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“It is not just the shape, there is more”: students’ learning of enzyme–substrate interactions with immersive Virtual Reality

Henry Matovu, ^a Mihye Won, ^b Roy Tasker, ^c Mauro Mocerino, ^d David Franklin Treagust, ^e Dewi Ayu Kencana Ungu ^f and Chin-Chung Tsai ^{gh}

Immersive Virtual Reality (iVR) can help students visualise and explore complex chemical concepts, such as protein enzyme structures and interactions. We designed a set of collaborative iVR-based learning tasks on the interaction between a protein enzyme and its substrate. We investigated how 18 pairs (36 students) in undergraduate chemistry courses changed their understanding of enzyme–substrate interactions through iVR learning tasks. Videos of pre- and post-interviews and student-generated diagrams were analysed. Before iVR, students had abstract models of the structure of a protein enzyme or its interaction with a substrate molecule. Over 90 per cent of the students (33/36) explained enzyme–substrate interactions using simplistic lock-and-key diagrams, exclusively focusing on the shape. Although many students employed key scientific terms like activation energy in their explanations, they were unsure how enzymes lowered activation energy or how catalytic reactions occurred. After iVR, all students discussed the inadequacy of 2D diagrams for representing complex enzyme–substrate interactions. About 90 per cent of students (32/36) used concrete ideas such as electron density and orientation of reactants in the active site to explain the probability of successful interactions between the enzyme and its substrate. Our findings provide evidence of how interactive iVR learning tasks can help students explore complex molecular structures, integrate ideas, and build a concrete understanding of challenging science concepts.

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One of the aims of science education is to help students develop a coherent and interdisciplinary understanding of phenomena in their daily lives (e.g., NRC, 2012). Achieving this goal is not trivial as it requires students to visualise submicroscopic interactions and integrate different pieces of information within and across disciplines (Franovic *et al.*, 2023). In this study, we designed interactive and collaborative learning tasks using innovative 3D visualisation technology called immersive Virtual Reality (iVR) to support students’ exploration of enzyme–substrate interactions. Developing a solid understanding of this concept is fundamental in chemistry, biology, and biochemistry as it allows students to grasp several phenomena,

such as enzyme kinetics, metabolism, and drug binding and inhibition (Srinivasan, 2021). For example, understanding the structure and function of the enzyme acetylcholinesterase is essential to elucidate how common pesticides and chemical weapons (e.g., sarin) induce paralysis in both pests and humans. Similarly, explaining the functioning of aspirin in pain management requires understanding aspirin as an irreversible inhibitor of the enzyme cyclooxygenase which mediates inflammation and pain (Srinivasan, 2021). Herein, we documented how students’ understanding of enzyme–substrate interactions changed through iVR-based learning.

Literature review

Students’ difficulties in learning enzyme–substrate interactions

Experimental studies suggest three essential considerations when predicting successful (acid–base and covalent) interactions between an enzyme and a substrate: geometric compatibility, electronic complementarity, and stereochemistry (Linenberger and Bretz, 2014). Geometric complementarity relates to the fit of the shapes of the substrate and the enzyme, electronic complementarity involves matching complementary

^a School of Chemistry, University of Sydney, Camperdown, NSW, Australia.
E-mail: henry.matovu@sydney.edu.au

^b Faculty of Education, Monash University, Clayton, VIC, Australia

^c School of Science, Western Sydney University, Penrith, NSW, Australia

^d School of Molecular and Life Sciences, Curtin University, Perth, WA, Australia

^e School of Education, Curtin University, Perth, WA, Australia

^f Institute for Pedagogical Innovation, Research & Excellence (InsPIRE), Nanyang Technological University, Singapore

^g Program of Learning Sciences, National Taiwan Normal University, Taipei, Taiwan

^h Institute for Research Excellence in Learning Sciences, National Taiwan Normal University, Taipei, Taiwan

electron densities, while stereochemistry pertains to the spatial orientation of the relevant reacting groups within the enzyme and substrate (Linenberger and Bretz, 2014). Understanding these factors requires students to visualise the 3D molecular structures and electron densities of an enzyme and its substrate and predict the resultant interactions. Yet, students often struggle to develop and apply 3D molecular visualisation skills, particularly when dealing with complex structures of biomolecules, such as proteins (Harle and Towns, 2013; Qin *et al.*, 2021).

Students' difficulties in learning enzyme–substrate interactions often result in students acquiring only a superficial understanding of the concept. For example, previous research has indicated that undergraduate students have difficulties explaining the role of electron density and molecular shape or the nature of the interaction between the substrate and the enzyme (Bretz and Linenberger, 2012; Linenberger and Bretz, 2014). Students also often have difficulties explaining the particulate-level mechanisms of the interactions between catalysts and reactants in chemical systems (Bain *et al.*, 2018). When explaining enzyme catalysis, students frequently invoked thermodynamics concepts, such as activation energy and transition states, but struggled to explain how these ideas could be used to explain enzyme–substrate interactions (Linenberger and Bretz, 2015). Recently, Franovic *et al.* (2023) reported that more than 70% of undergraduate chemistry and biology students were unable to successfully explain the preferential binding of a ligand (metal ion) to a protein active site. Such students' difficulties documented in the literature underscore the need for strategic interventions to help students visualise fundamental concepts, such as enzyme structures, electron densities, and 3D shapes, and integrate them into their explanations of enzyme–substrate reactions.

Teaching enzyme–substrate interactions

Common interventions for teaching enzyme structures and functions typically involve using diagrammatic representations of protein structures (*e.g.*, Richardson, 2000) and interactive 3D molecular visualisation programs on 2D computer interfaces, such as JSmol, Pymol, Chimera, or VMD (*e.g.*, Abriata, 2017; Fombona-Pascual *et al.*, 2022). However, these teaching approaches are limited in conveying spatial information in molecules, such as depth and scale, to students (Cassidy *et al.*, 2020; Doak *et al.*, 2020). Interpreting diagrammatic representations of complex 3D molecular structures, such as proteins, also requires a high level of conceptual understanding (Rau, 2017). As such, students without adequate background knowledge may struggle to construct useful mental images of complex molecules (Wu and Shah, 2004).

