In recent years, development of new technologies designed to enhance user experience have accelerated, often being used in modern media such as in films and games. Specifically, immersive experiences, such as virtual reality (VR) and augmented reality (AR), have redefined how digital media can be delivered, encouraging us to interact with and explore our environment. Reciprocally, as the power of these technologies has advanced, the associated costs to implement them have decreased, making them more cost-effective and feasible to deliver in a variety of settings. Despite the cost reduction, several issues remain with accessibility due to the knowledge base required to generate, optimise and deliver three-dimensional (3D)-digital content in both AR and VR. Here, we sought to integrate an AR-based experience into a level-4 biochemistry module in order to support the delivery of university lectures on protein structure and function. Traditionally, this topic would comprise two-dimensional still images of complex 3D structures. By combining a breadth of subject-specific and technological expertise from across the university, we developed an AR-enhanced learning experience hosted on the Zapworks AR platform. AR enabled full illustration of the complexity of these 3D structures, while promoting collaboration through a shared user experience. Assessing the impact of the AR experience via a formative test and survey revealed that despite only a modest increase in test performance, students overwhelmingly reported positively on the engaging nature and interactivity of AR. Critically, expanding our repertoire of content delivery formats will support the forward-thinking blended learning environments adopted across the higher education sector.

**Keywords:** augmented reality; structural biology; protein structure; biomedical science; biosciences; education; engagement

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Introduction

Augmented reality (AR) permits the superimposition of digital content on top of the real-world environment via the use of a smartphone, tablet or virtual reality (VR) headset (Carmigniani et al. 2011). Environmental triggers or geographical location serves as a physical reference point for the digital content to be mapped to (Berriman 2012). Prominent examples of this, in recent years, include the highly successful Pokémon GO game and the ‘live AR gig’ by mobile network operator EE, which appeared in a television advert (EE 2020; Paavilainen et al. 2017). These examples fundamentally showcased how technology could alter the way we interact with the environment and consume information. Uniquely, as the content is overlaid to the environment, the AR experience can be shared by many users simultaneously observing the same content, but from different perspectives (Phon, Ali, and Halim 2014). The ability to integrate three-dimensional (3D) models into AR provides the opportunity to create interactive activities that also foster collaboration (Phon, Ali, and Halim 2014). The potential of AR to revolutionise education is exemplified by Bower et al. (2014) who employed AR to access higher order thinking by asking students to design their own AR content (Bower et al. 2014).

Here, we used the ZapWorks AR platform to generate and deliver digital content into a higher education classroom (Zappar Ltd 2020). Software, such as Zapworks, have made the use of AR more accessible, which in the case of Zapworks comprises both a studio-editor and a smartphone/tablet app used in tandem. In addition, the widespread availability of hardware capable of displaying AR content, such as smartphones and tablets, has made it an exciting prospect for many industries, including the higher education sector. Previously, AR has been used in a range of educational settings for topics, including, but not limited to, human anatomy, chemical modeling and even surgical training (Bernardo 2017; Chen 2006; Thomas, William John, and Delieu 2010). We wanted to test whether AR could be used to in the delivery of biochemical content in a higher education setting. Previous pedagogical research has highlighted the benefit of using 3D-printed tactile objects in the learning environment to support object-based learning (Hannan, Duhs, and Chatterjee 2013; Smith 2016). This approach enables students to interact with and contextualise biological structures observed in nature, while also prompting dialogic learning through peer-to-peer interaction (García-Carrión et al. 2020). Crucially, AR presents an opportunity to deliver a ‘virtual’ object-based learning activity while supporting distance learning. Secondly, as the models are digital, it is possible to create multiple virtual objects and even place additional relevant information into the models, such as protein names. Ultimately, given the move towards remote and blended delivery in the wake of the disruption caused by the novel coronavirus (COVID-19) pandemic, technologies such as AR could contribute to creating a multifaceted and accessible learning environment.

In order to investigate the potential impact of AR on bioscience education, we specifically focused on the delivery of content pertaining to Structural Biology. Structural Biology is concerned with the molecular structure of biological macromolecules such as proteins (the molecular machines of the cell that perform a diverse array of important cellular functions) (Nelson and Cox 2017). Specifically, students are required to understand how highly specific 3D structures give rise to diverse protein functions and significantly forms the foundation for understanding how genetic mutations give rise to alterations in protein structure, which can ultimately lead to disease (Khan and Vihinen 2007). Moreover, this foundation is built upon subsequent topics,
including drug discovery (Surade and Blundell 2012). Crucially, *Structural Biology* can be conceptually challenging, not helped by the traditional media used to illustrate these 3D structures (i.e. 2D images displayed on presentation slides).

