

VISUALIZING THE PROTEIN-PROTEIN INTERACTIONS NETWORK IN
VIRTUAL REALITY AND MIXED REALITY ENVIRONMENTS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF INFORMATICS OF
THE MIDDLE EAST TECHNICAL UNIVERSITY
BY

BÜŞRA ŞENDERİN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE
IN
THE DEPARTMENT OF MULTIMEDIA INFORMATICS

AUGUST 2021

Approval of the thesis:

**VISUALIZING THE PROTEIN-PROTEIN INTERACTIONS NETWORK IN
VIRTUAL REALITY AND MIXED REALITY ENVIRONMENTS**

Submitted by Büşra Şenderin in partial fulfillment of the requirements for the degree of **Master of Science in Modelling and Simulation Department, Middle East Technical University** by,

Prof. Dr. Deniz Zeyrek Bozşahin
Dean, **Graduate School of Informatics**

Assist. Prof. Dr. Elif Sürer
Head of Department, **Modelling and Simulation, METU**

Assist. Prof. Dr. Elif Sürer
Supervisor, **Modelling and Simulation, METU**

Assoc. Prof. Dr. Nurcan Tunçbağ
Co-Supervisor, **College of Eng. and School of Medicine, Koç University**

Examining Committee Members:

Prof. Dr. Ahmet Oğuz Akyüz
Computer Engineering, METU

Assist. Prof. Dr. Elif Sürer
Modeling and Simulation, METU

Assoc. Prof. Dr. Nurcan Tunçbağ
College of Eng. and School of Medicine, Koç University

Assist. Prof. Dr. Burkay Genç
Computer Engineering, Hacettepe University

Assoc. Prof. Dr. Hüseyin Hacıhabiboğlu
Modeling and Simulation, METU

Date: 31.08.2021

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : Büşra Şenderin

Signature : _____

ABSTRACT

VISUALIZING THE PROTEIN-PROTEIN INTERACTIONS NETWORK IN VIRTUAL REALITY AND MIXED REALITY ENVIRONMENTS

Şenderin, Büşra

MSc., Department of Multimedia Informatics

Supervisor: Assist. Prof. Dr. Elif Sürer

Co-supervisor: Assoc. Prof. Dr. Nurcan Tunçbağ

August 2021, 67 pages

Thesis Abstract – Protein-protein interactions (PPI) define the physical contact of two or more protein structures. When these interactions are combined, the protein-protein interaction network (PPIN) is formed. The interactions between protein structures are distinct interactions—they happen in specific binding locations on proteins, and they have a specific biological function that they take on. With these networks, the processes within a cell or a living organism when healthy or diseased can be studied. In this thesis, a 3D visualization framework to envisage PPIs and PPINs in virtual reality (VR) and mixed reality (MR) environments will be developed. Detailed performance tests of the framework will be provided and analyzed for both VR and MR environments.

Keywords: Protein Interaction Networks, Virtual Reality, Mixed Reality.

ÖZ

PROTEİN ETKİLEŞİMLERİNİN SANAL GERÇEKLİK VE KARMA GERÇEKLİK ORTAMLARINDA GÖRSELLEŞTİRMESİ

Şenderin, Büşra

Yüksek Lisans, Çoklu Ortam Bilişimi Bölümü

Tez Danışmanı: Dr. Öğr. Üyesi Elif Sürer

Eş Tez Danışmanı: Doç. Dr. Nurcan Tunçbağ

Ağustos 2021, 67 sayfa

Tez özeti – Protein-protein etkileşimleri (PPE), iki veya daha fazla protein yapısının fiziksel bağlantı yapısını tanımlar. Bu etkileşimler birleştirildiğinde, protein-protein etkileşim ağı (PPEA) oluşur. Protein yapıları arasındaki bu etkiler belirgin etkileşimlerdir. Bu etkileşimler, proteinler üzerindeki belirli bağlanma yerlerinde meydana gelirler ve üstlendikleri belirli bir biyolojik fonksiyon bulunmaktadır. Bu ağlarla, sağlıklı veya hastalıklı bir hücre veya canlı organizma içindeki süreçler incelenebilir. Bu tezde, Sanal Gerçeklik (SG) ve Karma Gerçeklik (KG) ortamlarında PPEleri ve PPEAleri gözlemlemek için üç boyutlu bir görselleştirme sistemi geliştirilecektir. SG ve KG ortamları için sistemin ayrıntılı performans testleri sağlanacaktır ve analiz edilecektir.

Anahtar Sözcükler: Protein Etkileşim Ağları, Sanal Gerçeklik, Karma Gerçeklik.

To My Loving Family

ACKNOWLEDGEMENTS

First of all, I would like to thank my advisor Assist. Prof. Dr. Elif Sürer for shedding light on my path with her knowledge and experience on this long journey. I want to thank my co-advisor, Assoc. Prof. Dr. Nurcan Tunçbağ, for patiently answering my questions and not allowing me to float like a leaf in thousands of information.

I would like to thank my family for being with me in every decision and comforting me with my uncertainty. I could not have continued without their support and understanding. I am forever grateful to them for wrapping me in patience and love. Thank you too, number three, for being my source of joy.

I would like to thank Elif Hilal Korkut for helping me with her outstanding visual skills and İrem Karden Öztop for her support, time, and smiling face.

I would like to thank the participants for their time and invaluable feedback.

Finally, I thank myself for not giving up.

TABLE OF CONTENTS

ABSTRACT	iv
ÖZ	v
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	x
LIST OF FIGURES.....	xi
LIST OF ABBREVIATIONS	xii
CHAPTERS	
1 INTRODUCTION.....	1
1.1 Problem Statement and The Motivation of the Research	1
1.2 Aims and Contributions.....	2
1.3 Definition of Terms	3
1.4 Thesis Outline.....	4
2 LITERATURE REVIEW.....	5
2.1 Visualization Tools In Bioinformatics	5
2.2 The Use Of Immersive Technologies In Scientific Research.....	8
2.3 Immersive Technologies In Bioinformatics Visualization Tools.....	9
2.4 Summary and The Research Gaps	11
3 THE DEVELOPMENT OF BIONET-XR.....	13
3.1 Programming Language and Development Environment	13
3.2 Versions	14
3.2.1 Desktop Version with Two Viewer Modes.....	14
3.2.2 Virtual Reality Version	15
3.2.3 Mixed Reality Version	16
3.3 Software Feature Set.....	17
3.3.1 Supported File Formats	18
3.3.2 Calculating Network Parameters.....	19
3.3.3 Layouts	20
3.3.4 Finding Shortest Path	21
3.3.5 Finding Subnetwork	21
4 THE EVALUATION OF USABILITY	25
4.1 Participants	26
4.2 Procedure	29

4.3	Exercise Design	30
4.4	Data Collection Instruments	31
5	RESULTS AND DISCUSSION	33
5.1	Data Analysis of Usability Study	33
5.1.1	Analysis of SUS Questionnaire.....	33
5.1.2	Analysis of TAM Questionnaire	34
5.1.3	Investigation Into BioNet-XR’s First Impression	36
5.1.4	Investigation Into The Opinions of Participants About 3D vs. 2D	38
5.1.5	Investigation Into The Opinions of Participants About VR and MR Versions	39
5.1.6	Feedback	42
5.2	Network Construction Performance of The BioNet-XR.....	42
5.3	Discussion	44
6	CONCLUSION AND FUTURE WORK	47
6.1	Future Work	49
	REFERENCES.....	51
	APPENDICES	61
	APPENDIX A	61
	APPENDIX B	64
	APPENDIX C	66

LIST OF TABLES

Table 1: Summary of version features.	18
Table 2: Descriptive statistics of average SUS scores of groups.	33
Table 3: Descriptive statistics of average TAM scores of groups.....	34
Table 4: Independent samples t-test results for TAM evaluation of groups.	35
Table 5: Independent samples t-test results for TAM evaluation of those who have used a visualization tool and those who have not.	36
Table 6: Strengths and weaknesses of BioNet-XR.	38
Table 7: Network construction time of the PC and VR versions.	43
Table 8: Network importing time of the MR version.....	44
Table 9 Comparison of iCave, VRNetzer and BioNet-XR.	46

LIST OF FIGURES

Figure 1: Network creation steps of BioNet-XR.....	13
Figure 2: Example networks in the PC version.....	14
Figure 3: Example networks in VR with (a) desert and (b) blue wireframe theme...	15
Figure 4: Example networks in MR versions with (a) Single Circular Layout and (b) Force-directed Layout.	17
Figure 5: Example node selections and network parameters are shown.....	19
Figure 6: Visualization of the same network with (a) Single-Circular Layout, (b) Single Circular Layout with Louvain Community Detection, and (c) force-directed layout.	20
Figure 7: Screenshots of sample results of shortest pathfinding.....	21
Figure 8: Screenshots of sample subnetwork visualization by using (a) all-pairs shortest path, (b) Steiner tree, and (c) random walk in the same network with the same seed nodes. The angle was kept constant while the images were taken.	22
Figure 9: Education status of subjects.....	27
Figure 10: Distribution of the departments where (a) Bioinformatics group and (b) multidisciplinary group received their postgraduate education.	28
Figure 11: Participants' distribution of using visualization tools before. The chart was prepared from the answers to the question "Have you used any visualization software before?" in open-ended questions.	29
Figure 12: Participants' distribution of experiencing any VR/MR application before. The chart was prepared from the answers "Have you experienced any VR/MR application before?" in open-ended questions.	29
Figure 13: Adjective ratings that correspond to the SUS scores. The chart was prepared according to the study of Bangar et al. [44].	32
Figure 14: Histogram of SUS scores of groups.	34
Figure 15: Boxplots of average TAM score of the groups.....	35
Figure 16: The word clouds are composed of the 20 most frequent adjectives in the given answers to the question about the first impression as (a) Turkish and (b) English.	37
Figure 17: Distribution of the classified participants' responses given to the question, "Do you think it would be beneficial to examine protein interaction networks using virtual reality with BioNet, based on the video you watched?".....	40
Figure 18: Distribution of the classified participants' responses given to the question, "Do you think it would be beneficial to examine protein interaction networks using mixed reality with BioNet, based on the video you watched?".....	41

LIST OF ABBREVIATIONS

3D	Three-dimensional
2D	Two-dimensional
SDK	Software Development Kit
MR	Mixed Reality
VR	Virtual Reality
AR	Augmented Reality
XR	Extended Reality
SUS	System Usability Scale
TAM	Technology Acceptance Model
UI	User Interface
PIN	Protein Interaction Network
HMD	Head-Mounted Display
PPI	Protein-Protein Interaction
PIN	Protein Interaction Network
CAVE	Cave Automatic Virtual Environment
IoT	Internet of Things
VRML	Virtual Reality Markup Language

CHAPTER 1

INTRODUCTION

Visualization tools are basic research materials for bioinformatics studies. Visualization can be broadly divided into two as visualization of biological structures and biological networks. Biological structures such as proteins, DNA, and intracellular structures are three-dimensional structures. Biological networks identify the processes in biological structures and their interactions. For example, genetic networks are used for the gene expression process, signal transduction networks for a cell's stimulation-response process, and protein-protein networks for the interaction between proteins. Both structures and networks are diverse and complex. Hence, a thorough examination under many sub-topics such as gene expression, signal transduction, the structure and interaction of proteins, and the metabolic process is required.

Many bioinformatics studies involve finding information hidden in large amounts of data, so methods such as scanning or filtering data cannot be sufficient. Data visualization tools become important because processing data and obtaining information become easier processes, accelerating the research timeline. Due to the diversity of biological structures, their interactions and observation methods differ. Hence, visualization tools need to be customized for specific purposes in use. Hence, while developing a visualization tool for any topic, it is essential to determine the requirements and methods in line with the need to provide a helpful tool.

1.1 Problem Statement and The Motivation of the Research

Visualization in two-dimensional (2D) and three-dimensional (3D) space is common and essential. Many of the visualization tools support 2D visualization. Although it is common, it has some drawbacks that differ depending on the visualization. Visualizing structures in 2D is not sufficient for reducing complexity without information loss and presenting interaction [1]. Studying biological systems in 3D rather than 2D is better to understand and observe details.

In terms of biological networks, it may be sufficient to visualize them in 2D up to a certain level of complexity. Still, when visualization of a large-scale mesh is required, it becomes difficult to obtain an aesthetic and understandable appearance. Problems such as edge crossing and overlapping nodes need to be overcome. Edge-crossing is

the overlay of lines, making it challenging to understand which nodes are interacting when looking at a network.

However, moving it to 3D by adding depth to the mesh allows solving the abovementioned problems efficiently. 3D space provides mobility in the Z-axis. It makes it easier for the users to distinguish edges from each other at first glance and follow the edges by rotating the network [2]. Besides, features such as zooming and navigation in 3D space allow discovering networks quickly. Also, 3D stimuli are perceived and classified faster than 2D stimuli in object and face identification [3]. Therefore, supporting the visualization in 3D allows researchers to fathom the details more quickly and easily. However, it is also essential to discuss the use of flat computer screens in 3D visualization. Visualization as 3D on flat screens has inherent limitations compared to detecting the perception of a 3D object in the real world [4].

Studies have shown that people understand data better in virtual reality (VR) environments than in traditional 2D and 3D displays. Cassidy et al. [5] state that some details are difficult to observe as 3D and emphasize VR use because VR offers a broader field of view that is not clipped. Ware and Frank [6, 7] experimented to quantitatively measure whether information networks are better understood in viewing setup. Participants were asked to follow a short path between the two nodes on a network. Three viewing configurations were used, which are 2D view, 3D view, and 3D view with a head-tracking device that enables VR environments. The experiment showed that the graph is three times more understandable than using a 3D view with a head-tracking device. Also, Ratamero et al. [8] stated that immersion is essential for users to believe that such presences exist. It was highlighted that such an approach could have educational functions for researchers and the general audience to learn proteins.

In mixed reality, another immersive technology, virtual elements are placed in the real environment. Thus, VR provides benefits by isolating the user from the real environment, which MR does without disconnecting the user from the physical environment [92]. It makes navigation easier in mixed reality, and users can navigate without feeling unsafe.

This thesis addresses the lack of 3D visualization and immersion of the available tools for protein interaction networks. It also aims to include various analysis functionalities as well as visualization. Finally, this thesis aims to address the lack of assessment in the usability of the developed visualization tools. Thus, a thorough usability evaluation of the proposed visualization framework is presented in this study.

1.2 Aims and Contributions

This thesis focuses on the visualization of protein interaction networks and investigates the potential of immersive technologies. For this purpose, a visualization tool named BioNet-XR has been developed. The name BioNet is formed of combinations of the biological network terms. Since the software has VR and MR versions, the abbreviation of the extended reality (XR), which covers all immersive technologies, has been added to the end.

It has three versions: desktop, VR, and MR. In all these versions, networks are visualized in 3D. Desktop and VR versions offer analysis methods such as pathfinding and subnetwork finding. Thanks to exporting the network created in the desktop version, the MR version provides the opportunity to interact with a protein interaction network in a real-world environment. The desktop version works on Windows and Mac environments.

A journal article on this study is about to be submitted. The contributions of this thesis study can be listed as follows:

- The desktop version is one of the few tools which visualize protein interaction networks in 3D and offer some analysis functionalities.
- The VR version is an early example of visualizing protein interaction networks in 3D with analysis functionalities.
- The MR version is the first example of MR technology in bioinformatic visualization tools to the best of the author's knowledge.
- The contribution of immersive technologies in these tools was investigated via both VR and MR versions.
- The usability and technology acceptance of the desktop version is evaluated comprehensively and reveals a successful implementation and further improvement areas.

1.3 Definition of Terms

Virtual Reality (VR) is human-computer interaction in an artificial world that contains interactable virtual objects [2]. The user can move around this world, touch, or rotate objects. In this way, the user feels self in this world and forgets that such a world does not exist. To experience a VR application, advanced devices or cheap solutions are available. Advanced devices are more effective at increasing immersiveness. As a cost-effective solution, a Google Cardboard is sufficient with a smartphone.