Analogies, such as the lock-and-key model, and analogy-based activities are also commonly used to teach enzyme–substrate interactions. Educators employ props like plasticine, 3D printed models, or nuts and bolts to illustrate the concepts of enzyme specificity, substrate-binding, inhibition, or limiting substrate concentrations (*e.g.*, Kin and Ling, 2016; Friedman and Terry, 2021). Analogies can help students relate unfamiliar,

abstract concepts to what students already know (Orgill *et al.*, 2015), aligning with the constructivist view of learning (Jonassen, 1994). However, the transfer between the analogue and target concepts is not always spontaneous (Harrison and Treagust, 2006). Students need to recognise the connection between the attributes of the analogue and the target concepts, and thus, the limitations of the analogue (Harrison and Treagust, 2006). Moreover, in the context of enzyme–substrate interactions, these analogies may oversimplify the concept and fail to capture the intricacies of enzyme structures and mechanisms of the catalytic interactions.

Other educators utilise computer simulations (*e.g.*, Torres and Santos, 2017) or experimental tasks in laboratories (*e.g.*, Ramirez-Paz *et al.*, 2017) to help students explore properties of enzymes or collect quantitative data on the performance of enzymes in different reaction conditions. However, these approaches do not adequately help students visualise enzyme structure and function. Consequently, interpreting this quantitative data (*e.g.*, rate constants) or its representations (*e.g.*, kinetics graphs) remains challenging for many students (*e.g.*, Rodriguez *et al.*, 2019; Rodriguez and Towns, 2020).

Immersive Virtual Reality (iVR) for teaching enzyme–substrate interactions

Immersive Virtual Reality (iVR) technology offers a promising avenue to address the challenges in teaching enzyme–substrate interactions. Unlike mixed-reality or augmented-reality technologies which superimpose virtual elements onto the real world (*e.g.*, smartphone-based Pokémon Go and Apple Vision Pro), iVR completely replaces the learner's view of the physical world with a computer-generated world (Fombona-Pascual *et al.*, 2022), allowing learners to feel as though they are a part of the new environment and perceive virtual events as “real” (Slater *et al.*, 2022). By delivering stereoscopic images of otherwise inaccessible molecular environments, iVR helps learners to visualise intricate enzyme structures in concrete forms and enhance comprehension (Dede *et al.*, 2017; Matovu *et al.*, 2024). Students can also manipulate these structures using handheld controllers (*e.g.*, by dragging and connecting molecules) to test ideas, observe consequences, and revise their understanding (Dede *et al.*, 2017; Matovu *et al.*, 2024).

Several studies have explored the use of iVR for teaching enzyme–substrate interactions (*e.g.*, Bennie *et al.*, 2019; Won *et al.*, 2019; Cassidy *et al.*, 2020; Doak *et al.*, 2020; Qin *et al.*, 2021; Matovu *et al.*, 2024). However, many researchers tended to focus on iVR program development and interface evaluation rather than its benefits when implemented in chemistry units (*e.g.*, Cassidy *et al.*, 2020). Researchers who implemented iVR in their classes focused on enhancing students' engagement (*e.g.*, Doak *et al.*, 2020), exploring students' perceptions of the benefits of the technology (Bennie *et al.*, 2019; Bernholt *et al.*, 2019; Qin *et al.*, 2021), or documenting learners' collaborative interactions in iVR (Matovu *et al.*, 2024). Regarding learning benefits, researchers used multiple-choice questions to measure students' learning (Abbasi *et al.*, 2023). Considering that these instruments are often limited in the level of conceptual

understanding they assess (Breakall *et al.*, 2019), the value of iVR for enhancing understanding of complex enzyme–substrate interactions remains unclear. A previous study by our research group (Won *et al.*, 2019) discussed how the unique 3D visualisation capabilities, full-body engagements, interactivity features, and collaboration in iVR supported first-year university students' exploration of enzyme–substrate interactions. Through iVR, students appreciated being able to visualise and intuitively explore the complex 3D structure of an enzyme (Won *et al.*, 2019). However, it was not clear the extent to which students could apply the knowledge from the iVR experience to explain enzyme–substrate interactions.

In the present study, we aimed to systematically investigate the conceptual benefits of using iVR for learning enzyme–substrate interactions. To achieve this aim, we documented how undergraduate students explained the interaction between an enzyme and its substrate before and after exploring the concept with their peers in iVR. This study addressed the following research question:

- How do students explain enzyme–substrate interactions in relation to 3D structures, electron density, and energy before and after collaborative iVR-based learning activities?

Methods

Participants and study design

Participants were first- and second-year undergraduate students enrolled in chemistry-related programs (*e.g.*, Chemistry, Chemical Engineering, Biomedical Sciences, Food Science, and Nutrition) at a large public university in Australia. The iVR activity described here was the last of three mandatory iVR activities which were integrated into two chemistry units, Reaction and Function in Chemistry for first-year and Chemistry of Biological Processes for second-year students taught in semester 1, 2021. The first two iVR activities targeted the concepts of hydrogen bonds and stereochemistry and are detailed in our previous studies (*e.g.*, Matovu *et al.*, 2023a, b; Matovu *et al.*, 2024).

In total, 64 students participated in these iVR activities. The students worked in pairs, each student with the same peer over all three iVR activities. Each activity was organised in three parts: a pre-iVR session (15–25 min), an iVR-based learning session (25–40 min), and a post-iVR session (20–30 min). Any two iVR activities were spaced 2–3 weeks apart. Although completing the iVR activities was mandatory, participation in the research study was voluntary. Permission to conduct the study was granted by the institutional Human Research Ethics Committee (HRE2020-0081) and all students signed consent forms for their data to be used.