Fortunately, this topic lends itself naturally to integration in AR and VR due to the availability of 3D protein structures that have been solved using an X-ray crystallographic technique (Nelson and Cox 2017). It is possible using a computer suite and specialist software (such as pymol and UCSF chimera) to display this information as part of a workshop. However, using AR, we were able to deliver this content in a standard teaching classroom with minimal equipment (Pettersen et al. 2004; Schrodinger 2015). Additionally, as the content is hosted on an external server, any teaching space with a wireless internet connection can be used to deliver interactive content. Finally, we investigated the impact of this technology on both academic performance and perceptions of the technology via a formative test and survey, respectively.

**Methodology**

**Preparation of models**

Four protein crystal structures for proliferating cell nuclear antigen (PCNA), amyloid-β 1-42, collagen and green fluorescent protein (GFP) were downloaded in .pdb format from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank with accession numbers: 1AXC, 2MXU, 1K6F and 6FWW, respectively (accessed via https://www.rcsb.org/). Pymol was used to convert .pdb files to .obj using the ‘save’ command line function (> save ‘filename’.obj). Files were then optimised using Autodesk’s 3DS Max software. All 3D models are interpreted by the software as quads (squares) and/or triangle geometry. Imported models were triangulated with extremely high geometry counts (reducing performance). Optimisation was achieved by converting model geometry into quads and by lowering geometry count to ensure that mobile devices could render models efficiently when scanning a trigger image. Plain colour was assigned to each peptide of the model through 3DS Max’s Material Editor by selecting specific geometry sections. Text was added in a 2D format within the 3DS Max, which was then automatically converted to a 3D shape upon export. Attaching the text and smaller detached elements to the main model file ensured that upon exportation, the location of the text and protein model would remain consistent after importation into Zapworks Studio. This allowed models to be moved, scaled or rotated as one entity. Files were exported from 3DS Max in an FBX format - the .FBX file format was chosen for its broad range of data storage such as animation sequences and textures (colour), whereas .OBJ only stores the model itself.

**Importing into Zapworks**

The 3D-model files were imported into the AR platform following the video tutorials available on the application developers’ website (https://docs.zap.works/studio/3d-models/). The 3D-models were assigned to generic trigger images generated by Zapworks software. Various model scales were trialled to identify a satisfactory AR-projection and performance relative to the A3-sized trigger image used in the teaching session. Models with wider or taller dimensions were affected by the intensity of light projected onto the model. Projection of light was adjusted in Zapworks for each model following the software developers’ online tutorials.
AR-enhanced teaching session

Before the session, students were briefed on and provided with the opportunity to withdraw from the study. At the start of the session, participants were provided with the following instructions: (1) Pick an iPad, (2) open the Zappar application and (3) scan the trigger images around the room. Participants were then free to circulate around the room as they wished. During the teaching session, four stations were set up for each of the protein structures. Each station included a trigger image for the 3D model placed in the centre of the table and an identical trigger image affixed perpendicularly to the table on a nearby wall. At each station, students were prompted to consider the following: (1) How many peptides make up the protein? (2) What was the highest order of structure in the protein? and (3) What are the secondary structures that can be seen?

Results

In order to investigate whether 3D crystal structures of proteins could be presented in real time during a classroom setting, we initially picked four proteins with diverse crystal structures, which demonstrated key concepts and features covered during the associated lectures. This included proteins with secondary structures (α-helices, β-sheets and β-turns), as well as those that perform specific functions or have pathogenic properties (such as collagen as the structural component of connective tissue and amyloid-β 1-42 fibrils in neurodegeneration respectively). Table 1 details each of the structures used, their function and how they relate to the content from the lecture series.

Each of the protein crystal structures was downloaded from the RCSB protein data bank (accessed via https://www.rcsb.org/). Crystal structures were converted to .obj image files using the command line save function in Pymol. Models were then optimised for use in Zapworks AR-platform, including the addition of colour to peptide chains and features, as well as name labels. Each of the final structures is shown in Figure 1 (front and side views of each model are shown).