In augmented reality (AR), virtual objects are shown in the real environment rather than a virtual environment. A smartphone is enough to experience AR technology most of the time. Therefore, it is frequently used in games and phone applications. The most known examples are the augmented reality game Pokemon Go in which Pokemon characters are searched around with the help of the phone's camera and live filters applied to people's faces in social media applications. Since virtual objects are placed over the real environment, they cannot interact with the real world. Their interaction with the user is also limited. Since the virtual content is located on the surface of the real environment image, its distance to the user never changes [95].

In mixed reality (MR), a user can interact with virtual objects while still seeing the real environment [2]. AR and MR are confusing terms and often used interchangeably since virtual objects are shown in real-world environments. The main difference lies in how virtual content is placed in the real-world environment: in AR, the virtual

content overlays the real world, while in MR, the virtuality is placed into the real world. Thus, MR has not the same interaction restriction in AR. In MR, the virtual object can interact with both the user and the real environment since digital content in MR is 3D holograms integrated into the real environment. Unlike AR, MR has depth perception, which provides perspective [95].

These reality providers have three common characteristics: response to user actions, real-time 3D graphics, and a sense of immersion. Their primary purpose is to make a user feel “being there” (presence) [6]. Hence, they are also called “immersive technologies.” The term used in the literature, which covered all these virtual-real human-computer interactions such as virtual reality and augmented reality, is extended reality (XR) [91].

The immersive technologies are experienced by interacting with the help of special wearable devices such as a controller and a head-mounted display (HMD). An HMD is worn on the head and displays a separate image to each eye to present a 3D perception of a scene. Most HMDs have head-tracking capabilities, which follow the user’s head movements and display the relevant content while providing a 360-degree view.

1.4 Thesis Outline

This thesis has six chapters, including the current chapter. This Introduction chapter covers a short overview, problem statements and motivation of the study, and contributions. The contents of the following chapters are summarized as follows:

Chapter 2 - Literature Review: The state-of-the-art visualization tools, VR, and MR technologies are reviewed. The use of immersive technologies in terms of visualization tools is investigated. Last, the literature is summarized, and the gaps are specified.

Chapter 3 - Development of the proposed tool, BioNet-XR: The chapter provides information about the development and features of BioNet-XR.

Chapter 4 - Evaluation Methodology of Usability: The evaluation procedures of usability, the use of instruments, and the statistical analysis of data acquired are explained in this chapter.

Chapter 5 - Results and Discussion: The statistical analysis results are presented and discussed in detail. In addition, performance values are also included in this section.

Chapter 6 - Conclusion: Finally, the thesis is summarized with limitations, and some possible future improvements are introduced.

CHAPTER 2

LITERATURE REVIEW

The literature review has been divided into three sections, focusing on the visualization tools in bioinformatics, the use of immersive technologies in scientific research, and immersive technologies in bioinformatics visualization tools, respectively.

In the first section, the state-of-the-art and standard tools are presented. It provides information on which features are sought in visualization software and which challenges are present in general. Thus, the essential requirements and needs of the bioinformatics community are presented.

The second section focuses on immersive technologies like VR, AR, and MR and their use in research domains such as education, health, and architecture. Their areas of application and the benefits they provide are exemplified based on scientific research results so that a better understanding of the potential of these technologies is provided.

The last section converges on the visualization tools in bioinformatics and the use of immersive technologies. It covers the studies related to the main subject of this thesis. Recent advancements in the development and the use of visualization tools are summarized. The limitations and challenges, as well as which approaches and resources are used, are presented.

Finally, a thorough summary is provided in the Summary and The Research Gaps section. The gaps in the research area are highlighted.

2.1 Visualization Tools In Bioinformatics

Looking back to the 1950s and earlier, it is known that researchers physically modeled relevant biological structures. For example, Kendrew et al. [9] modeled the myoglobin molecule in three dimensions, using the image of the molecule obtained by the X-Ray method. First, they prototyped the molecule in three dimensions to get a sense of what it looked like. Subsequent studies have increased the number and variety of these models, and researchers requested and purchased them [10]. There were “molecular sculptors” who made such biological structures from different materials such as wood and iron.

Visualization with the computer has begun to replace physical modeling since the 1960s. Langridge et al. [11] succeeded in visualizing molecular structures in 3D and interactively in the study, which started in 1964. It is also one of the first examples of interacting with real-time 3D displays. It is emphasized that 3D structures should be visualized in 3D, even in this initial example of visualization software. Later, it was extended by visualizing the molecules in color by addressing the need to be more apparent of details [12]. Revealing details that other methods cannot observe is one of the main reasons for visualization. The advancements in computer science and technology have enabled the development of visualization software rapidly. They offer analysis capabilities besides visualization today.

That is why developing such tools is a hot topic in the bioinformatics field. However, the biological structures and their interactions with each other are pretty complex and diverse. That fact requires them to be examined and investigated under many sub-titles. Research on these sub-titles needs unique approaches and algorithms. For this reason, the visualization tools are developed specifically for the topic they address, and their use and features differ accordingly. However, they can be grouped under two general headings as visualizing biological structures and biological networks.

Visualizing molecular structures is essential to explore their nature and functions. Some of the numerous programs developed for displaying biological structures include: NGL Viewer [13] is a web-based visualization tool for macromolecular structures that can embed other websites. It helps to understand the relationship between protein structure and function. UnityMol [14], which is developed to present an “interactive virtual biology laboratory,” visualizes and simulates the molecular structures. Mol* [15] is a web-based visualization tool developed for the analysis of macromolecular structures. Besides, it provides a shared library that researchers and algorithm developers contribute to share resources and information in the community. Introduced in 1992 and continuing to be developed, VMD [59] assists researchers in the visualization and analysis of polymers. Pymol [58], another long-serving software, is a 3D molecule viewer introduced in 2002 and is still being refined. In addition to essential molecule display functions, it also has features such as animations and embedding molecule images into presentations. UCSF Chimera [60] is another desktop molecule visualization and analysis software such as VMD and Pymol. The most powerful feature of it is allowing developers to make additions. However, active development of it was terminated as of 2018 since such software must be sustainable and adaptable to innovations.

Visualization of networks is necessary to comprehend better how these structures interact with each other. It is helpful to examine the biological process and detect the interaction and functions of the structures in the process. Since there is no standard for methods used in network analysis [16], the results of the studies can be validated visually. Biological networks include protein-protein interaction networks, metabolic networks, genetic interaction networks, gene/transcriptional regulatory networks, and cell signaling networks [104]. For example, metabolic networks examine the biochemical reactions between metabolites and proteins [61]. Gene regulatory networks show the interaction between sections of DNA, which regulate genes copied into mRNA, and other intracellular elements [62]. Genetic interaction networks describe the interaction between genes to understand their functionalities [63].

The visualization of networks is based on graph theory [17], mathematical modeling of processes and relationships. It is widely used in many disciplines such as mathematics, physics, chemistry, and computer science. A graph is formed by a set of points naming vertices/node and their connections naming edges. In graphs, nodes and their interaction with edges represent biological structures such as genes, proteins, and metabolites.

Examples of popular tools for visualizing biological networks include: Omix [18] offers customizable visualization for metabolic networks. Arena3D [19] displays the biological networks as 3D with layers by combining the data from different databases. For example, it is possible to show the related genes and the relationships between proteins by adding a new layer. It is a compelling feature not found in many software. Its limitation is that it increases the visual complexity and requires a large amount of space to draw the mesh. Medusa [64] visualizes 2D biological networks with various layout options and has clustering functionality. It does not include network-specific methods and algorithms, as it is for general use. Cytoscape [21] offers a wide range of functionalities besides 2D visualization for biological networks. It allows users to add new algorithms and plugins, making it one of the most popular software among researchers. However, the software is complex to use and has a steep learning curve for the same reason. Osprey [65] provides 2D visualization by comparing different data sets to integrate information from different sources. Integrating with various data sources for visualization tools is notable demand and challenge. Examples can be expanded with Bio Layout Express 3D [66], which provides 3D visualization for gene expression data; PATIKA [67] that is developed for visualization of the cellular pathway and OmicsNet [72], which is a 3D web-based tool.

The main focus of this thesis is protein interaction networks. Protein interaction networks are frequently used in studies about many topics ranging from diseases to drug design. In addition to software such as Osprey, Medusa, and Cytoscape, a limited number of special visualization software has been developed for PINs. NetworkAnalyst [16], a web-based tool for PINs, makes it possible to create networks, visualize and analyze them. It addressed the lack of a single tool that can visualize and analyze a PIN. However, despite being web-based, it seems to be lacking in accessing and merging different data sources. In addition to visualizing protein interaction networks, ProViz [20] also has functions for analysis, such as comparing graphs, creating sub-graphs, and clustering proteins and interactions. It supports external data sources and the use of plugins. Other examples are PIVOT [68] and InterViewer [69]. What these tools have in common is that they visualize protein interaction networks in 2D. For researchers interested in 3D visualization, options are Arena 3D, BioLayout Express3D, and OmicsNet.

In summary, the features expected from network visualization tools are [23, 70, 71]:

- Aesthetic and clear network visualization
- Collections of layout algorithms
- Efficient graphical user interface

- Ability to manipulate and customize the network
- Collection of analysis functionalities
- Supporting integration of various data source
- Collaborative work

However, there are some difficulties in fulfilling these requirements. For example, layouts cannot provide an aesthetic appearance for large networks due to computational cost or long time and high hardware equipment requirements. Other reasons are that many of them provide limited interoperability, no data transfer between software, and different data sources use different file formats. Immersive technologies may be the appropriate solution to overcome some of these difficulties in visualizing biological structures and networks [22, 73].

2.2 The Use Of Immersive Technologies In Scientific Research

The emergence of immersive technologies like virtual reality dates back to the 1980s. There is a dependence on hardware to accomplish immersive technologies. As the hardware dependencies were developed, yielded better results, and became more affordable, these technologies became widespread. Although they were initially used for entertainment purposes, they are frequently used in scientific research. They are used in a wide range of applications such as education, health, education, and cultural heritage.

Many remnants of past civilizations are protected as cultural heritage today. However, it is not easy to preserve them in their original form. They move away from their original design due to changing environments and human conditions. Therefore, studies are carried out to use immersive technologies as a method of protecting cultural heritage. Unal et al. [24] contributed to preserving the cultural heritage of the ruined Roman Bath by developing an AR application in which they modeled via drones that ancient place. Gonizzi Barsanti et al. [74] turned the ancient Egyptian funeral ritual into an interactive game using virtual reality, thus aiming to introduce Egyptian funerary objects to people. Immersive technologies make it possible to experience the traditions and life of past civilizations, which have been accessed until now only by reading [75]. They also become widespread in museums and archaeological exhibitions to enhance visitor experience [76, 77, 78].

Immersive technologies are often used in educational applications. They cover a wide range: teaching musical genres in primary school music education [79], developing social skills of children with autism [80], ensuring that students get maximum efficiency from the Computer Graphics course [81], medical students learn the anatomy of the human arm [82] and training of surgeons in robot-assisted surgery [25].

They are also utilized in engineering studies, which is another area where data visualization is needed. For instance, one of the issues that come up with the Internet of Things (IoT) field developments is smart cities which require analyzing city data.

Zhang et al. [26] developed an AR application where Toronto was modeled. Various data were visualized on the model with properties such as different colors and heights. In the user study in which the desktop and AR versions are compared, results show that AR is more satisfying in most aspects such as natural interaction, flexibility, and visual quality. As another example, Rozmi et al. [83] emphasized that 2D/3D software has disadvantages such as cost and unreliability. In contrast, VR and AR have benefits such as sustainability and design simulation in developing renewable energy sources. ARchemy [84], an AR-aided mobile application, visualizes molecules and animates the chemical reactions for educational purposes.

Mixed reality applications are prototyped to provide experience for physicians and students for surgical operations and anatomy to provide more realistic cases [93]. It can also assist robotics research by providing a safer testing environment (for example, robots interact with virtual humans in the physical world) and making debugging easier [94]. The results of the Situated Multimedia Arts Learning Laboratory application, which was built for a high school chemistry class, revealed that the students were more cooperative and motivated [96]. Collaboration is one of the important potential uses of mixed reality. There are studies in which this potential is questioned, such as visiting museums [97], participating in archaeological excavations [98], or making teleconferences [99]. XR technologies offer a solution to touristic activities that are not possible during the pandemic [100]. In addition, these technologies can be adapted to areas such as libraries and offer new and different means of access to information [101]. Their benefits have been demonstrated in architectural designs and simulating how daylight will be used beneficially in them [102, 103].

As a result, VR and MR are just as common in research as well as entertainment. Since many studies have revealed their benefits, focusing on them in areas not used before would be beneficial. A protein interaction network (PIN) visualization tool, which is developed using immersive technologies, can be helpful when their positive contributions in education and engineering are taken into consideration.

2.3 Immersive Technologies In Bioinformatics Visualization Tools

Jonathan et al. [27] emphasize the benefit of the immersive effect of the sense of touch interaction, noting that physical models of the biological structures were used a long time ago. Today, it can be achieved using immersive technologies.

Although HMDs are affordable today, there are studies using web-based VR which promise more accessibility. Web-based VR allows experiencing virtual reality applications through the browser due to APIs and frameworks provided. It has broad hardware compatibility, and there is no need to download any software or plugins. The web-based VR applications work on many operating systems and VR devices. ProteinVR [5] is a web-based 3D protein structure visualization tool that enables the stylization of the molecule's components. Also, it allows scientific collaboration by sending URLs and showing potential benefits for using such a tool for education. VRMol [28] visualizes macromolecular structures by web-based VR and offers some analysis functions, integrating multiple databases specifically related to drugs.

However, web-based VR tools are not frequent because of the limited VR support of the browsers [22]. Also, the HMDs are still needed for the virtual reality experience if they are not developed as a phone application using cardboard VR glasses.

Another method used is CAVE (Cave Automatic Virtual Environment) [29], which was developed in 1992 for scientific visualization purposes. In CAVE, the person is in a room where images are projected onto their walls. She interacts with virtual objects by wearing glasses with head-tracking capability and the controllers she holds in her hands. Since it can still overcome some difficulties, CAVE is used today. For example, when compared with HMDs, CAVE is faster in displaying surroundings. Also, it is safer to move in a CAVE environment, thanks to the awareness of the real environment. iCave [30] is a 3D tool that visualizes biomolecular networks via computer screen, stereoscopic 3D display technology, and CAVE.

In stereoscopic 3D display technology, users can wear stereoscopic display glasses and examine the network by connecting to the computer screen whose stereoscopic display mode is on. The use of stereoscopic 3D display technology is an important choice made in the development of iCave, given that VR technology has limited “portability” because of the hardware limitations of personal computers. It has been emphasized that iCave is entirely “portable” thanks to stereoscopic display. The importance of the study in terms of using VR is as follows: it also offers visualization by using the CAVE (Cave Automatic Virtual Environment) via an immersive 3D method. Since personal computers and VR headsets are more accessible than CAVE, the limitations of VR technologies, such as portability, are overcome. Another tool based on CAVE, MetNet3D [31], displays a metabolic network of genes of interest to the user with animations. However, it can use it on different VR platforms and even desktops, thanks to its infrastructure. It highlights the flexibility of interaction in VR. While the user tries to reduce complexity by manipulating the network on the computer, it happens spontaneously in VR, such as moving around it. The use of CAVE is disadvantageous in terms of daily use and access, as it creates a dependency on an adequately designed physical space. In terms of collaborative work, when compared to CAVE and HMDs, it was revealed that the task was completed faster in HMDs, and there was no difference in accuracy [87].