Pre-interviews sessions. Students (in pairs) were introduced to the learning tasks and interviewed to evaluate their prior understanding of enzyme–substrate interactions. For instance, students were asked to illustrate their understanding of enzyme–substrate interactions and explain why the breakdown of a substrate by its enzyme was much faster than a similar laboratory reaction without an enzyme. Students were also

introduced to the interaction between the enzyme acetylcholinesterase and its substrate acetylcholine as the context for the iVR-based learning session. This reaction was chosen because it is one of the fastest enzyme reactions known. The reaction also plays a fundamental role in regulating key physiological functions in humans and other living organisms. The chemical acetylcholine is a neurotransmitter in our bodies, and it helps in facilitating muscle contraction. The enzyme acetylcholinesterase breaks down excess acetylcholine into its products (choline and acetic acid) which allows muscles to relax. Moreover, compared to most other enzymes, acetylcholinesterase is a relatively simple protein molecule, yet complex enough for students to benefit from the unique 3D representation of molecules offered by iVR technology. The pre- (and post-) interview prompts that were employed are provided in the Appendix. When students were unsure of enzymes and their interactions, the researcher scaffolded their understanding by giving extra prompts related to catalysts, reaction rates, and the importance of electron densities and molecular shapes in chemical reactions.

iVR-based learning sessions. After pre-interviews, participants were moved to a specially organised room and trained on using hand-held iVR controllers to manipulate virtual objects. They were encouraged to discuss ideas with peers and reach an agreement before moving from one task to another. The participants were also encouraged to walk around the virtual space to explore 3D objects from different angles. They were instructed to immediately report any dizziness or discomfort during iVR. To complete the iVR-based learning tasks, students wore HTC VIVE Pro Eye headsets with wireless adaptors and handheld controllers. Two students shared the virtual space (4 m × 4 m) and together completed a set of tasks involving acetylcholinesterase (enzyme) and acetylcholine (substrate). In iVR, students saw each other's avatars (floating headsets and controllers) in the virtual space, manipulated shared objects, and talked to one another. At each step in iVR, students also received audio and text instructions to encourage their exploration and collaborative discussions.

In iVR, students completed several tasks involving a substrate and its enzyme. The tasks included exploring different representations of the protein enzyme structure (Fig. 1a) while discussing the pros and cons of each form (Won *et al.*, 2021a). Students were also prompted to scale (zoom in and out) the protein structure to change their perspective of the structure and explore the structure as a whole or its intricacies (Won *et al.*, 2021b). For instance, students were prompted to zoom in, walk into the protein, and explore the catalytic triad inside the active site. When students touched each amino acid of the triad with their hand-held controllers, the program displayed the name of that amino acid. In other tasks, students also explored the optimal orientation of the acetylcholine molecule at the entrance of the enzyme (Fig. 1b) and dragged and placed the acetylcholine molecule in the active site for the reaction to occur (Fig. 1c; Won *et al.*, 2021c). These learning tasks required students to apply their understanding of fundamental chemistry concepts (*e.g.*, electron density and

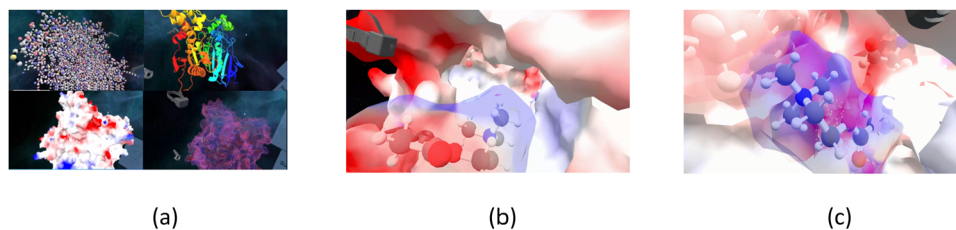


Fig. 1 (a)–(c) Sample iVR-based learning tasks. (a) Exploring representations of a protein enzyme structure. (b) Exploring the best orientation of the substrate molecule at the entrance of the enzyme structure. (c) Placing the substrate molecule in the enzyme active site for the catalytic reaction to occur.

3D molecular shape) integrally. For instance, when orienting the substrate at the entrance of the enzyme (Fig. 1b), students observed the substrate molecule bouncing back or getting stuck at the enzyme entrance and not getting through when the orientation was not right even though the electron densities were aligned correctly. The iVR session ended with students watching a one-minute step-by-step 3D animation of the mechanism of the breakdown of the substrate and the restoration of the enzyme's active site to its original state. In this task, students could play, pause, and replay the individual steps if they wished to. The development of and reasons for including the different tasks are described in detail in our earlier publication (Won *et al.*, 2019).

Post-interview sessions. After iVR, students were asked to explain what they had learned in iVR. They were also asked to critique their pre-iVR diagrams and revise their explanations of the difference in reaction rates with and without enzymes. Students' interactions in all pre/post-iVR sessions and iVR-based activities were audio and video recorded. During and after each interview, the interviewer also made initial notes regarding the ideas discussed by the students.

Data analysis

Before analysis, all pre/post-interviews were transcribed. Students' understanding of enzyme–substrate interactions was first categorised based on the researchers' notes, student-generated diagrams, and interview transcripts. Two initial categories emerged: students uncertain about what enzymes were, could not illustrate enzyme–substrate interactions in any way, and required a structured introduction to enzyme reactions (28 students) and students with a basic understanding of fundamental concepts in the context of enzyme–substrate interactions (36 students). The nature of support provided to student pairs who did not have adequate prior knowledge varied considerably depending on the pair. Therefore, this study focuses on the progress of the 36 students who exhibited a basic understanding of enzymes. Before iVR, these students explained their ideas with reasonable confidence, drew diagrams to illustrate enzyme–substrate interactions, and did not require introductory teaching. As described later in the Results section, these students' models of enzyme–substrate interactions were simplistic and focused on shape (geometric) compatibility between the enzyme and its substrate. Further analysis aimed to investigate how iVR-based learning transformed their

understanding of molecular interactions and the application of fundamental chemistry concepts in the context of enzyme–substrate interactions.