Table 1. Protein crystal structures optimised for use in augmented reality alongside key structural and functional features.

| Protein name                                | Structure and function                                                                 | Reference                        |
|----------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------|
| Proliferating cell nuclear antigen (PCNA)    | Demonstrates a quaternary ‘doughnut’-like structure formed from three peptides. Functions in the replication of DNA. | (Gulbis et al. 1996)            |
|                                              | Demonstrates the pathogenic potential of misfolded proteins. Amyloid protein aggregates are important in Alzheimer’s disease. | (Xiao et al. 2015)              |
| Amyloid-β 1-42                               | Demonstrates structural role of fibrous proteins. It is used in the formation of connective tissue, and mutations that alter structure are implicated in connective tissue disorders (such as Ehlers-Danlos syndrome). | (Berisio et al. 2002)           |
| Collagen                                     | Demonstrates structures that give rise to unique properties (i.e. fluorescence). Used to study cell biology by tagging proteins with a fluorescent marker. Isolated from a species of jellyfish (Aequorea victoria). | (Gil-Garcia et al. 2018)        |
| Green fluorescent protein (GFP)              |                                                                                         |                                  |
In order to ensure the relevance of this content in the context of the wider degree programme, an AR session was scheduled to be delivered following a lecture on Protein Structure as part of a series of four lectures looking at the biochemistry of proteins from amino acids and peptide formation to hierarchy of structure and protein function. The structural and functional features of the proteins selected complemented the key concepts conveyed in the lecture series.

The design of the AR-enhanced teaching session included four stations each with an A3-sized trigger image placed in the centre of a table. Chairs and other obstructions

| Protein name           | Front view | Side view | Original publication          |
|------------------------|------------|-----------|-------------------------------|
| PCNA                   | ![PCNA Image](image1.png) | ![PCNA Side Image](image2.png) | Gulbis et al. (1998)         |
| Amyloid β1-42 fibril   | ![Alpha Image](image3.png) | ![Alpha Side Image](image4.png) | Xaio et al. (2015)           |
| Collagen               | ![Collagen Image](image5.png) | ![Collagen Side Image](image6.png) | Berisio et al. (2002)        |
| Green fluorescent protein | ![Fluorescent Image](image7.png) | ![Fluorescent Side Image](image8.png) | Gil-Garcia et al. (2018)     |

Figure 1. 3D-protein models used for AR session. The figure shows the 3D models obtained from the protein databank for use in the AR session. Additional information such as the protein name was also integrated into the model. Colour schemes for peptides and formatting were added to optimise performance in AR. Images shown include front view and side view of each model alongside the reference for the original crystal structure.
were removed to allow free movement around the stations. Participants in the study were given freedom to move around the models, while additional explanation regarding the content in Table 1 was offered. Although the proposed technology will work with any smartphone/tablet with internet access, tablets were provided during this session to ensure that AR content was accessible to all participants. Figure 2 shows examples of how the models were integrated into the session at key stations.

The technology enables each student to view the 3D protein structure from a unique angle, allowing them to move closer or further from the model to investigate features, such as subunits, domains and functional groups (such as the chromophore in GFP that grants its fluorescent properties). Simultaneous visualisation of the protein structures prompted discussion between participants and prompted additional questions pertaining to the structural and functional features of the macromolecules. Integration of AR content in this context was designed to facilitate active discovery by allowing them to take the path they choose to achieve the learning outcomes, rather than following a prescriptive path (Kolb 2015). This was encompassed in the teaching session in which students were free to navigate the learning environment at their own pace and in an order of their choosing. Within this format, the students gain control and are provided the opportunity to take ownership over their learning (Light, Cox, and Calkins 2009).

In order to evaluate the impact of this technology both on the academic understanding of participants and on their perceptions of the technology as a measure of overall engagement, we designed a study as shown in Figure 3.
Twenty participants from the 2019-2020 cohort who currently enrolled on a level-4 biochemistry module were divided evenly into two groups using a random group generation function hosted on our virtual learning environment (Moodle). Group 1 were given 15-minutes to complete formative test comprising 13 multiple choice questions designed to test their knowledge and understanding of concepts in Structural Biology. After which, participants attended a 20-min AR-enhanced teaching session, comprising the models shown in Figures 1 and 2 hosted in a teaching classroom. At the end of the session, students were asked to complete a short survey, which included three Likert scale questions and three open-ended free-text questions. Reciprocally, group 2 students were invited to participate in the 20-min AR session first before being asked to complete the formative examination. From the submissions received, two students from group 2 did not complete the formative examination questions despite attending both parts of the study. At the end of the study, group 2 students were also asked to complete the survey. Therefore, both groups would participate in the AR-session; however, only group 2 would have completed the AR session prior to completing the formative test. A third control group, group 0, was included to assess the likelihood of achieving a score based on guesswork. This group was comprised of support staff who did not have a biosciences background and had not attended any of the lectures or the AR session. Figure 4 shows a Box-and-Whisker plot of test results by group shown as a percentage. Although there was no statistically significant difference in test performance between groups 1 and 2 (those having completed the AR-session first versus the taking the quiz first), there was a significant difference in test performance between group 0 (no lectures and no AR session) and group 2, but not group 1. This suggests that despite the modest upward trend in performance between each group, there is a move towards significance with the combination of both lectures and the AR session. Critically, it is likely that more extensive
testing on a larger number of participants will reveal true differences in academic performance by enriching education with AR.