Therefore, the use of HMDs is more common and effective in immersive technologies. UnityMol-APBS [32], the customized version of UnityMol, aims to offer a more immersive user experience by developing the VR version as well as the desktop version. It visualizes the interaction between organic and inorganic molecules by calculating the electrostatic between them. BioVR [33] visualizes biomolecular sequences with the power of data integration. Three-dimensional visualization of molecules is essential for drug development. Molecular Rift [34] enables interaction with molecules such as proteins, protein-ligand complexes in a virtual environment for drug designers. It is one of the studies that emphasizes the potential of VR to be used for educational purposes. Another tool introduced by Ratamero et al. [8] animates the interactions between drugs and proteins using VR. It has been observed that VR increases immersion and presence perception compared to tools that make visualizations in 2D and 3D. Therefore, the tool is more informative and detailed. CellexalVR [35] is for visualizing and analyzing single-cell RNAseq. It makes collaborative work possible by providing multi-user support. PyMol [58] is a desktop

software, but the data of the displayed molecule can be exported as virtual reality markup language (VRML). In this way, it is possible to see the molecule in a virtual reality environment through software and web services that support VRML import. VRNetzer [85] is a biological network visualizer that uses virtual reality to solve the complexity of large networks. It provides analysis functions along with a customizable interface. Aouaa et al. [86] pointed out that 2D visualization is insufficient to handle large amounts of data and aimed to get a more effective result by combining the new layout approach they developed with virtual reality.

Given that few studies use the potential of AR or MR, multi-user application in AR and MR can be considered for future studies [22]. Hoffman et al. [4] used Hololens to display molecules. The geometric data file of a molecule downloaded from Chimera is uploaded to OneDrive and displayed in Hololens. However, the study presents a set of steps to follow, not a tool. Müller et al. [36] investigated the hardware capacity of Hololens in molecular visualization and highlighted its natural usage close to the real world, like its navigation. ChemPreview [88] is an AR tool that allows interacting with and modifying molecules at the atomic level.

Software developed using these technologies for biomolecular structures is more common than those for biological networks. Existing infrastructure and services to visualize these structures with immersive technologies lack biological networks. A few software visualizes networks using VR, such as MetNet3D and VRNetzer, while none use AR or MR.

2.4 Summary and The Research Gaps

Although the visualization tools are diverse and numerous, the researchers got stuck in using specific tools because a visualization tool has many challenges to overcome, both in terms of performance and functionality. Overcoming all of these challenges is only possible with a community-supported development continuum, as in Cytoscape. It should be noted that even Cytoscape currently only offers 2D visualization and suffers from its disadvantages. Therefore, the main focus of the thesis is to provide 3D visualization of protein interaction networks. However, 3D visualization on a flat-screen is not enough to give the feeling of 3D and immersiveness. A few software for biological networks offers visualization with VR and none with AR or MR environments.

BioNet-XR, a PIN visualization tool with three versions (PC, VR, and MR), is presented in this thesis to overcome the abovementioned shortcomings. In addition, it has analysis functions that offer different layout options, calculate network parameters, find shortcuts, and find subnets. Although its user-friendliness is one of the primary considerations, the methodological evaluation of visualization tools is generally the missing point. Previous studies have not taken a thorough approach in this regard. Therefore, an evaluation method focused on usability has been followed.

CHAPTER 3

THE DEVELOPMENT OF BIONET-XR

BioNet-XR was developed in four steps: the data processing step, the graph data structure construction step, the network visualization step, and the user actions step, as seen in Figure 1. In the data processing step, the file uploaded by the user is read and parsed. As a result of data processing, protein names and connections between them are obtained. A graph data structure is created based on the parsed information. In the graph data structure, nodes represent proteins, and connections represent edges. In the network visualization, 3D objects are visualized as spheres and pipes for nodes and edges, respectively, and the corresponding layout and visual settings are applied. In this way, the network creation process is completed. Responding to the user actions involves executing modifications and functionalities after the network is created.

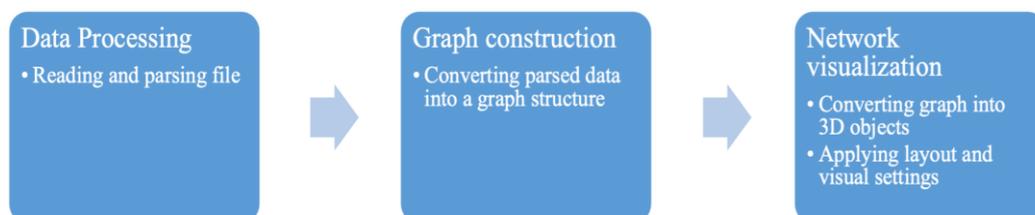


Figure 1: Network creation steps of BioNet-XR.

3.1 Programming Language and Development Environment

BioNet-XR was developed using the Unity3D game engine with the C# programming language. Unity3D is one of the dominant game engines in the game industry. It supports development in 2D and 3D via cross-platform support. It also offers the possibility to use plugins and toolkits for other disciplines such as artificial intelligence, VR, and AR. It has also been used in many academic studies that developed a visualization tool for Unity3D desktop, VR, and MR platforms [8, 32, 34, 35]. Therefore, Unity3D has been the preferred framework during the development of BioNet-XR.

The VR version is compatible with the HTC Vive. However, HTC Vive should be connected to the computer when developing an application, and it does not offer an emulator. Hence, the Vive Input Utility [37] in the Unity Asset Store was used to deal with this challenge. The Vive Input Utility provides both development and emulator

support for the HTC Vive. The desktop version (for Mac and Windows) and VR version were carried out on the Mac.

Some system features require an MR or AR application compatible with Microsoft Hololens, a lightweight and affordable headset. The Mixed Reality Toolkit is provided to develop applications for the Hololens. It is available for Unity3D, too, but it has some system requirements. A computer with Windows 10 (Education, Pro) operating system is essential to use the toolkit. For this reason, the MR version was developed on a computer that meets these requirements. It was tested by deploying the build from the Unity3D to the emulator and Hololens 2.

3.2 Versions

BioNet-XR has three versions; a desktop version for Windows and Mac, VR, and MR. VR and MR versions are compatible with HTC Vive and Hololens headsets, respectively.

3.2.1 Desktop Version with Two Viewer Modes

The desktop version of BioNet-XR does not need the installation process after the software is downloaded. Users just click and run the application. Two viewer modes are provided for the user to examine the network. These are called ‘Default Viewer’ and ‘First-person Viewer.’

In the Default Viewer mode, the user can examine the network with the classical viewpoint. The mouse wheel can zoom in-out and move the network up-down and left-right with WASD or arrow keys. It can rotate the network by holding down the left button of the mouse. Figure 2 shows the example networks created in Default Viewer mode. Furthermore, the user can move back and forth within the network with the WS or arrow keys.

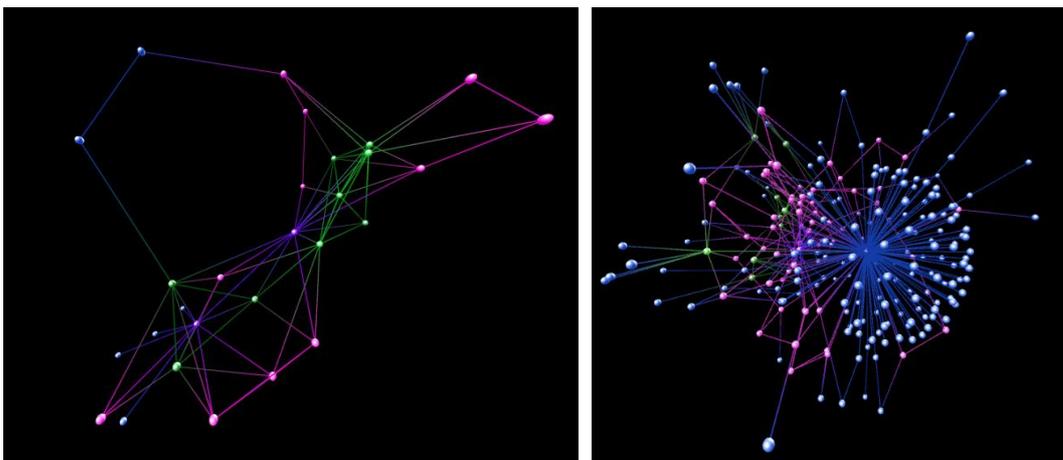


Figure 2: Example networks in the PC version.

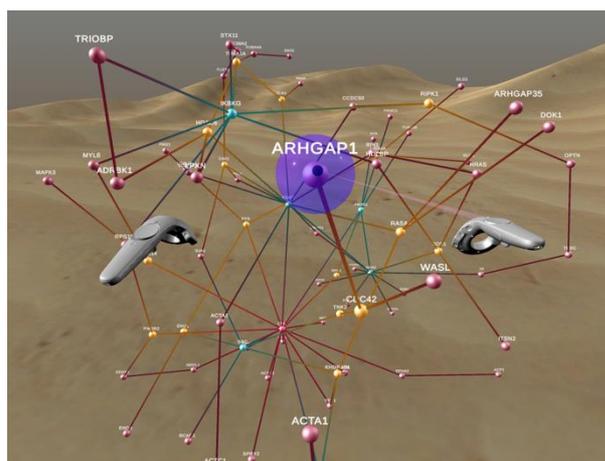
First-person viewer mode is an integrated version of the first-person camera used in games. The difference between the default mode and this mode is the camera’s mobility. In default mode, the camera is fixed, and the network has mobility. The

network rotates and moves closer and further away from the camera. In the first-person viewer mode, the network no longer needs to move because the camera is like a placeholder of the user and has mobility. The user rotates the head and navigates through the network as the camera. It means that when the camera rotates, the users feel as if they are turning their heads. Hence, they get the impression of being inside the network environment. Sample figures for this mode are not provided intentionally. Such figures can cause misunderstanding that both modes are the same because no avatar represents the user positioned as the camera.

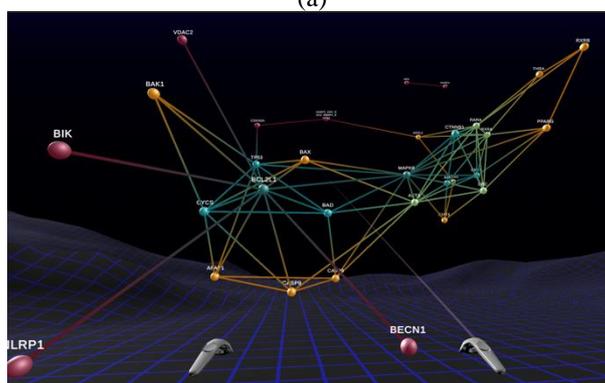
All analytical methods are available in this version. Users can find the shortcut between the two nodes and find the subnetwork with their selected nodes have selected. They can perform various modifications on the network, such as selecting the node, moving it, and viewing neighbor nodes.

3.2.2 *Virtual Reality Version*

The VR version has been developed to make the BioNet-XR more immersive. It has 3D viewing and almost all features offered by the desktop version. It has been designed to work with the HTC Vive, as shown in Figure 3.



(a)



(b)

Figure 3: Example networks in VR with (a) desert and (b) blue wireframe theme.

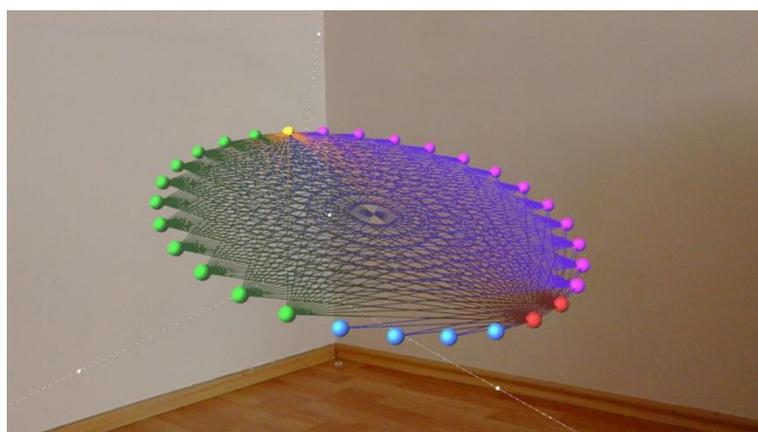
The user can turn her head and have a 360-degree view, thanks to the HMD. The user can navigate in the network by teleporting to the nodes with hand controls. Teleporting enables the user to examine the network from many viewing angles that the PC version

cannot offer. In the VR version, three different virtual environment theme options are available. The user can choose a theme initially and use it in the selected virtual environment.

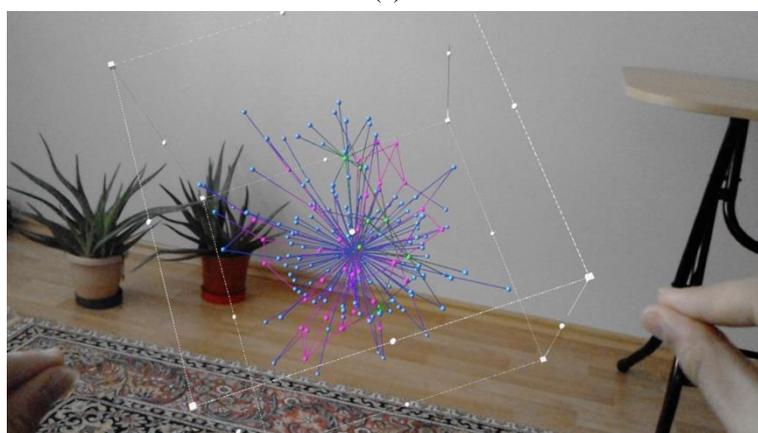
3.2.3 *Mixed Reality Version*

The most crucial feature of mixed reality is supporting the real-world environment instead of a completely virtual environment. In other words, it offers the opportunity to view the created network in a real-world setting. MR is a newly emerging technology in visualization tools, and there are very few studies. An MR version displaying protein networks has also been developed by considering its capability. The version works in harmony with the HoloLens.

After a network is loaded in the desktop version, some information about the relevant 3D network model is saved in a JSON format when the E key is pressed. The data consists of the positions, colors, and sizes of the spheres and lines representing the nodes and edges. When the exported file is loaded to the MR module of the software, the import process performs. It parses the JSON file and creates the relevant spheres and lines using position, color, and size information. Thus, users can visualize the same 3D network model in MR with the layout and aesthetics we have obtained in the desktop version seen in Figure 4.



(a)



(b)

Figure 4: Example networks in MR versions with (a) Single Circular Layout and (b) Force-directed Layout.

The MR version does not have the analysis functionalities of the desktop and VR versions. It can be considered a protein network viewer to move, resize, and rotate using hand gestures. Thus, the network can be examined from different directions and perspectives while the real-world environment is still being observed. At the same time, it offers a natural interaction with real-world objects. In other words, when the user moves around, the angle and distance she examines the network can change accordingly: When the user steps towards the network, she will realize that she is approaching the network. Alternatively, when she rotates around the network, the network angle will shift in that direction. The MR version of BioNet-XR provides immersive interaction with a virtual network in the real world.

3.3 Software Feature Set

The software has features such as calculating network parameters, finding the shortest path, and finding subnetworks by offering different algorithm options. These features are essential to analyze the network in detail. Table 1 shows a summary of these features in terms of versions.

Table 1: Summary of version features.

Desktop Version	VR Version	MR Version
Rotating network		
Zoom in/out		
Ability to move the network in four directions (up-down, right-left)	-	-
Controlling by mouse and keyboard	Controlling by hand controllers	Controlling by gestures
First-person viewing mode	-	-
Moving node	-	-
Exporting network	-	Importing network
-	Three themes	-
-	Moving into the network by teleporting between nodes	-
Node selection and showing network parameters		-
Finding shortest path		-
Finding subnetwork		-
Visual settings (Such as node color, font size.)		-
Three layout options		-
-		Resizing network

3.3.1 Supported File Formats

Files in the local source can load into the application via a file browser. Users can upload files with .txt, .csv or .tsv extensions where each line represents the connection between a pair of proteins. The comma character separates protein names. A network file given as input is simply a list of protein links.