Although students were interviewed together, most of them tended to provide individual diagrams and explanations. Consequently, individual students' data were analysed. When students built on each other's ideas (one pair before iVR, and five pairs after iVR), the combined explanation was coded and assigned to each of the contributing students. Students' data (verbal explanations, hand-drawn diagrams, and gestures) before and after iVR was analysed following an inductive approach (Thomas, 2006; Merriam and Tisdell, 2015). Due to the multi-modal nature of students' communication (Jewitt, 2013), the analysis was iterative with researchers going back and forth between the transcripts and videos of the interviews to confirm their interpretations. For example, in terms of the specificity of enzyme–substrate interactions, before iVR, students verbally described the specificity of enzymes while pointing at their 2D diagrams. This pattern reflected that the students conceptualised the interaction in a simplistic way. However, after iVR, students used a lot of gestures to describe a complex enzyme structure and explain how the substrate approached the enzyme entrance and catalytic site in a 3D space.

Before or after iVR, students' ideas of how the substrate is bound to the enzyme did not differ much among individual students. For instance, before iVR, all students were aware of the presence of active sites and explained how the binding of the substrate to an enzyme depended on the molecular shape of the substrate. However, students' explanations of the fast rate of an enzyme-catalysed reaction varied considerably among students, especially after iVR. A combination of deductive and inductive approaches was used to identify the main ideas in students' explanations of the rate of enzyme-catalysed reactions. Before iVR, most students were unsure of the explanations, while some mentioned the idea of an alternative reaction pathway in the context of lowering the activation energy. After iVR, students used ideas including electron density, the positioning of reactants (substrate and amino acid residues) in the active site, and the mechanism of the reaction between the enzyme and substrate to explain the probability of enzyme reactions. Each student's explanation was coded based on the key ideas presented to identify common patterns and generate categories. The codes and categories generated from the data were checked and agreed upon by four authors (HM, MW, MM, and RT).

Findings

Through iVR-based learning, students' conceptions of enzymes and enzyme–substrate interactions changed significantly from simplistic models based on the idea of molecular shape (before iVR) to complex models involving different ideas, such as electron density, positioning and spatial orientation of reactants in the active site, and reaction mechanism (after iVR). We have organised the results in two parts, A and B, to describe students' conceptions before and after iVR, respectively.

Part A: students' conceptions of enzyme–substrate interactions before iVR

Binding of a substrate to an enzyme. All 36 students emphasised the binding of the substrate molecule to an active site of an enzyme before the substrate molecule is broken down. However, most students had a simple model of the interaction between an enzyme and a substrate and did not explain what happened after the substrate was bound to the enzyme. All students, except three, depicted the enzyme–substrate interaction through drawings illustrating the lock-and-key analogy. These drawings, together with supporting verbal explanations, showed that students recognised that the substrate-binding process depended on the relative shapes of the enzyme and substrate molecule (geometric compatibility). Only a substrate of a complementary shape could bind to the enzyme. This consideration successfully explained why enzymes were very specific in terms of the molecules they bind. For example, Ellen drew Fig. 2a and described an enzyme–substrate interaction based on the concept of fit between the substrate and the enzyme active site. Ellen's diagram and verbal explanation explained how the substrate was bound to the enzyme but not how the substrate was broken down. Below is an excerpt of the explanation provided by Ellen.

Ellen: “We have our enzyme and a substrate. And they come together, and it fits nicely, like the shape of the... active site. That's where the reaction occurs, the substrate goes in there and it forms this complex, and then the enzyme speeds up the reaction, and in this case, it forms two products. The enzyme stays the same afterwards, just the drawing doesn't [clearly] show that.”

Three students explained that, to bind the substrate molecule, the enzyme changes its conformation to accommodate the substrate molecule (e.g., Fig. 2b). The students referred to this

process as the “induced-fit” model, which is likened to the fitting of a hand in a glove (Koshland Jr 1995). These explanations also emphasised the importance of molecular shape. For instance, Tim was aware of the lock-and-key mechanism but disputed it in favour of the induced-fit model, as illustrated in the excerpt below:

Tim: “... I don't wanna just do the lock and key because ... [it] isn't right. Um, it's kind of hard to draw ... but say you have like a substrate here and then you have like a larger enzyme here. Then this [substrate molecule] will be able to go into here, then once it's in there, the shape of this active site might change... The handshake model is the preferred ... it's like lock and key, but when the key goes in, it changes the shape of the [lock] ...”

Only three students (e.g., Nate) mentioned the role of both molecular shape (geometric compatibility) and electron density (electronic compatibility) in influencing an enzyme–substrate interaction. Below is an excerpt of the explanation provided by Nate when referring to his diagram of the ‘lock-and-key’ model.

Nate: “[I have drawn] like a site there, kind of that this part of the reactant fits into... Your reactant kind of fits into that site and then they, yeah, ... like, it's [the enzyme] got a, the pocket is kind of in a particular shape and then charge and polarity and stuff like that, that it orients it [the substrate molecule] the correct way ...”

Students' conceptions of the structure of an enzyme. Despite mentioning the presence of active sites in enzymes, most students seemed to have very abstract models of enzyme structures. When asked about their mental models of enzymes, 20 students admitted that they did not have a clear idea of what enzymes looked like. Some students described an enzyme as a structure with the shape of a physical ball (e.g., Ellen), or a Covid-19 virus (e.g., Albert). Another student Charles described an enzyme as a bowl or doughnut with an active site in the middle. Below are excerpts of some students' responses:

Ellen: “Like I think of it as some sort of like, physical kind of ball (*gestures a spherical ball*)”

Charles: “Picture a large bowl, it's, it's not realistic like a bowl we eat in, but more like a doughnut with the whole field in the centre. The centre or the divot within an enzyme is the activation site, which is where molecules will come and interact with the enzyme. This way, just like, yeah, just like that [lock-and-key model] diagram.”