In order to provide an indication of academic longevity following the session, participants from both groups were invited to re-sit the formative test 4-weeks later in order to assess whether they had retained the information. Ten participants completed the re-sit attempt, which showed results comparable with those of group 2 4-weeks earlier. Further investigation with a larger cohort of participants will be required to fully tease out impact of AR-enhanced education on test performance by participants. Additional questions of variable difficulty may also help to identify the levels of comprehension and pinpoint whether participants utilise higher order thinking with respect to spatial recognition of structures. Repeated investigation with future cohorts may also reveal year-on-year variation in test performance in response to this format of learning.

In order to capture the perceptions of participants on the use of AR, students were asked to respond to three statements and questions on a Likert scale of 1–5 (Figure 5a and b), indicating whether they strongly disagreed, disagreed, neither
1. I think that AR has helped my understanding of the material covered in the lecture (20 responses)

2. Did you find AR content more engaging than the lecture alone? (19 responses)

3. Would you want to use AR in class in future? (20 responses)

| Strongly disagree | Disagree | Neither agree or disagree | Agree | Strongly agree |
|-------------------|----------|---------------------------|-------|---------------|
| 1                 | 2        | 3                         | 4     | 5             |

A one-sample Wilcoxon signed rank test for a null distribution ($\mu = 3$)

| Q   | Mean value | P-value by normal approximation | 95% Confidence Interval |
|-----|------------|---------------------------------|-------------------------|
| 1   | 4.17       | <0.001                          | 4.49-5.00               |
| 2   | 4.25       | <0.001                          | 4.00-4.99               |
| 3   | 4.52       | <0.0001                         | 4.5-5.00                |

Figure 5. Responses to survey questions. (a) The figure shows the three statements or questions that were posed at the end of the AR session for both groups 1 and 2. The number of responses that were received to each statement or question is marked in parentheses. (b) Responses were recorded on a Likert scale graded from 1 to 5 (strongly disagree through to strongly agree). (c) The chart to show the responses recorded. Percentages for overall agreement (response 4 or 5), disagreement (response 1 or 2) or indifference (response 3) is shown. (d) The table shows mean scores and the outcome of a non-parametric one-sample Wilcoxon sign rank test for a null distribution ($\mu = 3$), including probability values and 95% confidence intervals.
agreed or disagreed, agreed or strongly agreed to each statement or a question. The proportion of responses (1-5) for each statement or question are plotted in a bar plot shown in Figure 5c. About 80% of respondents agreed or strongly agreed that AR had helped them to understand the material covered in the lecture (100% response rate). A further 89% agreed or strongly agreed that they found the AR content more engaging than the lecture alone (95% response rate) and 90% agreed or strongly agreed that they would want to use AR in class in future (100% response rate). Collectively, this indicated overwhelmingly that participants found the use of AR engaging, confirmed that it supported their learning and is something they would like to make use of in future. It is likely that such interactive experiences may also prompt students to continue their study of these traditionally niche topics outside of the classroom.

Finally, study participants were prompted to provide open-ended responses to questions regarding their ‘thoughts on the use of AR’, what they felt were the ‘positives of AR’ and any ‘limitations of AR’. Free-text responses on participants’ thoughts of AR were recorded, and sentiment analysis was performed to extract the overall sentiment of each response. Responses were analysed using the tidytext R package to screen responses for matches to the ‘Bing’ sentiment lexicon (Hu and Liu 2004; Silge and Robinson 2016). After removal of stop words, matches to the lexicon were scored as either ‘positive’ or ‘negative’ for each word. The overall sentiment of each response was made by calculating the sum of the positive words (+1) and negative words (-1). Figure 6a shows each of the responses recorded and the overall sentiments (all responses to free-text questions are shown in Figure S1).