While the network is loading, besides the file selection, it is selected layout and visual settings. Visual settings consist of settings such as font size, node size, and color. The appearance of the representations is tunable with parameters such as node color and node size.

A graph data structure is created by parsing the selected network file. Each protein name corresponds to a node in the graph created, and the link between two proteins corresponds to an edge. When the operation is completed, 3D object representations of the nodes and edges in the data structure are created. Nodes (proteins) are visualized as spherical objects, and edges (bonds between proteins) are visualized as lines between two related nodes. During the creation of 3D objects, the settings mentioned above are also considered. The selected layout algorithm is applied to the 3D graph.

At this point, it is worth mentioning what effect some settings have on visualization. Font size is related to the size of protein names seen on nodes. The default node color is blue and changeable. It is also used in the Random Walk algorithm when forming the color palette. Nodes are assigned according to their degree based on a predefined color palette if the node degree affects the node color. Similarly, when the node degree affects the node size, the node's size is determined in direct proportion to its degree.

In addition, the network creation time varies according to factors such as the size of the network and the selected layout algorithm. How long the network was created is included in the performance data in the Results and Discussion Chapter.

3.3.2 Calculating Network Parameters

When clicking on a node, incident edges and adjacent nodes are highlighted in red, and the edges are pink. Network parameters of this node are shown in the box that appears at the right bottom, as seen in Figure 5. These parameters are node degree, closeness centrality, and betweenness centrality.

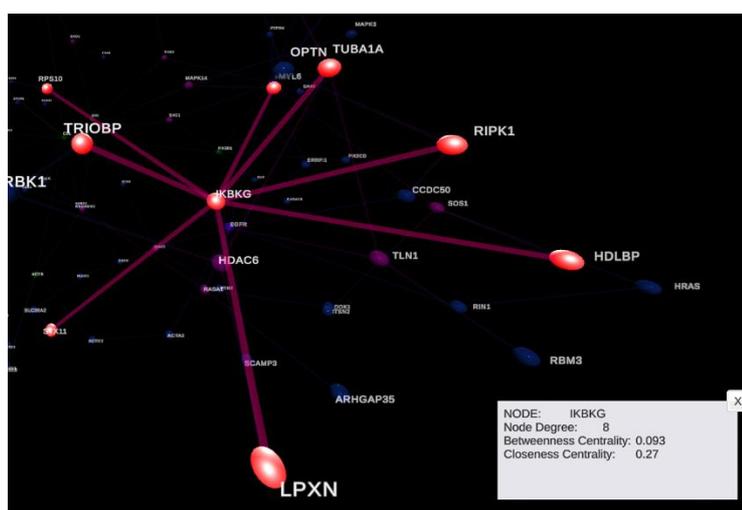


Figure 5: Example node selections and network parameters are shown.

- Node degree: It is the number of adjacent nodes [90]. Nodes with high degrees are called hub nodes. Hubs are a group of highly connected nodes.
- Closeness Centrality: The total length of the shortest paths of a node to all other nodes is calculated. Closeness centrality is the inverse of the average of the total length. Nodes with higher closeness centrality degrees are closer to other nodes, so they are more central than others [90].
- Betweenness Centrality: The ratio of how often a node is in short paths between other nodes [90]. While calculating this measure, the Brandes algorithm [89] is used: the shortest paths between all nodes are found using the Breadth-first Search algorithm. It gives the value on how many of these short paths the associated node is located. It is significant as it acts as a bridge for the flow of information on the network. Nodes with high betweenness degrees are called bottlenecks.

3.3.3 Layouts

When a graph is visualized, it is needed to place nodes and edges properly to get a clear and understandable view. Hence, the layout algorithms are used to determine how a network will lay out in the space. Various layout algorithms have different advantages and disadvantages. Therefore, offering more than one layout network layout is a common feature of visualization tools. BioNet-XR includes three layouts to show the placement of nodes and edges neatly and aesthetically, as seen in Figure 6.

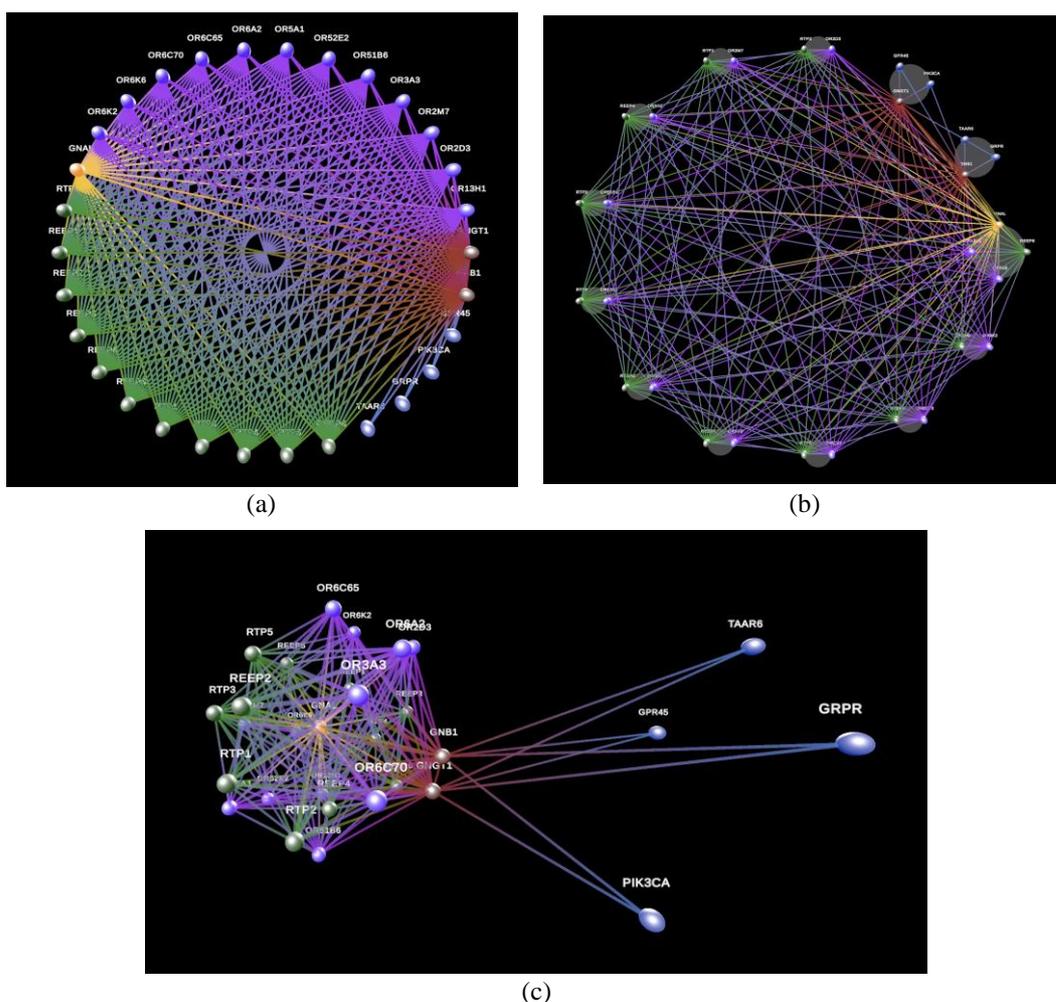


Figure 6: Visualization of the same network with (a) Single-Circular Layout, (b) Single Circular Layout with Louvain Community Detection, and (c) force-directed layout.

- Force-directed Layout

In this layout, the graph is placed by modeling the nodes as if there is a physical push-pull force between them. Fruchterman and Reingold [38] developed an algorithm for 2D graphs, which is adapted to 3D. In a nutshell, nodes are assigned random positions initially. The push-pull force on the nodes is calculated in each iteration, and how much the node will move is computed considering the “temperature” value. The “temperature” value affects the maximum amount the node can move and decreases over time. (The number of iterations can be changed over the Unity Editor.) This

algorithm gives a successful result in terms of aesthetics. The force-directed layout is commonly included in most visualization tools of biological networks [39]. The disadvantage is that in each iteration, the push-pull forces between all nodes are calculated. The applying time can increase as the number of nodes and iterations increases. It can be time-consuming for large graphs [23].

- Single Circular Layout

The used single circular layout is based on the barycenter heuristic [105]. Implementing a barycenter heuristic allows nodes to be placed as close as possible to the nodes they are connected to, rather than randomly around a circle. It reduces overlapping nodes and edges and provides a more aesthetic and understandable appearance. However, since the diameter of the circle increases as the number of nodes increases, the space required for drawing can be pretty large.

- Single Circular Layout with Louvain Community Detection

The combination of Louvain Community Detection and Single Circular Layout is presented as a third layout. First, the clusters in the network are determined with Louvain Community Detection. Then, the single circular layout is applied to the clusters, and the final layout of the network is obtained.

3.3.4 Finding Shortest Path

The shortest path between a start and end node specified by the user on the interface is found using the breadth-first search algorithm. The breadth-first search algorithm finds the shortest path from a start node to an end node by browsing the graph gradually over the neighbors. When a route is found, nodes as red and edges as pink are visualized in Figure 7. If there is no short path, only the start and end nodes are marked with red color. As seen in Figure 7(b), the transparency of the rest of the network is reduced. Thus, the result of finding the shortest path is highlighted.

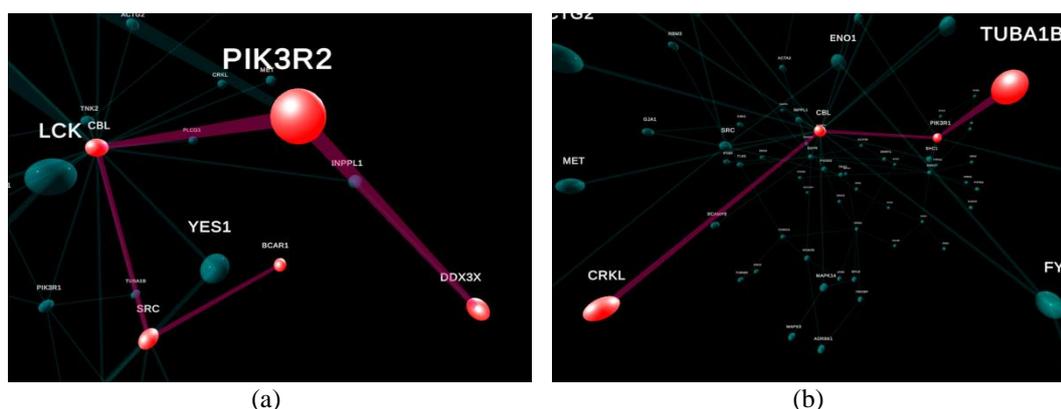


Figure 7: Screenshots of sample results of shortest pathfinding.

3.3.5 Finding Subnetwork

A subnet is a smaller network within a network. Subnetworks help identify genes, pathways, specific processes that have a role in a disease, a process, or an interaction

[107]. Extracting only subsets containing relevant structures and processes from large networks increases the efficiency and speed of research [108]. Extracting only subsets containing relevant structures and processes from large networks increases the efficiency and speed of research [107, 109] because it is easier to work with smaller networks to interpret them to validate experimental data. Determining the critical subnets is also crucial in determining the target structures for diagnosing and treating diseases such as cancer [110, 111, 112].

In BioNet-XR, subnets are used to focus the interaction of specific nodes within the network. These nodes that the subnet is intended to contain are called seed nodes. In order to find a subnet, the seed nodes are determined and given as input to the algorithms. There are two ways to identify seed nodes in BioNet-XR: the user selects them from the node list or uploads a file containing the seed node names and allows it to be selected automatically. The second method makes the selection process more straightforward when it comes to a large number of nodes. BioNet-XR includes the three options for finding a subnetwork: All-Pairs Shortest Path, Steiner Tree, and Random Walk. Figure 8 shows example subnetwork outputs of them, respectively.

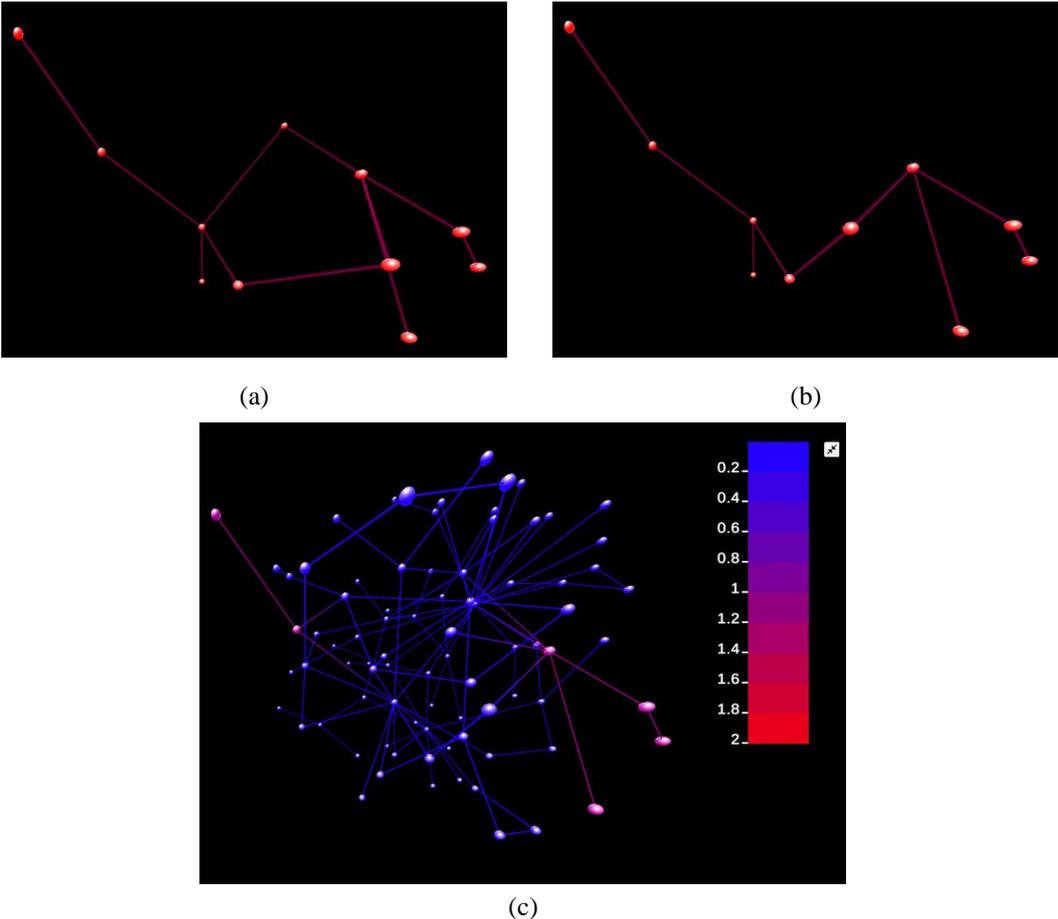


Figure 8: Screenshots of sample subnetwork visualization by using (a) all-pairs shortest path, (b) Steiner tree, and (c) random walk in the same network with the same seed nodes. The angle was kept constant while the images were taken.

- All-Pairs Shortest Path via Floyd-Warshall Algorithm

All-pairs shortest path means the shortest path between all nodes in the network. It is a problem solved thanks to the Floyd-Warshall Algorithm [40]. That is why their names are often used interchangeably. When finding a subnetwork, only the nodes given as input are taken into account, and the shortest paths between them create the subnetwork. Non-seed nodes that are on the path between seed nodes are part of the network. Figure 8(a) shows an example subnetwork found with it.

- Steiner Tree via Kou's Algorithm

A Steiner tree is a problem that interests finding a tree containing a specific node subset in a graph and is another solved problem by Kou's Algorithm [41]. The algorithm first finds a minimum spanning tree in the given graph. Then, unlike the All Pairs Shortest Path, it removes other nodes until all remaining leaf nodes are seed nodes. That is why it provides a more pruned result than the All-Pairs Shortest Path method. For example, the subnetwork found by All-Pairs Shortest Path in Figure 8(a) has more nodes and connections than the subnetwork found by this method in Figure 8(b).