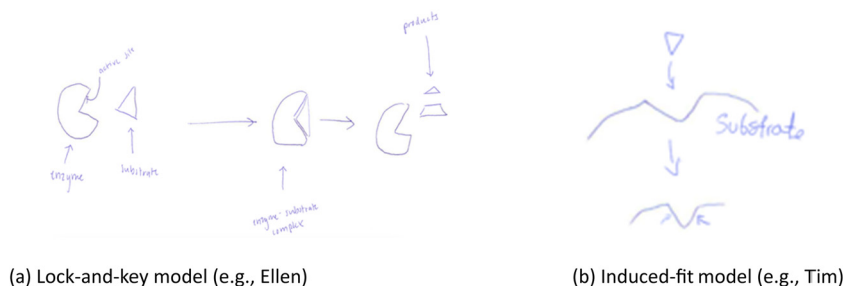


Fig. 2 (a) and (b) Examples of students' diagrams to explain enzyme–substrate interactions.

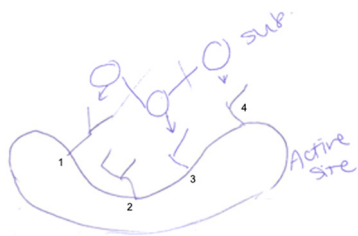


Fig. 3 Ella's illustration of the enzyme-substrate interaction.

Albert: "Uh, well I'm not [sure], I don't remember seeing a proper one, but I just think of the virus Covid. I just imagine it looking a bit like that."

Only two students (Ella and Tim) recognised that an active site in the enzyme had constituent functional groups that helped to orient the substrate molecule in the right position or cleave the bonds in the substrate molecule. For instance, Ella drew Fig. 3 and explained that

"the active site has amino acids (labelled 1–4), a catalytic triad – one of the amino acids holds the substrate into place based on charge, and the others break the bonds in the substrate".

Explaining the fast rate of an enzyme-catalysed reaction. Before iVR, all 36 students described enzymes as catalysts for biological reactions and identified typical properties of catalysts. However, as shown in Table 1, when they were asked to explain why the breakdown of acetylcholine in the presence of its enzyme was much faster than the hydrolysis of acetylcholine in the laboratory (without an enzyme), more than one-third of the students ($n = 13$; e.g., Tamir) admitted that they were unsure

of the explanation. About 50% of the students ($n = 17$; e.g., Kelly) mentioned that the enzyme provided an alternative reaction pathway which required a lower activation energy. However, when prompted to elaborate, the majority (11 out of 17) were unable to explain how the enzyme facilitated the alternative reaction pathway. Five of the 17 students (e.g., Peter) hypothesised that the enzyme lowered the activation energy by providing a surface where a substrate molecule could meet and react with other molecules. These students did not recognise that the enzyme actively breaks down the substrate molecule or explain further how the enzyme held the substrate molecule. Only one student (Tim) attempted to elaborate on how an enzyme could provide an alternative reaction pathway of lower activation energy using ideas such as steric effects or ionic effects. Below are excerpts of the explanations Tamir, Kelly, Peter, and Tim provided before iVR.

Tamir: "I don't really know. I know the general influence that a catalyst has on a reaction, it speeds it up like we just said, but at a biochemical level, I'm not entirely sure what's happening ..."

Kelly: "I dunno, in this particular scenario, but ... the point of putting a [enzyme] catalyst in there is that ... it literally, it short circuits the [slow] step. So, it provides a path of lower activation energy or less resistance to, to make that [reaction] faster."

Peter: "It [the enzyme] lowers the activation energy ... it's able to guide two molecules into place, either like next to each other or something to then react to where they meet. So rather than just waiting for them to randomly bump around [in]to one another."

Table 1 Categories of (and key ideas incorporated in) students' explanations of enzyme-catalysed reactions before and after iVR

Category/key ideas	Descriptor	Number of students	
		Before	After
Unsure	Student is uncertain of the explanation	13	
Hard to categorise	Student presents incoherent ideas or provides no response after the peer has explained	2	2
Electron density	Student suggests that electron density of the enzyme facilitates the breakdown of the substrate		2
Activation energy	Student explains that the enzyme provides an alternative reaction pathway requiring a lower activation energy	17	
Positioning of reactants	Student explains how the spatial orientation of the substrate molecule relative to amino acids in the active site enhances the chances of the catalytic reaction	2	14
Positioning of reactants + reaction mechanism	Student explains how the spatial orientation of the substrate relative to amino acids in the active site facilitates the catalytic reaction, and tries to explain the reaction mechanism		2
Electron density + positioning of reactants	Student explains how the electron density and 3D structure help to position the substrate close to amino acids within the active site to facilitate the catalytic reaction	2	10
Electron density + positioning of reactants + reaction mechanism	Student explains how electron density and 3D structure help to position the substrate within the active site to facilitate the catalytic reaction, and tries to explain the reaction mechanism		6

Tim: “An enzyme provides an alternative pathway with lower activation energy ... A great example is the idea of the, of say like an oxy-anion hole. I just imagine that this is like a contributing part of an enzyme. Say, if you're trying to break this double bond because you want this carbonyl to react, then it [the enzyme] can provide strain on the carbonyl, which means that it more readily is going to enter the charged tetrahedral formation ... which then can encourage, say like a double bond to be broken.”

Only two students (e.g., Herbert) mentioned how the electron density of the enzyme facilitated the catalytic reaction by orienting the substrate molecule in place, rather than relying exclusively on random collisions between the substrate and other reacting molecules. However, it was not clear from these students' explanations how exactly the substrate molecule was broken down. For example, Herbert explained that

“The enzyme holds molecules in the right place based on electron density so you will not need to count on chance”.

Part B: students' understanding of enzyme–substrate reactions after iVR

Structure of an enzyme and the binding of a substrate molecule to an enzyme. After iVR, students highlighted different aspects that surprised them about the enzyme and its interaction with the substrate. Twelve students explained how, before iVR, they had never imagined the protein structure to be so complex, or the fact that it was made up of amino acid residues. For instance, after iVR, Jill explained, *“I was not sure about the scale ... the scale was really surprising. I didn't realize it'd be so big”*. Another student, Milly, explained that

“[I now know] it's made out of different compounds ... I think before we [had] like an imagination of what enzyme is, but that one showed us that it's all made of molecules, carbon and nitrogen. ... it's complicated”.