Overwhelmingly participants responded positively when prompted to offer their ‘thoughts on the use of AR’. To explore these sentiments in more detail, participants were also asked to provide free-text responses regarding what they thought were the positives and negatives of AR. To summarise these responses, word clouds were generated using a free online word-cloud generator accessible at https://www.wordclouds.com/. Figure 6b and c shows the word clouds generated from participant responses, all words excluding stop words such as ‘and’, ‘the’ and ‘or’ are shown with more frequently occurring words appearing larger.

Predominantly, when asked for their thoughts on AR, participants mentioned how the technology helped them to better understand and visualise the protein structures:

‘Much easier to see the structure as a 3D model which makes it easier to visualise and understand’.
‘I struggle to understand this topic, as its not stuff you can see, but this definitely helped to a certain extent’.
‘It is a useful tool to visualise complex structures’.

When asked to comment on the ‘positives of AR’, students commented predominantly its use as an aid to visualisation:

‘Brings the protein to life, helps you to see the peptides and how they join to make larger structures’.
‘You can actually see what you study about’.

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Figure 6. Participant free-text responses on the use of AR. (a) The figure shows sentiment analysis of participant responses to their ‘thoughts on the use of AR’. Analysis was performed using the tidytext R-package and the ‘Bing’ lexicon published by Hu and Liu (2004). Positive (+1) and negative (−1) sentiments were totalled for each response to provide an overall sentiment for responses received. (b) Statements regarding the ‘positives of AR’ and (c) the ‘negatives of AR’ were used to generate a word cloud – all words excluding stop words such as ‘and’, ‘the’ and ‘or’ are shown with more frequently occurring words appearing larger. Word clouds were generated using wordclouds.com. The number of responses recorded for each question (excluding NA or blank responses) is noted below each word cloud.
Finally, when asked to comment on the potential ‘negatives of AR’, participants highlighted their practical issues, such as the requirement for good internet connection, available space with larger classes and learning depth:

‘Sometimes it loses connection but may be due to wifi’.
‘Space may be an issue in larger classes’.

Taken together, it is clear that participants found the use of AR to be interactive and engaging. Furthermore, participants recognised the value of this technology in facilitating their understanding of complex 3D biological models in relation to their function.

Discussion
Traditionally, bioscience topics such as structural biology have predominantly been taught using static 2D images of what are extensively complex 3D structures. Previously, researchers have attempted to address this shortfall by employing object-based learning in order to provide students with tactile objects or models of the structures
they are studying (Smith 2016). This approach has key advantages to students over 2D still images, including enabling them to manipulate the object in 3D space, explore protein-protein interactions and stimulate peer-to-peer discussions, thereby facilitating dialogic learning (Da Veiga Beltrame et al. 2017; García-Carrión et al. 2020). This is further illustrated by the work of Gillet et al. (2004) who combined 3D-printed models with AR to overlay additional information on physical models, thereby demonstrating the interactions mediated by proteins and small molecules (Gillet et al. 2004). Despite the significant advantages of this active learning approach, it remains logistically challenging to prepare because specialist equipment, such as 3D-printers, are required to generate models along with an inordinate amount of time to generate these. In this study, we used AR to flexibly create and introduce several 3D protein crystal structures at scale that students could dynamically explore in the classroom. Through administration of a formative quiz and a survey, we sought to capture the impact of this activity on learning. Crucially, we identified that despite only a modest impact on quiz performance, students overwhelmingly found the activity unique and engaging.

Ultimately, AR has the potential to enrich bioscience education and may serve as an effective teaching aid where visualisation of 3D models is central to the learning outcomes. Effective expansion of the learning environment is paramount, considering the new challenges faced in higher education in a post-COVID19 world, whereby remote learning is fortified by engaging and interactive content. As a result, it is likely that AR will become a more widespread tool for delivery of digital content. Importantly, care needs to be taken to ensure the relevance of AR-based activities in relation to the key concepts being conveyed and the learning outcomes stipulated. Although further investigation is required to determine whether AR aids overall student attainment, significant opportunities remain to expand the classroom in many subject areas beyond Structural Biology.

Author contributions
L.R. prepared AR content and helped to carry out the study session, R.A.B. designed the study and wrote the manuscript, E.B. helped to carry out the study session and aided with data collection, M.B helped to identify and develop the content generation pipeline, A.S. provided guidance on the use of digital content, I.T. and M.G. provided access, instruction and guidance to both facilities and specialist equipment required for the study.

Open data and ethics statements
Data collected during the study will be made available on request. Ethical approval was obtained prior to the start of the project in line with Solent University’s Ethics Policy. Participants were provided with the opportunity to withdraw from the study at any point.

Conflicts of interest
The authors declare no conflicts of interest.
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