- Random Walk

It is one of the variations of network propagation. In network propagation, the biological signal between genes/proteins interacting is strengthened. Transfers between adjacent nodes will be more frequent, so the signal between them is more robust. It helps determine disease genes, genetic modules, and drug targets [42]. For example, it is incorrect to deduce that neighbors of disease-related proteins are also associated with the disease. Such a method can lead to false results. These proteins may not be associated with the disease, or they may not have an immediate neighborhood with the proteins related to the disease. As an approach, the distance and paths of proteins to disease-associated proteins can be considered. However, the problem here is that many proteins can be at the same distance and have paths. Network propagation tackles the difficulties associated with the disease. However, it is not being detected by considering all possible pathways between genes.

Random Walk enables finding subnetworks by scoring signals of nodes. A certain amount of signal (fluid, heat) is spread from the seed nodes in the network. This signal propagation is repeated a certain number of times. In BioNet-XR, the user can set that iteration number. An equal signal is transferred to the neighbors in each iteration. The amount of signal on the node at the end of the iterations is its score. The only subnetwork is displayed in the first two methods. Unlike this method, the whole network is shown, and the nodes are colored based on their scores. Coloring is done according to a gradient color palette that corresponds to score ranges. The color palette is created using colors determined in the network construction and subnetwork method selection.

CHAPTER 4

THE EVALUATION OF USABILITY

The International Organization for Standardization (ISO) usability definition highlights the importance of enabling users to perform targeted work effectively and satisfactorily [43]. A usable software thus also has a smoother learning curve. Users do not want to use a system that they do not quickly understand how to use. Because it both annoys them and requires spending time learning to use. Therefore, usability is a criterion that is frequently considered and evaluated in software developed.

In very few studies about visualization tools, their usability results were presented by evaluating them. Hence, there is no consensus and standard for testing the usability of tools in the bioinformatics field [44]. The methods and instruments used are so diverse that it is not easy detecting a common approach to set an example for other studies. For example, the usability of the visualization tool called Omix [18] was evaluated as follows: Users that exercised the tool were rated one to ten on how easy it was to use and then completed a survey. As a result, only the average was specified as the usability score, and no additional statistical method was applied. In other words, usability was evaluated but not with advanced statistical methods. In another study [44], a usability evaluation was carried out by applying think-aloud and observation methods when researchers worked on their actual tasks in their work environment. While this is illuminating in understanding real use-cases, it is a challenging method to implement. Bolchini et al. [45] conducted a study about the usability of web-based bioinformatics applications. For this purpose, inspection and task-based thinking aloud methods were conducted for four online applications of biological databases. However, usability was not measured because the study aims to detect general usability problems of existing web tools and propose solutions. Another online service, PlantGenIE, was taken into maintenance by applying usability design principles, and the effect of renewal was investigated [46]. The study used different evaluation methods: the remote test by conducting a survey, quantitative data collection using Google Analytics, task-based user study by conducting an interview, and usability experts assessments. The critical point is that all participants except the last one are plant biologists and familiar with previous versions. It may have been influential in the participants' evaluations. The evaluations may have occurred in evaluating

different versions of the same system, as in A/B tests. The task-based user study by applying an interview method was used for precisely this intention by Javahery et al. [47] to measure the usability of two different designs of a visualization tool. Since not every developed tool is subjected to usability evaluation in the bioinformatics field, such studies are minimal. Although there is no common usability assessment language, these studies have shown that they all prefer a task-based approach [48]. A task-based approach was preferred in this study. The instruments in serious games are referenced because usability is frequently measured in the studies of that domain. The methods and instruments used are explained in more detail in the following sections.

Usability tests measured not only the BioNet-XR's usability to the target audience but also its overall usability. Since BioNet-XR can be used both by researchers in clinical studies and by universities for educational purposes, thanks to the suitability of immersive technologies for collaborative use. Therefore, it should be suitable for both the target audience and the general audience. The tests were conducted with both audience samples. Most available tools are not intended for general audience use and are not easy to use and learn. As the development of visualization tools requires a correlation between disciplines, this challenge is also a barrier to testing existing visualization tools and examining user habits and needs for researchers outside the Bioinformatics field.

Only usability tests of the PC version were performed. Usability testing of VR and MR versions could not be conducted as planned because the participants had to use the headset and hand controllers. Due to the pandemic, hygienic conditions were not suitable for it, and no way was found to decontaminate these devices. Also, the participants were unwilling to come to the institute where the tests would be held. There was no alternative way to perform the tests for the MR version. There were mobile-based and web-based alternative methods for VR, but these were also ineffective and had shortcomings. In mobile-based VR, the user can self-test at home with a smartphone and Google Cardboard. However, interaction is minimal and only possible with gazing. It is challenging for the user to select from the interface since there is no hand control or advanced method to detect movements as in HTC Vive and HoloLens. Since the display is provided from the phone screen, the resolution is low, and the field of view is restricted. These reasons keep it away from being a solution. Web-based VR allows users to test from their homes, but in this case, the user must own a headset. Most of our participants reported that they did not have such a device.

4.1 Participants

Subjects have been invited into the online tests by advisors via email, and participation has been requested. They have enrolled in the experiment by submitting the registration form that requires some information convenient for them to participate. Thirty people have participated in the online tests. Of the participants aged between 24 and 40, 14 (46,67%) were female, and 16 (53,3%) were male. As seen in Figure 9, nearly half of the participants (46,7%) were MSc candidates. The remainder (53,3%) consisted of people with an MSc graduate and further education.

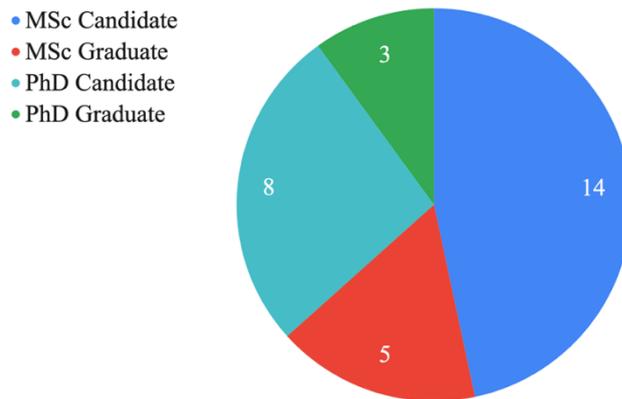
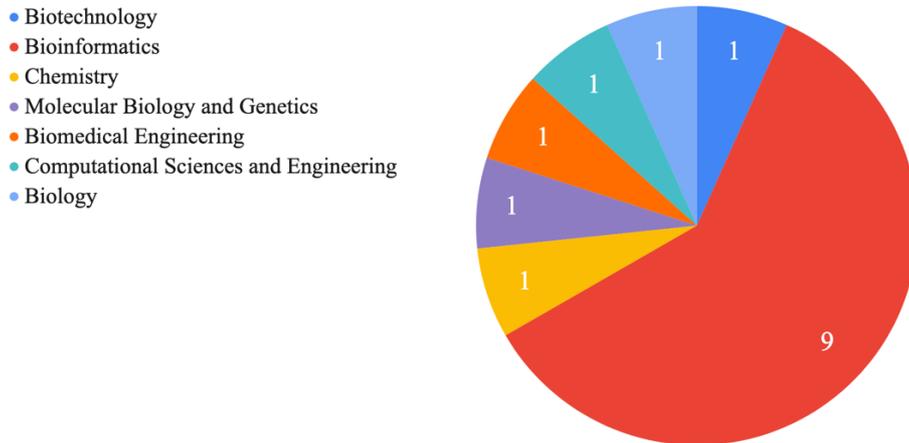


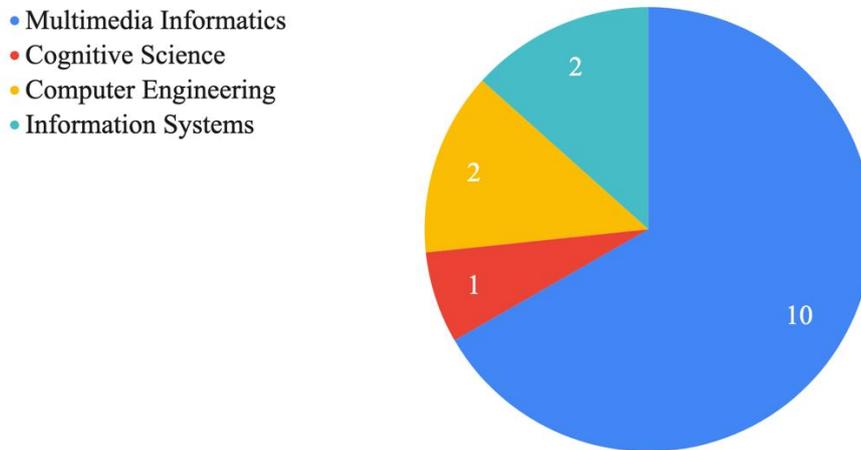
Figure 9: Education status of subjects.

Participants were invited in a planned way to form two groups of 15 each, Bioinformatics participants and multidisciplinary participants. Participants who studied or graduated from one of the departments related to life sciences formed the Bioinformatics group.

Figure 10a shows the department distribution of the Bioinformatics group who received postgraduate education. The participant who has a master's degree in Computational Science and Engineering is in this group because she has a doctorate in bioinformatics and is a lecturer in this field. Those from other disciplines formed the multidisciplinary group. As seen in Figure 10b, 66.6% of the group is from Multimedia Informatics. While the Bioinformatics group is familiar with the visualization tools and target audience, the multidisciplinary group represents a general audience.



(a)



(b)

Figure 10: Distribution of the departments where (a) Bioinformatics group and (b) multidisciplinary group received their postgraduate education.

Since all participants have proficiency in English and Turkish to read and understand, the language used in the exercise, in the SUS and TAM questionnaires, and the software is English. Open-ended questions were prepared in Turkish.

In the open-ended questions, the participants were asked whether they had used visualization tools before and if they did, which ones they used, and how often. In total, 43.3% (N = 13) of the participants stated that they used it and 56.7% (N = 17) did not. As seen in Figure 11, while most in-field participants used such a tool, most out-of-field participants did not as expected. The available software is reported as follows: Cytoscape, Networkx (a package for Python), VMD, Pymol, Gephi, NeoVis, Chimera, and Autodock. According to the number of times they were stated, Cytoscape, VMD, and Pymol were the three software most used by the sample in this study.

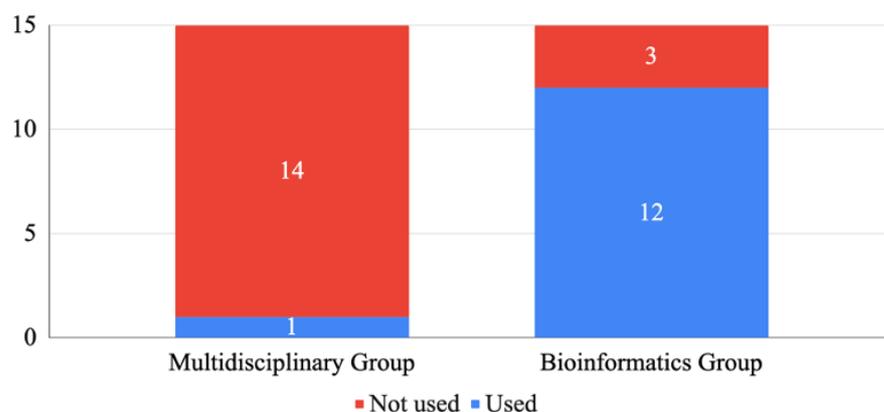


Figure 11: Participants’ distribution of using visualization tools before. The chart was prepared from the answers to the question “Have you used any visualization software before?” in open-ended questions.

In addition, the participants were asked whether they had ever experienced a VR/MR application before. In total, 40% (N = 12) of participants reported that they had not tried VR or MR before. The remaining 60% (N = 18) stated that they experienced it. In Figure 12, it is seen that the number of experienced and non-experienced persons is equally distributed. Participants could not test the BioNet-XR’s VR and MR versions due to the pandemic process. For this reason, it was of great importance to at least watch the usage videos in test sessions to give them an idea. In open-ended questions, participants were asked whether they thought VR/MR versions would be helpful based on the videos they watched.

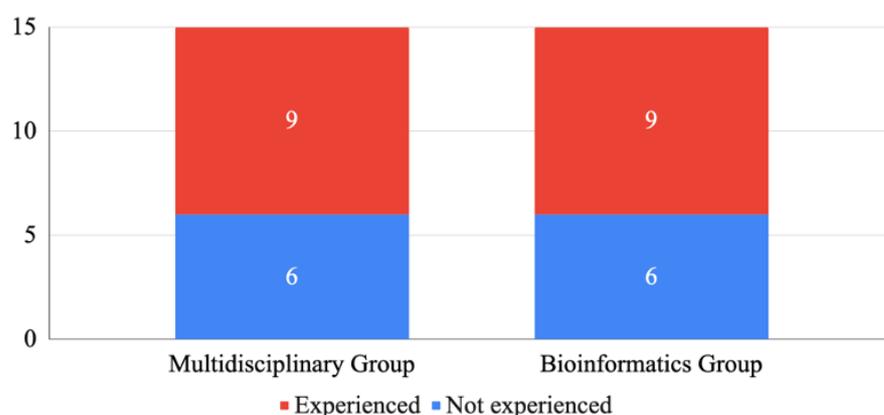


Figure 12: Participants’ distribution of experiencing any VR/MR application before. The chart was prepared from the answers “Have you experienced any VR/MR application before” in open-ended questions.

4.2 Procedure

The procedure was administered online via the Zoom application. Each online test session was one by one in the date and time the participant had been chosen. Before the test date, download links for the software and user guide had been sent to the subjects. It was not asked to use software or read the user guide before the tests. A brief description of the test procedure was described at the beginning of the test.

Subjects were asked to follow four steps in a specified exercise with given data files within an hour. Such an exercise was needed so those, especially non-domain users, would not feel lost while using the software.

During the test session, they could use the user guide and ask for help where they got stuck. The subjects also shared their screens during the exercise, so their use was observed. The test sessions were recorded for later analysis when the participant allowed. The completion time of exercise varied depending on the participant. The most extended test duration was 17 minutes 36 seconds, and the shortest time was 1 minute 60 seconds. The average completion time is 5 minutes and 13 seconds.

After the exercise was completed, they watched videos of VR and MR versions to have an idea. Finally, they were asked to fill questionnaires online. The tests took approximately one hour, including completing the exercise, watching the videos, and completing the questionnaires. Access links for the user guide and videos are available in Appendix B.

4.3 Exercise Design

In the task-based method, the user is asked to complete specific steps. It is widely used in testing visualization tools, which was also applied in this thesis. It provides that the participants did not feel lost, and the usability tests were efficient. For this, an exercise was prepared in such a way as to ensure that each participant tests at least the following primary functions of the software: installing the network, controlling and manipulating the network, finding short paths and subnets.

It is also essential that the prepared task is as close as possible to an actual use case. Hence, the case study in the exercise was designed based on Chen et al. [49] constructing and analyzing PIN for heroin addiction. Such a case study also helps non-field participants better understand the purpose of the software and have a clearer idea of what it will be used for. Additionally, some helpful information and definitions were given, especially for them in the exercise. Further, it did not contain a question to be answered and did not ask for any inferences to be made. Thus, the user can focus only on the software without being under pressure and stress.

The study on which the exercise is based was provided information about a network created for heroin addiction. It provides information about the proteins and connections contained in the network and about these proteins. A data file suitable for BioNet-XR was created using provided information and given to the participants at the beginning of the test. Thus, the participants reproduced the network in 3D in BioNet-XR.