Twenty students commented that, through iVR, they clearly understood the specificity of the enzyme–substrate interaction. The students highlighted how electron density and molecular shape influenced the binding of the substrate to the enzyme in a 3D space. For instance, Tamir explained:

“The orientation as we were talking earlier about how it fits in, that's new. Um, how that (points at the model of acetylcholine) reacts with the enzyme surface and the electron-rich, electron-poor parts of the surface and how that [electron density] is used to kind of induce an orientation for it to go in (gestures the substrate molecule entering through the gorge in a very specific angle)”

Twenty students explained that iVR helped them learn about the presence of the catalytic triad or understand its role in the catalytic process (e.g., Tim). Moreover, sixteen students explained that they had learned more about the reaction resulting in the breakdown of the substrate molecule. For instance, despite knowing about the involvement of multiple amino acids in an enzyme reaction before iVR, like many of her peers, Ella imagined all enzyme reactions to be surface reactions. However, through iVR, Ella recognised that the reaction

occurred in a site that is buried inside the protein. Below are excerpts of the explanations by Tim and Ella after iVR:

Tim: “It [iVR activity] was useful to understand how the catalytic triad worked because I, I knew about it, there were lectures about it, but, um, yeah, but it was hard to visualize [before iVR].”

Ella: “[Before iVR] I thought it was just like a crater, and it [the catalytic reaction] happened in the crater rather than it being within the protein. So, it [iVR] very much touched on that point. Like you have the protein (*gestures a big structure*), and you have the reaction occurring, to some extent like, within the interior (*gestures the hand forward to illustrate the active site buried in the gorge*) of it rather than on the outside of it.”

Recognition of the strengths and limitations of 2D diagrams for representing enzyme–substrate interactions. After iVR, many students were quick to dismiss their initial diagrams based on the simplicity or two-dimensionality of these diagrams. Yet, when the interviewer encouraged them to improve the diagrams, the students explained that it was hard to represent the enzyme reaction accurately. The students then relied on gestures and their verbal explanations to comment on their diagrams. For instance, Ella explained:

“My drawing [Fig. 2c] seems very mediocre now (laughs). [But] I don't think I can change it. Trying to represent 3D would be hard. ... So, this would be like a 2D side view of what you're looking at. But in reality, it'd be like a whole circle and then that would be into the page. So, I can't really draw it better than that”.

All students explained that their initial diagrams provided a basic idea of the binding of a substrate to an enzyme reaction based on the concept of molecular shape, with comments such as *“It still explains that it needs to be a certain shape ... only a certain shape will fit.”* The students further explained that these 2D diagrams did not reflect the importance of electron density in the catalytic reaction. In addition, the diagrams were limited in showing where the catalytic reaction occurs, the actual amino acid residues involved in the catalytic reaction, or the complexity of the reaction once the substrate enters the active site. For example, Charles and Craig explained as shown below:

Charles: *“... the [electron density] regions were basically the most important thing, whereas like these models that we drew ... they don't really take into account the electron densities of the different regions and amino acids and their functional groups. So, it's just, it was kind of a lot more complex.”*

Craig: *“[the diagram] doesn't demonstrate how the substrate enters the enzyme at a specific orientation (gestures the molecule entering the catalytic site). And... especially when I drew this, I had no idea how the reaction actually took place when it went inside. I kind of just imagined, like it pulled it apart. But I know that's not what happens. But yeah, obviously that drawing ... doesn't have any indication of the, uh, catalytic triad that's in there because I didn't know that existed ...”*

Integrating concrete ideas to explain the rate of enzyme-catalysed reactions after iVR. After iVR, all students provided some form of explanation of enzyme reactions. About 90% of the students (32 out of 36) incorporated key ideas

(e.g., positioning/spatial orientation of the substrate and relevant amino acid residues in the active site, electron density, and the mechanism of the reaction) to explain why an enzyme-catalysed reaction was much more likely and fast. The students contrasted this reaction with a laboratory reaction which relied exclusively on random and unlikely collisions between reactant molecules.

After iVR, eight of the 13 students who were initially unsure how to explain the rate of enzyme-catalysed reactions recognised how the positioning of the substrate molecule in the active site allowed it to successfully interact with the relevant amino acid residues which are also aligned correctly. For example, Tamir explained:

Tamir: “[without the enzyme] you have to align all these (*points at the models of different amino acid residues*) perfectly, but the enzyme is a machine . . . it is designed to form, orient them [the substrate] in such a way that they [the ACh] can be pulled apart.”

Thirteen of the 17 students who had used the term ‘activation energy’ to explain enzyme catalysis before iVR (e.g., Job) explained that the enzyme positioned the substrate in the correct orientation relative to the amino acid residues in the active site to facilitate the reaction. Of these 13 students, five students (e.g., Kelly) also recognised the importance of electron density in facilitating the orientation of the substrate in the active site. According to the students, these factors reduced the degree of randomness in the reaction and increased the chances of a successful reaction between the amino acids and substrate. Below are the explanations provided by Job and Kelly after iVR:

Job: “Um, well, I think the enzyme, the different amino acids are in fixed positions. So, when the substrate comes in, it’s got a specific orientation, but without an enzyme, if you just mixed all the amino acids in a solution, you’d have to wait till all three of them line up in a very specific way. But with the enzyme, it’s already ready. It just, you just need the substrate to go in. So, you don’t have to wait for the [random] collisions to occur.”

Kelly: “It (*points at the model of tryptophan*) helps by putting it [substrate] in a specific orientation with electron density. . . and all, it’s all [other amino acids] right where it already needs to be. There’s none of this, like, let’s hope that these [reactants and substrate] collide and, and the right point hits the right thing, and they make the right reaction. Here, there’s none of that. It’s all right there and ready to go.”

