 3D auxiliou os estudantes a compreender o conteúdo de química de proteínas; porém, ficou evidente que os alunos tinham dificuldades de compreender alguns conceitos químicos.
- a utilização da barata *N. cinerea* como modelo alternativo foi aceita pela maioria dos estudantes, e permitiu que eles compreendessem de forma satisfatória os mecanismos de inibição enzimática *in vivo* e *in vitro*.

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