Additionally, the study shows common proteins that are also effective in alcohol, cocaine, and amphetamine addictions. These proteins were prepared as appropriate data files for seed node selection while finding subnetwork in BioNet-XR. The aim is to direct the participant to find a subnet of proteins effective in heroin and another addiction using the corresponding file. The exercise is available in Appendix A.

4.4 Data Collection Instruments

The instruments used are System Usability Scale (SUS), Technology Acceptance Model (TAM), and open-ended questions. The opinions and suggestions that the participants expressed verbally during the tests were also noted.

In task-based user studies, data such as the user's completion time are also often evaluated. However, it was observed that the completion time was not suitable for making an inference in terms of usability. The long or short duration of tests varied in the direction of the participant's desire and interest. For example, while it took a long time to complete because one user had difficulty, it took a long time for another because the user dived into the case study and tested it more thoroughly. Alternatively, it took a short time because one user was in a hurry to complete it, whereas the other was familiar with using such tools. Due to these unpredictable and uncontrollable bidirectional effects, it was not used in the analysis. Contexts of the instruments are available in Appendix B.

- System Usability Scale (SUS)

John Brooke introduced System Usability Scale in 1986 [50] to measure the usability of systems as context-independent. Being context-independent makes it easy to use and flexible. It can be applied commonly in a wide range from hardware to software. Since it is a fast, easy and reliable test, it is also broadly used in academic studies. It is a 5-point Likert scale (1=Strongly Disagree, 5=Strongly Agree) with ten items. Odd-numbered items are positive, and even-numbered ones are negative in terms of syntax and semantics. The contribution of the score of the items to the total score ranges from 0 to 4. It requires a particular calculation to obtain the total SUS score.

The steps are followed to calculate the total SUS score: The scores of odd-numbered items are added, and five is subtracted as in equation 4.1.

$$s_o = \left(\sum_{i=1}^{i=n/2} p_{2i-1} \right) - 5 \quad (4.1)$$

The sum of the scores of the even-numbered items is subtracted from 25.

$$s_e = 25 - \left(\sum_{i=1}^{i=n/2} p_{2i} \right) \quad (4.2)$$

The odd and even number sums are added and multiplied by 2,5. In this way, the range is converted from 0-40 to 0-100.

$$(s_o + s_e) * 2,5 \quad (4.3)$$

A value in the 0-100 range is not considered a percentage value. It is a normalization operation to obtain percentile rankings [106].

The higher than 68, which is in the 50th percentile, is considered satisfactory in usability [50]. Bangar et al. [51] presented adjective ratings to make SUS scores more understandable. Seven adjectives correspond to the score ranges in the adjective scale,

as seen in Figure 13. From the lowest usability rating to the highest, these adjectives are: worst imaginable, awful, poor, ok, good, excellent, and best imaginable.

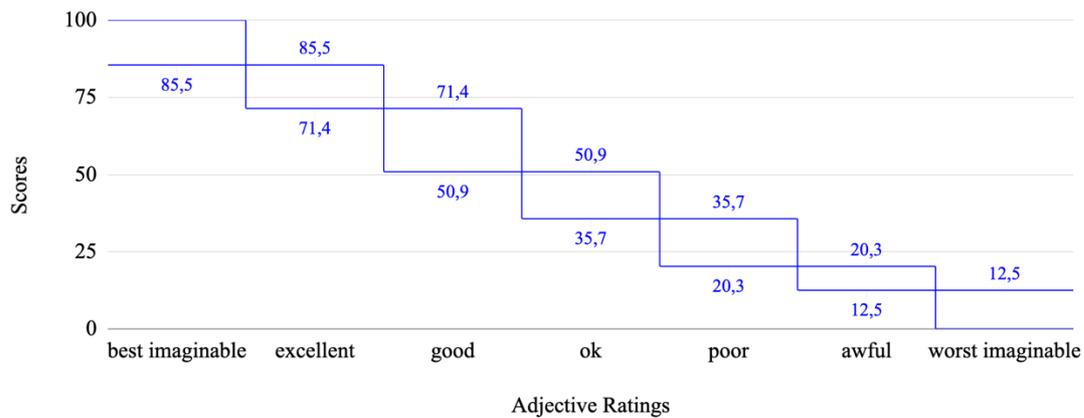


Figure 13: Adjective ratings that correspond to the SUS scores. The chart was prepared according to the study of Bangar et al. [44].

- Technology Acceptance Model Questionnaire

Technology Acceptance Model (TAM) [52] is a framework for assessing information systems. It enables us to understand the target audience’s technology acceptance level of a system. It consists of various assessments that measure multiple aspects of how an information system is used. The main assessment measures are perceived usefulness, perceived ease of use, attitude toward using, and actual current usage of the system. They are extended in the subsequent studies that are for the improvement of the TAM. The used questionnaire consists of 14 questions and is scaled between (0 = Strongly Disagree, 10 = Strongly Agree). All items are semantically positive except for the third item, “During the test, I felt pain and/or discomfort.” For this reason, the best score for the third item is 0, while for the others, it is 10.

- Open-Ended Questions

While preparing open-ended questions, questions used in similar studies were taken as examples [53, 54]. These questions are grouped under several headings (Demographic Questions, General Questions, Questions About Appearance, Questions About Exercise, Questions About VR & MR) and collect feedback on these headings. The Questions About Appearance heading included questions providing feedback in terms of user interface and appearance. In the Questions About Exercise, the questions aim to evaluate the perceived difficulty of the exercise in general. The Questions About VR & MR part provides information about participants’ previous experiences on VR and MR technologies and their impressions about these versions of BioNet-XR.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Data Analysis of Usability Study

Data collected by SUS and TAM questionnaires during usability tests were used for quantitative analysis. The answers to the open-ended questions were used in both qualitative and quantitative analysis. Open-ended questions are Turkish, and participants answered in Turkish. When needed quoting, they translated English. Statistical analyzes were performed using JASP [55], which is an open-source statistical analysis tool.

5.1.1 Analysis of SUS Questionnaire

SUS scores calculated as explained in Chapter 4. The overall application usability is scored as 80,8. These averages for multidisciplinary and Bioinformatics groups were 76,3 and 85,3 respectively, as seen in Table 2. Usability was evaluated as an ‘Excellent’ rank in general and groups specific.

Table 2: Descriptive statistics of average SUS scores of groups.

	Average SUS Score	
	Multidisciplinary group	Bioinformatics group
Mean	76,333	85,333
Std. Deviation	15,694	10,431
Minimum	45	62,5
Maximum	95	100

Figure 14 shows the SUS score distributions by the adjective scale. Although the ratings were the same between the groups, it was observed that there were very few differences in the score distributions. The lowest usability evaluation of the Bioinformatics group was ‘Good,’ while the multidisciplinary group was ‘Ok.’ The score stack is at the highest usability rating, ‘Best Imaginable,’ for the Bioinformatics group. In the non-domain group, scores were nearly evenly distributed among ‘Good,’ ‘Excellent,’ and ‘Best Imaginable.’ As a result of SUS, BioNet-XR showed it has good usability.

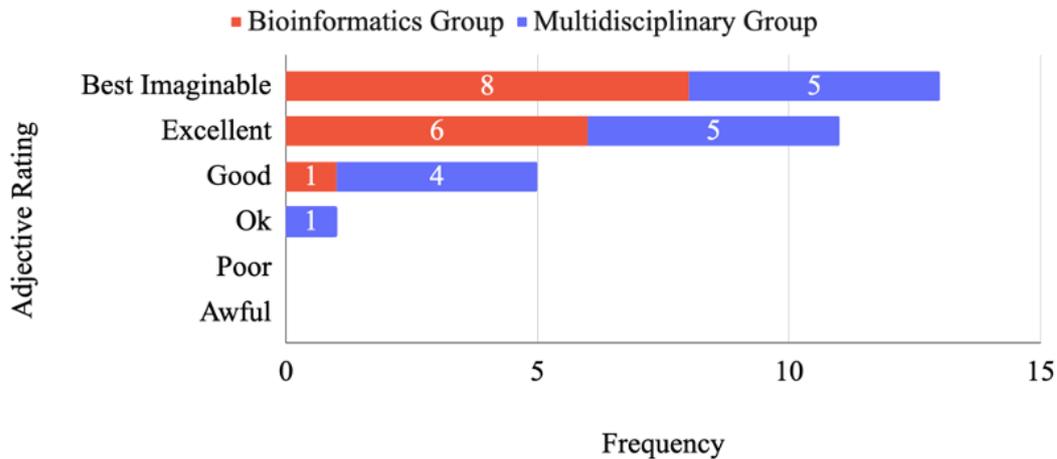


Figure 14: Histogram of SUS scores of groups.

According to the result, the PC version of BioNet-XR was sufficient and satisfactory in terms of usability. Since the usability was found to be acceptable by participants from different disciplines, it demonstrates the potential for BioNet-XR to appeal to general users. Since advanced and popular tools are intended for professional users with domain knowledge, it may be difficult for users other than these to learn and use. Offering such ease of use paves the way for its use for educational purposes.

5.1.2 Analysis of TAM Questionnaire

The TAM score was calculated by averaging the scores assigned to the items. It is important to note that the 3rd item scores were used by subtracting the maximum scale score of 10 since it is a reversed-scaled item. Before analyzing TAM scores, reliability analysis was applied to the questionnaire to evaluate the internal consistency of the scale items. Cronbach’s alpha (α) was calculated, which expresses reliability when a result above .70 is obtained [56]. The alpha for the total scale was 0,843, which is acceptable reliability. However, the individual item statistics showed that its reliability would increase without the third item, which is “During the test, I felt pain and/or discomfort.” Hence, it is indicated as a reversed-scaled item. Because, unlike the others, 0 is the best score that can be given, while 10 is the worst score in the item. As a result, the absolute reliability of the scale is $\alpha = 0,886$.

After validating the reliability of the questionnaire, descriptive statistics were applied. As seen in Table 3, that shows descriptive analysis results, the mean TAM score of the Bioinformatics group ($\mu = 8,143$) is higher than the multidisciplinary group ($\mu = 7,910$). The overall average is 8,026, which is a high score considering the maximum possible score (10).

Table 3: Descriptive statistics of average TAM scores of groups.

	Average TAM Score	
	Multidisciplinary group	Bioinformatics group
Mean	7,910	8,143
Std. Deviation	1,310	1,196

As seen in Figure 15, the concentration in the Bioinformatics group is in the upper scores than seven. In the out-of-area group, the distribution is more dispersed, and some gave low scores. However, looking at the mean and score distribution, it is not sufficient to interpret the technology acceptance of the groups is the same or different. Therefore, independent samples t-test were conducted, which reveals whether the difference between the means of the two samples is statistically significant.

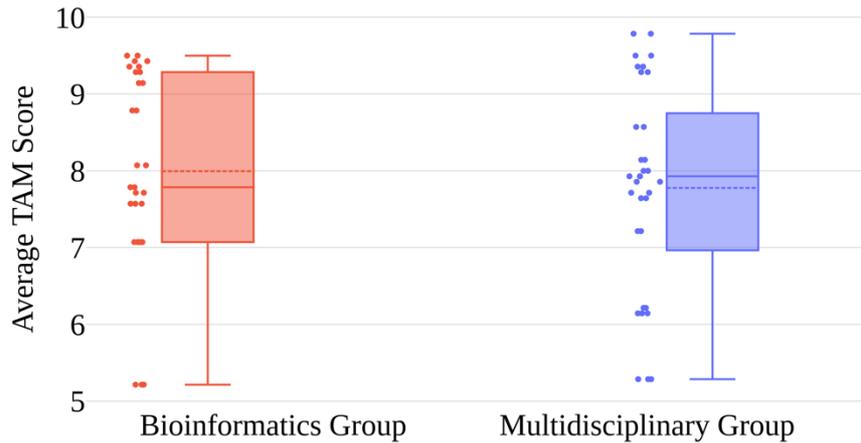


Figure 15: Boxplots of average TAM score of the groups.

The null hypothesis to reject and the two-tailed alternative hypothesis are as follows, respectively:

$H_0: \mu_1 = \mu_2$, which means there is no difference in technology acceptance levels between the groups.

$H_a: \mu_1 \neq \mu_2$, which means the technology acceptance levels of groups are not identical.

The confidence interval is determined as 95%, and the p-value should be less than .005 to reject any null hypothesis. As seen in Table 4, the Bioinformatics group ($M = 7,910$, $SD = 1,310$, $N = 15$) compared to the multidisciplinary group ($M = 8,143$, $SD = 1,196$, $N = 15$) in the terms of technology acceptance level and did not demonstrate significantly different, $t(28) = -.509$, $p = .614$ and rejecting the null hypothesis is failed.

Table 4: Independent samples t-test results for TAM evaluation of groups.

						95% CI for Mean Difference	
	t	df	p	Mean Difference	SE Difference	Lower	Upper
Average Score	-0,509	28	0,614	-0,233	0,458	-1,172	0,705

Acceptable by the Bioinformatics group is an essential indicator for BioNet-XR to be presented to the target audience. Such software is generally challenging to appeal to users outside the domain. The fact that there is no difference between the out-of-field and in-field groups indicates that it has overcome this challenge.

It was also analyzed whether there was a difference between those who used the visualization tool and those who did not. Some participants from the in-field group did not use it. Data on subjects' use of the visualization tool was collected through open-ended questions. The null hypothesis and the two-tailed alternative hypothesis is as follows, respectively:

$H_0: \mu_1 = \mu_2$, which means there is no difference in technology acceptance levels between ones who have used a visualization tool and ones who have not.

$H_a: \mu_1 \neq \mu_2$, which means their technology acceptance levels are not identical.

In the results shown in Table 5, the null hypothesis was not rejected because the p-value is greater than the confidence interval threshold of .005. TAM scores of the group which do not use ($M = 7,966$, $SD = 1,265$, $N = 17$) and the group group using ($M = 8,104$, $SD = 1,349$, $N = 13$) is not significantly different, $t(28) = -0,288$, $p = .768$.

Table 5: Independent samples t-test results for TAM evaluation of those who have used a visualization tool and those who have not.

					95% CI for Mean Difference		
	t	df	p	Mean Difference	SE Difference	Lower	Upper
Average Score	-0,298	28	0,768	-0,138	0,464	-1,088	0,812

As with the previous test, there was no difference in technology acceptance levels between these groups. In other words, it is a useful and sufficient software for those who have used similar software and those who have not.

5.1.3 Investigation Into BioNet-XR's First Impression

Participants were asked these questions to get their views on BioNet-XR:

- What are your first impressions of BioNet-XR?
- What do you think was the strongest and weakest aspect of BioNet-XR?

Using Voyant Tools [57] from the answers given to the first question, word clouds were created from the 20 most frequent adjectives seen in Figure 16. Since participants answered the questions in Turkish, Figure 16(a) was produced from the participants' original answers, and Figure 16(b) was produced from the translation of the answers into English. The side effects of using positive adjectives in negative sentences and negative adjectives in positive sentences have been eliminated. There was no answer where negative adjectives are used in positive sentences. However, two responses used positive adjectives in the negative sentence:

“It is fun. However, the purpose of the tool was not very clear.”

“Very useful but can be made more user friendly. Maybe a few more functions can be added.”

Taking into account the other negative adjectives used, the negative meaning in the first answer has been changed to a negative adjective which corresponds to the adjective “belirsiz” in Turkish:

“Fun. Nevertheless, the purpose of the tool was unclear.”

In the second response, by taking into account the user’s comment about increasing the functions, it was changed as follows, that the adjective “zahmetli” was used in Turkish:

“Very useful but onerous. Maybe a few more functions can be added.”

Their frequencies are low, like most other negative adjectives. Therefore, they are not included in word clouds.

The most frequent adjectives consist of useful, simple, easy, positive, fun, different, simple. They are all the positive as semantic. These positive words for first impressions show that BioNet-XR has a positive effect on participants.