Interestingly, after iVR, students did not explicitly refer to the concept of activation energy in their explanations and only eight of the 36 students (e.g., Ellen) attempted to explain the reaction mechanism involving the enzyme. Below is an excerpt of the explanation provided by Ellen:

Ellen “. . . And like those R groups provide, like molecules necessary for the different reactions to occur, like the first step like to this, to this carbon here (*points at the carbonyl carbon in a model of acetylcholine*), like adds another O that came from the amino acids and then that allowed that [ester bond of acetylcholine] to be split. And then I could really understand like, how, like, when you have another O connected to here [the carbonyl carbon], how that forms the [acetate] . . .”

Discussion

Science educators recognise the value of 3D visualisation and actively design tools to aid students in comprehending molecular structures and interactions (Wu and Shah, 2004). In this study, we investigated how completing interactive learning tasks with cutting-edge 3D visualisation technology, iVR, impacted students’ conceptual understanding of enzyme–substrate interactions. Before iVR, most students visualized enzyme–substrate interactions as simple reactions and tended to focus on the role of molecular shape in influencing enzyme–substrate interactions. Most students described enzyme–substrate interactions using the lock-and-key analogy. This was not surprising considering that this analogy is widely used in high school and university contexts to explain enzyme specificity. However, the students could not explain how the catalytic process occurred, suggesting that they had difficulties visualising the structure of an enzyme and its interaction with a substrate. Previous studies also found that students tend to focus on individual factors influencing enzyme–substrate interactions (Linenberger and Bretz, 2014). Our findings remind us of the difficulties that students have in developing coherent explanations of scientific phenomena.

The findings of the present study revealed that students’ understanding of enzyme–substrate catalytic interactions starkly improved through collaborative iVR-based learning tasks. This enhanced understanding manifested in several ways. First, after iVR, students critiqued their initial diagrams in terms of the simplicity of the diagrams and explicit focus on the concept of shape without highlighting the role of electron density and the relevant amino acid residues inside the catalytic site. This finding suggests that the iVR activity enhanced students’ understanding of enzyme–substrate interactions as well as their understanding of the strengths and limitations of 2D diagrams for explaining the concept. Educators (e.g., Wu and Shah, 2004; Rau, 2017) argue that improving students’ representational competency in science is as important as improving students’ knowledge of concepts.

Secondly, after iVR, most students also integrated concrete ideas (such as electron density and the spatial orientation of the substrate and amino acid residues in the active site) to explain how the enzyme enhances the chances of the catalytic reaction. Our findings demonstrate how a single iVR activity was able to help students recognise electronic and spatial aspects in molecular interactions and integrate fundamental ideas to explain complex molecular interactions. However, in their explanations after iVR, very few students explained the mechanism of the enzyme-catalysed reaction or linked the mechanism to activation energy as commonly discussed in textbooks. This is likely because the concept of activation energy was not highlighted in iVR. We had anticipated that allowing students to play, pause, and replay the steps of the 3D animation of the reaction mechanism would encourage them to analyse key aspects like reaction intermediates and activation energy without further prompting. However, most students played the animation once without reflecting much on the specific details and, thus, missed an opportunity to understand

how the enzyme fosters a reaction with a lower activation energy. Prior research also indicated that simply watching dynamic animations does not guarantee learning (Lowe, 2003). In addition, in the iVR environment, students may not have always focused on the specific areas that required attention at each step of the 3D animation. Besides, the reaction between acetylcholine and its enzyme is also a complex multi-step reaction which might have been difficult for the students to grasp within the limited time of exposure.

Overall, the enhanced conceptual understanding observed in this study contributes to the growing body of evidence on the value of iVR for enhancing students' visualisation of abstract chemistry concepts (e.g., Dede *et al.*, 2017; Matovu *et al.*, 2023a, b). By exploring and interacting with the 3D structures of an enzyme and its substrate in iVR, students recognised the complexity of an enzyme structure. Students could also walk into the enzyme active site to experience the journey of the substrate themselves, observe the spatial orientations of amino acid residues in a 3D space, and fit the substrate molecule in the active site. For instance, when the substrate molecule was not correctly aligned at the protein entrance, it would not fit through the gorge into the active site. This prompted students to experiment with different 3D orientations of the substrate and appreciate the role of molecular shape. In addition, in iVR, regions of high and low electron densities in the enzyme and substrate were explicitly and consistently represented as red and blue areas to help students recognise the role of electron density in an enzyme–substrate interaction. These concrete representations and physical interactions provided opportunities for students to explore and improve their understanding of the role of fundamental ideas in enzyme–substrate interactions. Other scholars also predicted that learning media could promote learning when they explicitly highlight the concepts to be learned (Wu and Shah, 2004), support embodied processes (e.g., Beier and Rau, 2022), and aid students in constructing knowledge (Won *et al.*, 2023). Overall, our study shows that, by carefully utilising the possibilities of iVR, such as 3D visualisation and interactivity, educators can provide relevant contexts for students to explore challenging concepts, apply prior learning, and integrate concepts to develop a coherent understanding of phenomena.

Implications and limitations

The findings from this study have implications for the teaching of enzyme–substrate interactions, in general, and for using iVR to support students' learning. Regarding chemistry teaching, the difficulties expressed by students in explaining enzyme-catalysed reactions suggest that when teachers explain the concept, there needs to be a deeper discussion around ideas such as the associated reaction mechanisms and activation energy. A more explicit focus on the roles of electron density and spatial orientation of reactants in enzyme–substrate interactions could also enhance students' understanding.

The results also remind us of the need to explicitly caution students about the benefits and limitations of analogies when

teaching abstract concepts. In this study, many students used the lock-and-key analogy to explain enzyme–substrate interactions before iVR. This analogy adequately highlights the importance of molecular shape. However, the model does not explicitly highlight the concept of electron density, the spatial orientation of the reacting groups, or the mechanism of the reaction between the enzyme and its substrate. Without cautioning students on the limitations of the analogies used, students' understanding of the concepts could be superficial, as reflected in students' pre-iVR conceptions of enzyme–substrate interactions. Indeed, researchers (e.g., Treagust *et al.*, 1998; Orgill and Bodner, 2004) have also warned that analogies in science teaching are double-edged swords. If not well explained, students may use an analogy as an algorithm to solve problems without fully understanding the abstract concept behind it (Orgill and Bodner, 2007).

The findings also have implications for designing and implementing iVR to support chemistry learning. Unlike previous studies which focused on improving students' learning experience when exploring enzyme–substrate interactions (e.g., Bennie *et al.*, 2019; Qin *et al.*, 2021), the present study provides tangible evidence of the conceptual benefits of using iVR for learning the abstract topic. The existing literature indicated that such positive educational outcomes as those in the present study are not realised by simply using iVR headsets. Educational outcomes depend on how the technology is designed, implemented, and evaluated (Matovu *et al.*, 2023a, b). While iVR-based learning can be motivating and engaging, it may also overwhelm students and hinder productive engagement with the content (e.g., Parong and Mayer, 2018). In the present study, our research team took advantage of the key features of iVR, such as interactivity and 3D visualisation capabilities (Dede *et al.*, 2017), and research-informed educational principles (e.g., collaborative learning) to design the iVR application. In particular, allowing students to explore concrete representations of concepts (3D molecular structures and electron density), and providing opportunities to test, share, and revise their ideas in iVR enhanced students' understanding. Using similar design considerations, other educators may wish to explore iVR for teaching other challenging science concepts.

Despite the learning benefits observed, this study had some limitations which could be addressed in future studies. Firstly, for most students in the present study, there was little evidence that they learned much from the 3D animation of the enzyme–substrate reaction. Further research on the mechanisms for supporting students' learning from dynamic animations in this cutting-edge visualisation technology is recommended. For instance, prompting students to focus on specific areas in iVR (Zacharia *et al.*, 2015), or encouraging thoughtful discussions about the animations may enhance students' comprehension (Wu and Shah, 2004). Secondly, in the iVR application used, we simplified the concept of enzyme–substrate binding and used static models of the enzyme and substrate structures to highlight the importance of 3D structure and electron density in these interactions. As other educators (e.g., Rau, 2017; Talanquer, 2022) noted, changing the visual representations

displayed or the concepts or properties highlighted may influence students' reasoning and learning. Future studies may wish to explore how different forms of molecular representations and properties highlighted in iVR influence students' understanding of enzyme–substrate interactions. Thirdly, the present study focused on the progress made by the 36 students who already had a basic understanding of enzyme–substrate interactions. As students' understanding in a regular chemistry class is more diverse, the benefits observed in this study may not exactly be replicated for all students. Our sample size was also relatively small which could limit the generalisability of the findings. A more detailed study to document students' learning with a larger sample size, and in a general chemistry class is needed.

Moreover, in the present study, we demonstrated how a collaborative and interactive iVR design where two students worked in a shared virtual space and interacted with shared virtual objects in real-time supported students' learning. However, common designs involve individual students interacting in iVR (e.g., Parong and Mayer, 2018; Bennie *et al.*, 2019) and have produced contradicting findings regarding the conceptual benefits of iVR in science education. In future, researchers may want to investigate whether iVR designs in which students work individually can produce learning gains similar to those observed in our study.

Conclusion

Teaching the concept of enzyme–substrate interactions is often challenging due to the complexity of the molecules involved. In this work, we systematically investigated students' learning of enzyme–substrate interactions using innovative 3D visualisation technology, iVR. Through the analysis of student-generated diagrams and explanations of enzyme–substrate interactions before and after iVR, this study illustrates how iVR helped students change from simplistic models to a more comprehensive and integrated understanding of the catalytic process involving an enzyme. Improvements in students' understanding of enzyme–substrate interactions were facilitated by the unique opportunity students had to explore complex 3D molecular representations in iVR. However, with the iVR application used, most students did not intuitively grasp the mechanism of the enzyme reaction and its relationship with the associated activation energy. Future studies may want to explore how iVR can be leveraged to enhance students' understanding of such abstract ideas as activation energy and reaction mechanisms.

Data availability

Following research ethics guidelines, the data will not be made available.

Conflicts of interest

The authors have no known conflict of interest to declare.

Appendix

Pre-interview prompts

1. In today's collaborative activity, we are going to explore an important enzyme reaction in our bodies. What do you know about enzymes?

2. How do enzymes work (or break down substrates)? Please illustrate what you mean.

3. The breakdown of substrate molecules by enzymes is a very fast reaction. Some enzymes can break down up to 20 000 molecules of the substrate per second. However, the same reaction, if carried out in a laboratory, takes hours. Why do you think this is the case? How can you explain this difference in reaction rates with and without an enzyme?

4. Have you heard about the enzyme acetylcholinesterase before? What does it do? (*The researcher further explains the importance of acetylcholinesterase*).

5. In iVR, we will explore the structures of acetylcholine and acetylcholinesterase and use fundamental chemistry concepts to explore the interaction between the two. Before we go to the VR, let's first go through a short activity. I have a model of acetylcholine (*Researcher gives students a molecular model of Acetylcholine, ACh*)

(a) Explore the model. What features of this molecule do you notice? Discuss with your peer.

(b) What functional groups do we have in this molecular structure?

(c) Which part of the acetylcholine molecule is electron-rich, and which part is electron-poor? Why is that?

(d) As a protein, the enzyme acetylcholinesterase has many other amino acids; some actively participate in breaking the ester bond in ACh, and others only offer a supporting role. Based on electron density, how do you think ACh will interact with the other amino acids in the enzyme active site?

Post-interview prompts

1. How was the experience? Why was that?

2. What do you think you have learnt or reinforced from the iVR activity?

3. Please look at your diagram of the interaction between an enzyme and substrate again. Are you still happy with it? Why or why not? How would you improve it? Do you want to draw an improved diagram?

4. Before iVR, I asked you about the differences between an enzyme-catalysed reaction and a similar reaction conducted in the lab without an enzyme. The reaction between the enzyme and acetylcholine is very fast. The breakdown of acetylcholine in the laboratory takes hours. Having completed the iVR activity, how can you explain the difference in rates of reaction in the two conditions?

5. You have studied complex molecular systems (such as enzymes) in your (bio) chemistry classes before. How does studying these concepts in iVR compare to the ways used in your chemistry classes?

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