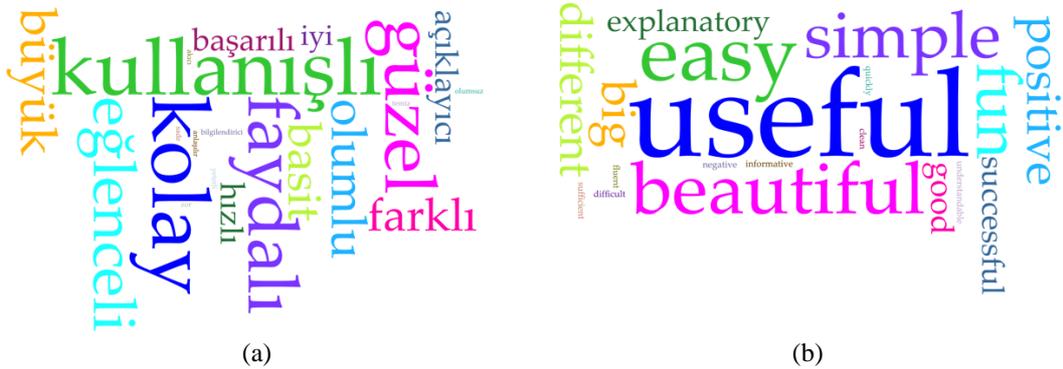


Figure 16: The word clouds are composed of the 20 most frequent adjectives in the given answers to the question about the first impression as (a) Turkish and (b) English.

Table 6 has been prepared from the stated strengths and weaknesses. Although they have not tried it, they mention VR/MR integration as its powerful feature. In addition, being 3D is one of the strongest features mentioned. However, it is stated as a weak feature that it does not offer as wide a range of features as Cytoscape. While true, it is not a fair comparison. Because, thanks to the Cytoscape community, it has gained many features over the years, and new features are still being added. Considering the SUS and TAM results, its weaknesses are not fatal to disrupt the user experience.

Table 6: Strengths and weaknesses of BioNet-XR.

Strengths	Weaknesses
A clear and understandable visualization	Responsiveness and performance problems
Different camera features	Not giving feedback on a transaction
Finding subnets using different algorithms	Being less customizable
Being 3D	Minimal bugs
Easy to interact	The high sensitivity of mouse interactions
Usability in VR and MR environments	Limited actions and manipulations on the network
Being easy to learn	Needs UI enhancements (Not able to search the nodes, difficult file selections, lack of progress bar)
Color representations and clear connections	Being far behind in terms of features compared to a frequently used tool like Cytoscape
Portable, easy to install, and start to use	Not giving output that can be used in publications
Simple user interface	Close button not showing in Windows OS

5.1.4 Investigation Into The Opinions of Participants About 3D vs. 2D

The following question was asked to find out the opinions of the participants on 3D displaying: Do you think that examining in three dimensions provides an advantage over two-dimensional examination?

The advantages listed below are those expressed similarly by more than one participant:

- It provides a clear and understandable visualization.
- It is easier to see connections between objects than in two dimensions.
- Biological networks are complex structures and visualize their local features in 3D better.
- It provides convenience in huge meshes where the overlapping of elements is inevitable, if not in average-sized meshes.
- It enables the perception of details and patterns that cannot be noticed in the two-dimensional examination.
- It is more helpful in looking at subnets in crowded protein networks.

- It is easier to navigate the network than 2D.

One of the participants mentioned an important point other than these. The answer of the out-of-field respondent emphasizing that 3D visualization increases focus is as follows:

“I think it is more advantageous to study in three dimensions because it allows us to have a map of these networks in our minds, and it can enable us to make connections more clearly and accurately about the subject we are researching. It is also very valuable for increasing the researcher’s attention to the subject. Even me, although I have been unfamiliar with the subject. I followed the testing phase with great interest.”

Almost all of the answers from the participants are about the benefits of 3D and how the difficulties in 2D can be solved in 3D. These can cause them to tend to prefer 3D visualization tools rather than 2D. These may lead them to prefer 3D visualization tools over 2D whenever possible. Three-dimensional visualization has been instrumental in making BioNet-XR’s positive impression.

5.1.5 Investigation Into The Opinions of Participants About VR and MR Versions

The responses were collected from the following questions for VR and MR:

- Do you think it would be beneficial to examine protein interaction networks using virtual reality with BioNet, based on the video you watched?
- Do you think it would be beneficial to examine protein interaction networks using mixed reality with BioNet, based on the video you watched?

They were classified into three categories as yes, no, and maybe, according to their context. Responses with semantically positive expressions such as “I think it would be beneficial” and “It will provide easy-to-use” were categorized as yes. Answers with semantically negative phrases such as “It is not more useful than 3D” and “No, it cause motion sickness” were categorized as no. Other than these, answers such as “I do not know,” “I am not sure,” “Maybe,” which do not have a clear positive or negative meaning, were grouped under the category of maybe.

As a result, 63,3% of them favor the use of VR will be beneficial. While 17,7% abstained from it, 20% had a negative view of its use. By looking at the intergroup classification in Figure 17, it can be said that the in-field group has a more moderate approach.

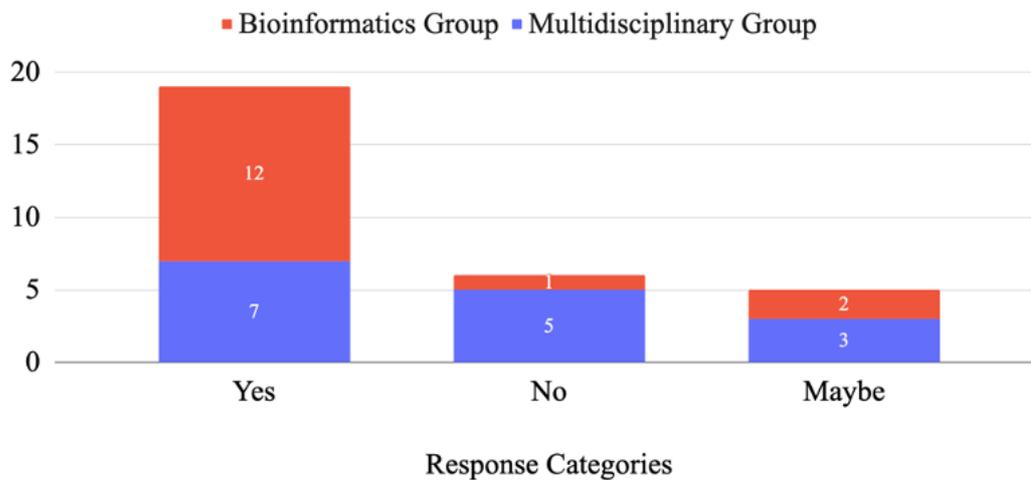


Figure 17: Distribution of the classified participants' responses given to the question, "Do you think it would be beneficial to examine protein interaction networks using virtual reality with BioNet, based on the video you watched?"

Participants think VR will be beneficial in the following ways:

- Making information easy to read
- Clearer visibility of nodes and interactions
- Removing the field of view limit for networks that cannot be visualized due to their size
- Fully experiencing the feeling of 3D interaction
- Interact much easier than in a PC environment
- Being able to experience the interior of an invisible structure

The main reasons for the negative or abstention of the participants are as follows:

- Thinking it will not be any different from the desktop experience
- Not being able to foresee the benefit of experiencing more immersive
- Thinking that protein structure would be more beneficial than protein interaction
- Considering that it can be tiring in long-term use
- Not being sure without experience

In terms of the use of MR in visualization tools, 50% are positive, 20% are negative, and 30% are abstaining. Figure 18 shows the distribution of these results between groups. Although the majority of them look positive for MR, it is seen that there is more hesitation when compared to VR.

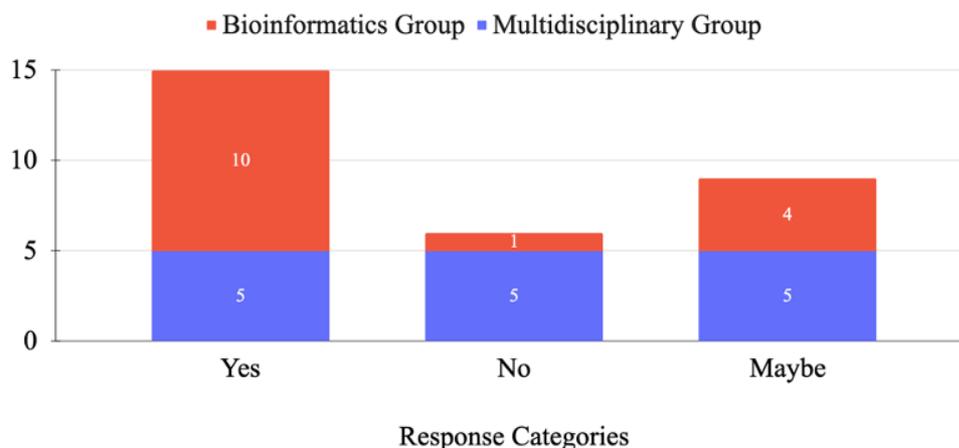


Figure 18: Distribution of the classified participants' responses given to the question, "Do you think it would be beneficial to examine protein interaction networks using mixed reality with BioNet, based on the video you watched?"

What the participants stated about the benefits of MR can be listed as follows:

- Suitable to work in a lab environment
- Visually more understandable networks
- Increasing the working efficiency because there is no need for extra tabs
- Providing entire 3D interaction since some points can be understood in 2D in the desktop version
- Using in collaborative works, especially about network discussion
- Seeing the real world instead of a virtual one so that using other study materials as well

Among the reasons why the participants remained negative and abstained from the use of MR are as follows:

- Thinking it will not be any different from the desktop experience or thinking it is not as helpful as VR
- Considering that it will be more useful on PC, but not thinking that it will be as successful as virtual reality to only focus on the task because of seeing the real environment
- Considering that the real world creates difficulties in visualizing complex three-dimensional structures
- Not being sure without experience
- Thinking more functionality is needed

The fact that users do not experience VR and MR versions affects their doubtful viewpoints. Therefore, for those who have used a VR or MR application, the fact that their previous experiences have been good or bad may have affected their interpretation. For those who have not used it, watching it as a video on the flat screen was insufficient to give a sense of how they would see and feel in virtual reality or mixed reality.

Also, some reasons are a positive justification for one user, whereas a negative justification for another user. For example, some users thought that seeing the real environment would be beneficial than seeing the virtual environment, whereas others thought it would be useless. It is a shortcoming that users did not use these versions and makes inferences difficult.

5.1.6 Feedback

The tests were not conducted as a think-aloud session. However, participants were free to ask questions and, therefore, sometimes voiced their requests and suggestions. They referenced and gave an example of regular uses from other software such as Cytoscape. These opinions are significant in understanding the general usage habits and identifying the deficiencies. They were noted for consideration and reference for future studies.

One of the participants gave the following feedback:

“MR may be better than VR. Because you do not see your surroundings in VR and you have to remove and re-wear the headset to check something. However, since you can see around in MR, you can return to your notes, search for something on the internet, and review again. However, it needs to add more functionality.”

One participant that is abstaining from the use of VR and MR pointed to motion sickness as a reason:

“VR and MR will not be good for long-duration use. I have experienced that it causes symptoms such as nausea and dizziness.”

In these technologies, such effects can be seen when the rendering is not fast enough to respond to the speed of turning a person’s head. Although the motion sickness caused by MR is at a very minimal and less disturbing level compared to VR, it seems to be a point to consider. However, the solution can be provided by advancements in these technologies. It ensures the necessary adaptation by following the developments that need to be made in visualization tools.

5.2 Network Construction Performance of The BioNet-XR

One of the main challenges that visualization tools face is that it becomes more challenging to make an aesthetic and understandable visualization as the network grows. This process requires computational power and time. As the size of the network increases, the network construction time increases since the computational processes increase. Dealing with it may be possible by developing new layout algorithms or

improving existing ones. At the same time, the hardware strengthening of computers will help to shorten these times. Since the challenge is due to different factors, different layout options are offered to users in visualization tools. Thus, the user is not stuck in a single layout and can choose more suitable for different network sizes and topologies.

By creating networks of different sizes with different layouts, these times are presented in Table 7. Single Circular Layout, which is based on arranging the interacting nodes around a circle as close to each other as possible, promises the shortest times at this point. Thus, Single Circular Layout can easily visualize large-size meshes. The only problem is that the circle radius increases when the mesh grows. Hence, it requires considerable space to visualize. Although the single circular layout provides short times, the construction time increases when combined with the Louvain Community Detection algorithm since it requires additional operations as finding clusters and applying the single circular layout to each cluster on its own. It makes the combination more preferable for small networks. The force-directed layout can create the network in relatively better times. However, considering that many users do not wait long, it is more suitable for networks that are not large.

Table 7: Network construction time of the PC and VR versions.

Node Count	Edge Count	Layout	Time	
			PC Version	VR Version
210	250	Force-directed Layout	3 s	3 s
		Single Circular Layout	0.6 s	0.6 s
		Single Circle Layout with Louvain	17 s	17 s
347	500	Force-directed Layout	9 s	10 s
		Single Circle Layout	1 s	1 s
		Single Circle Layout with Louvain	87 s	89 s
616	1000	Force-directed Layout	18 s	20 s
		Single Circle Layout	1.5 s	2 s
		Single Circular Layout with Louvain	over 300 s	over 300 s

These results are obtained on a computer with the following specifications: Intel Core i7-9750H CPU @ 2.60 GHz 2.59 GHz Processor, 16. GB RAM, 64-bit Windows 10 operating system.

There is no creating graph data structure and applying layout steps in the MR version. Therefore, the network creation time cannot be mentioned. Instead, the network created in the PC version is exported as a JSON file. By importing the JSON file, the network is created in MR. Networking by import does not require complex computation like layouts. Objects are created only based on information such as the relevant position, color, size. It makes it possible to view the network in a short time, as seen in Table 8. In this case, layouts do not affect the import time. As the network

grows, the time increases again, but it will be considerably shorter than the PC and VR versions.

Table 8: Network importing time of the MR version.

Node Count	Edge Count	Layout	Time
30	212	Single Circular Layout	0.03
184	250	Force-directed Layout	0.04
31	106	Single Circular Layout	0.02
21	106	Force-directed Layout	0.02

5.3 Discussion

According to the SUS result, the usability of BioNet-XR both in general and within groups is sufficient and satisfactory. When considered in terms of the bioinformatics group, it shows that BioNet-XR supports general usage habits of individuals familiar with such software are compatible. The multidisciplinary group's usability rating as 'Excellent' indicates that it is easy to learn and use for the general user group.

TAM questionnaire, which is applied to understand the acceptability of BioNet-XR, resulted in the software positively affecting both in general and within groups. Because the bioinformatics group had domain knowledge, they were more knowledgeable about what the software meant and its purpose. The multidisciplinary group performed the tests with the information provided in the exercise and the user guide. Hence, the independent t-test was applied to understand whether domain knowledge affects technology acceptance. A statistically significant difference was not observed between the groups. It means that BioNet-XR is not difficult to use for a user outside the field and can make them understand the purpose of the software. In addition, the effect of being familiar with such software was investigated by applying an independent t-test, and the results showed that it was not. Then, BioNet-XR has a sufficient feature set when considering participants who use more feature-rich software find it acceptable and usable.

BioNet-XR's simple interface provides ease of use and easy learning. Being 3D provides clean and understandable network visualization. Another advantage is that it offers different algorithm options. However, some bugs stated by users need to be fixed, and interface elements that are difficult to use need to be developed. These do not create such problems as to damage the user experience. Possible solutions and improvements for the points that respondents indicated need improvement are:

- Taking images of network for publications
- Searching in user interface components that lists the node names
- Changing layout and visual settings without need reload

- Giving feedback for successful and unsuccessful or ongoing transactions
- Providing flexibility to assign controls to the user as in games
- Providing improvements and new algorithm options for performance and responsiveness problems
- Utilizing parallel programming for long-running processes

One of the significant aspects noted by participants is the availability of VR and MR versions. When their opinions regarding VR and MR are analyzed, the argument that it is beneficial for both is dominant. However, especially in the answers given for the MR version, negative and abstaining opinions are substantial. Receiving feedback via video has provided a foresight for future studies, but it does not seem sufficient. Therefore, participants need to experience the VR and MR versions to give healthier feedback.

Although the functions in the VR version were not available, the development of the MR version was important. Because in VR, users may not feel safe while moving and walking around for fear of hitting something because they cannot see their real surroundings. In MR, on the other hand, the user feels much more comfortable as the real environment is visible for navigation and navigating around. Also, it is easier and more efficient to collaborate with more than one user in MR for the same reason.

As the size of the network increases, the computational cost increases; therefore, the time to create the network is prolonged. The three layout algorithms BioNet-XR offers different network generation times in the different network sizes. However, it is necessary to add new algorithms and improve existing algorithms. Thanks to the import-export process, the MR version has not been a challenge at this point. The import-export process is the right approach considering the limited hardware power of HMD devices.

Instead of comparing BioNet-XR with advanced standalone visualization tools such as Cytoscape, it would be more appropriate to compare it with tools that visualize biological networks using immersive technologies. It can be another helpful research tool that enables solving some of the challenges that they cannot solve and have features that they cannot offer. Hence, it is better to compare it with the available VR visualization tools that can be used for the protein interaction networks: iCave [30] and VRNetzer [85].

Table 9 Comparison of iCave, VRNetzer and BioNet-XR.

	iCave	VRNetzer	BioNet-XR
Method	CAVE based	Computer-based	Computer-based
Intended use	Biological networks	Biological networks	Protein interaction networks
Compatibility VR device/setup	CAVE	HTC Vive & Oculus Rift	HTC Vive
Layout Options	Available	Available	Available
Analysis Functionalities	Available	Available	Available
Integration to external databases	Available	Available	Not available
Customization	Available	Available	Available
Extendibility	Not available	Available	Not available
Desktop version	Available	Available	Available

The disadvantage of iCave is that it is a CAVE-based VR application, as mentioned before. For this reason, a room with the necessary setup is needed to use it. It obliges researchers to go to the location of the room. Such physical dependence keeps it from being portable and used anywhere it is needed. Moreover, headsets are more affordable than the CAVE setup.

VRNetzer has an advanced infrastructure and software architecture. VRNetzer is more advanced and offers more options in terms of common features such as analytics and customization. There is also a standalone version, but it does not offer all the features of VR. Its installation is a bit cumbersome as it requires installing external software and libraries such as MySQL. Its usability assessment has not been made.

CHAPTER 6

CONCLUSION AND FUTURE WORK

Bioinformatics focuses on the processing and analysis of biological data and information with the help of computer science. Biological data covers a large number of complex structures and their interaction with each other. Accessing the information stored in the data is requisite in studies such as discovering the molecules in diseases and developing drugs. It is not easy to examine such massive raw data and to find and extract useful information. It makes visualization an essential method for researchers. For example, it is not easy to identify a protein that makes the most connections in a data sheet consisting of thousands of rows. However, when it is visualized, that information can be easily obtained. Hence, developing such software and producing solutions to overcome challenges in visualizing is a popular and dynamic research topic. It is known that 3D models of biological structures were prepared from various materials and demanded by researchers in the 1960s. Advances in technology and computer sciences contribute positively to these studies.

Many sub-topics deal with different structures and interactions. Various methods, algorithms, and techniques are used while researching them. It requires each visualization tool to have specific requirements and features relevant to the subject matter. Since the study focuses on protein interaction networks, requirements have been determined in that direction. There are many visualization tools developed for protein interaction networks. Cytoscape, which is the most popular and advanced among the tools, has the disadvantages of 2D rendering. Displaying in 3D reduces complexity and makes it easier to detect details. While the layout algorithms in 2D have difficulty presenting an aesthetic and understandable network as the size increases, that complexity decreases in 3D. Since the human eye is accustomed to seeing in 3D, it can analyze the complexity in 3D more easily than in 2D [6]. These advantages of 3D visualization are undeniable. However, the lack is to visualize and experience on a three-dimensional flat screen. Since it does not provide a 360-degree field of view, it causes the displayed area to clip and cripples immersiveness. Virtual reality and mixed reality offer a potential solution since they are more effective in seeing details from 3D and being immersive. It is also important to make people feel the existence of these biological entities, which cannot be seen with the naked eye, especially in education [8]. The use of these technologies is still sparse in such tools.

In light of these points, this study presents a tool called BioNet-XR that enables the visualization of PINs. It supports files of extensions .txt, .tsv and .csv and has both PC, VR and MR versions. The PC version has three different layout algorithms and visual adjustment options to create a network. The layout algorithms are force-directed, single circular, and single circular with Louvain community detection. The user can view the network parameters, find the shortest path between two nodes, and find subnets with three algorithms: all-pairs shortest path, Steiner tree, and random walk. It also has third-person and first-person camera modes. The first-person camera mode aims to make the user feel inside the network. The VR version has almost the same features as the PC version. For example, first-person camera mode is not needed in VR, so it is not available. A network created in the PC version can be viewed in the MR version with the help of the export-import mechanism. In the MR version, the user has navigation features such as moving, rotating, approaching the mesh, but analysis features are not available.

A usable system provides the user with maximum benefit and efficiency. Hence, usability tests are conducted to understand how effectively users can use the developed system. In this study, the usability evaluation of BioNet-XR was made. However, since VR and MR versions can be used with headsets, data acquisition could not be performed due to pandemics. The desktop version of the software was tested by a homogeneously distributed group of 30 people consisting of bioinformatics-related fields and other disciplines participants. While the in-field group was familiar with visualization tools, the number and frequency of use were very low in the out-of-field group. Thus, it has been tested by both a group of researchers familiar with visualization tools and a general audience of users.

Test sessions were conducted online with a task-based approach. An exercise file which is a sample use case and contains the steps to be completed, was sent to the participants. After completed the exercise, they watched VR and MR usage videos to give an idea. SUS, TAM, and open-ended questions were used as test instruments. The difficulty with the tests was that the participants attempted to test independently of the exercise. In such cases, the participant was not intervened. If they were in-field participants, they generally had no problems due to familiarity. However, most multidisciplinary participants who tried to continue this way were lost in the application and needed guidance. A more efficient way would be to prepare the instruction steps in the software rather than on paper.

According to the result of the SUS evaluation, the overall average, in-field average, and out-of-field average are 80,8, 85,3, and 76,3, respectively. These scores correspond to excellent in the adjective rating. It shows that BioNet-XR is easy to use for participants familiar with other tools and does not force their usage habits. Also, it has a smooth learning curve for others who do not know how to use such tools. Overall average TAM score is obtained as 8,026 out of 10. The average TAM scores of multidisciplinary and Bioinformatics groups are 7,910 and 8,154, respectively. The independent t-test was performed on TAM scores to see if the difference between groups was statistically significant. Results showed that the technology acceptance level of participants from different disciplines and studying in bioinformatics is the same. Also, the participants were grouped as those who had used such visualization software and those who did not, and a t-test was applied. There is no difference in this

regard, too. It means using or not using such software did not affect the technology acceptance level of the participants.

The participants were asked for their opinions on whether VR and MR versions would be helpful based on the videos they watched in open-ended questions, and the feedback was positive. Considering the current challenges, they focus on the benefits and solution possibilities of immersive technologies. However, one of the reasons for the participants who expressed negative and abstaining opinions was that they could not test these versions.

In the light of user feedback and open-ended questions, BioNet-XR has left a positive impression. Participants also indicated their strengths and weaknesses in their responses to verbal and related questions. Being 3D, VR/MR integration, simple interface, and clear visualization are some of its powerful features. The source of most weaknesses is the lack of familiar actions from other software such as customization and functionality.

The existing community may be distant from the use of VR and MR technologies. However, considering the technological habits and tendencies of the next generation that will be added to the community, it can be predicted that they prefer the use of such tools. Therefore, researching the potential of immersive technologies in information visualization tools make a significant contribution.

6.1 Future Work

Such tools are well suited not only for scientific studies but also for collaborative work. In addition, it provides the opportunity to learn not only through paper and images but also by interacting, so they can be used as educational material. Hence, it is planned to add existing functions to the MR version and collaboratively offer multi-user support. Also, usability tests of VR and MR versions will be measured scientifically and gather the users' demands, requests, and suggestions.

The usability research revealed that the present capabilities of BioNet-XR are adequate. However, users desire the functionality that they are accustomed to seeing in other software. Versions' feature set needs to be expanded.

The existing headset devices need improvement and have adverse effects in developed applications. However, they are becoming more minimized, more performance, and affordable with hardware innovations. Therefore, while their negative effects are gradually minimized, their potential becomes more exposed. It should be kept in mind that more remarkable results will be obtained by putting these studies on with more advanced devices in the future.

REFERENCES

- [1] Martinez, X., Chavent, M., & Baaden, M. (2020). Visualizing protein structures—tools and trends. *Biochemical Society Transactions*, 48(2), 499-506.
- [2] Zheng, J. M., Chan, K. W., & Gibson, I. (1998). Virtual reality. *Ieee Potentials*, 17(2), 20-23.
- [3] Purcell, D. G., & Stewart, A. L. (1991). The object-detection effect: Configuration enhances perception. *Perception & psychophysics*, 50(3), 215-224.
- [4] Hoffman, M. A., & Provance, J. B. (2017). Visualization of molecular structures using HoloLens-based augmented reality. *AMIA Summits on Translational Science Proceedings*, 2017, 68.
- [5] Cassidy, K. C., Šefčík, J., Raghav, Y., Chang, A., & Durrant, J. D. (2020). ProteinVR: Web-based molecular visualization in virtual reality. *PLoS computational biology*, 16(3), e1007747.
- [6] Ware, C., & Franck, G. (1996). Evaluating stereo and motion cues for visualizing information nets in three dimensions. *ACM Transactions on Graphics (TOG)*, 15(2), 121-140.
- [7] Ware, C., & Franck, G. (1994, October). Viewing a graph in a virtual reality display is three times as good as a 2D diagram. In *Proceedings of 1994 IEEE Symposium on Visual Languages* (pp. 182-183). IEEE.
- [8] Ratamero, E. M., Bellini, D., Dowson, C. G., & Römer, R. A. (2018). Touching proteins with virtual bare hands. *Journal of computer-aided molecular design*, 32(6), 703-709.

- [9] Kendrew, J. C., Bodo, G., Dintzis, H. M., Parrish, R. G., Wyckoff, H., & Phillips, D. C. (1958). A three-dimensional model of the myoglobin molecule obtained by x-ray analysis. *Nature*, 181(4610), 662-666.
- [10] Martz, E., & Francoeur, E. (2004). History of visualization of biological macromolecules. *University of Massachusetts Amherst. Sep.*
- [11] Levinthal, C. (1966). Computer construction and display of molecular models. In IBM Scientific Computing Symposium on Computer Aided Experimentation. IBM.
- [12] Langridge, R., Ferrin, T. E., Kuntz, I. D., & Connolly, M. L. (1981). Real-time color graphics in studies of molecular interactions. *Science*, 211(4483), 661-666.
- [13] Rose, A. S., & Hildebrand, P. W. (2015). NGL Viewer: a web application for molecular visualization. *Nucleic acids research*, 43(W1), W576-W579.
- [14] Doutreligne, S., Cragolini, T., Pasquali, S., Derreumaux, P., & Baaden, M. (2014, November). UnityMol: interactive scientific visualization for integrative biology. In 2014 IEEE 4th Symposium on Large Data Analysis and Visualization (LDAV) (pp. 109-110). IEEE.
- [15] Sehna, D. R. A. S., Rose, A. S., Koca, J., Burley, S., & Velankar, S. (2018, June). Mol*: towards a common library and tools for web molecular graphics. In Workshop on Molecular Graphics and Visual Analysis of Molecular Data. The Eurographics Association (Vol. 10).
- [16] Xia, J., Benner, M. J., & Hancock, R. E. (2014). NetworkAnalyst-integrative approaches for protein-protein interaction network analysis and visual exploration. *Nucleic acids research*, 42(W1), W167-W174.
- [17] Singh, R. P. (2014). Application of graph theory in computer science and engineering. *International Journal of Computer Applications*, 104(1).
- [18] Droste, P., Nöh, K., & Wiechert, W. (2013). Omix—a visualization tool for metabolic networks with highest usability and customizability in focus. *Chemie Ingenieur Technik*, 85(6), 849-862.
- [19] Pavlopoulos, G. A., O'Donoghue, S. I., Satagopam, V. P., Soldatos, T. G., Pafilis, E., & Schneider, R. (2008). Arena3D: visualization of biological networks in 3D. *BMC systems biology*, 2(1), 1-7.
- [20] Iragne, F., Nikolski, M., Mathieu, B., Auber, D., & Sherman, D. (2005). ProViz: protein interaction visualization and exploration. *Bioinformatics*, 21(2), 272-274.
- [21] Kohl, M., Wiese, S., & Warscheid, B. (2011). Cytoscape: software for visualization and analysis of biological networks. In *Data mining in proteomics* (pp. 291-303). Humana Press.

- [22] Martinez, X., Chavent, M., & Baaden, M. (2020). Visualizing protein structures—tools and trends. *Biochemical Society Transactions*, 48(2), 499-506.
- [23] Agapito, G., Guzzi, P. H., & Cannataro, M. (2013). Visualization of protein interaction networks: problems and solutions. *BMC bioinformatics*, 14(1), 1-30.
- [24] Unal, M., Bostanci, E., & Sertalp, E. (2020). Distant augmented reality: Bringing a new dimension to user experience using drones. *Digital Applications in Archaeology and Cultural Heritage*, 17, e00140.
- [25] Rajanbabu, A., Drudi, L., Lau, S., Press, J. Z., & Gotlieb, W. H. (2014). Virtual reality surgical simulators—a prerequisite for robotic surgery. *Indian journal of surgical oncology*, 5(2), 125-127.
- [26] Zhang, L., Chen, S., Dong, H., & El Saddik, A. (2018). Visualizing Toronto city data with HoloLens: Using augmented reality for a city model. *IEEE Consumer Electronics Magazine*, 7(3), 73-80.
- [27] Hirst, J. D., Glowacki, D. R., & Baaden, M. (2014). Molecular simulations and visualization: introduction and overview. *Faraday discussions*, 169, 9-22.
- [28] Xu, K., Liu, N., Xu, J., Guo, C., Zhao, L., Wang, H. W., & Zhang, Q. C. (2021). VRmol: an integrative web-based virtual reality system to explore macromolecular structure. *Bioinformatics*, 37(7), 1029-1031.
- [29] Cruz-Neira, C., Sandin, D. J., DeFanti, T. A., Kenyon, R. V., & Hart, J. C. (1992). The CAVE: audio visual experience automatic virtual environment. *Communications of the ACM*, 35(6), 64-73.
- [30] Liliashvili, V., Kalayci, S., Fluder, E., Wilson, M., Gabow, A., & Gümüş, Z. H. (2017). iCAVE: an open source tool for visualizing biomolecular networks in 3D, stereoscopic 3D and immersive 3D. *GigaScience*, 6(8), gix054.
- [31] Yang, Y., Engin, L., Wurtele, E. S., Cruz-Neira, C., & Dickerson, J. A. (2005). Integration of metabolic networks and gene expression in virtual reality. *Bioinformatics*, 21(18), 3645-3650.
- [32] Laureanti, J., Brandi, J., Offor, E., Engel, D., Rallo, R., Ginovska, B., ... & Baker, N. A. (2020). Visualizing biomolecular electrostatics in virtual reality with UnityMol-APBS. *Protein Science*, 29(1), 237-246.
- [33] Zhang, J. F., Paciorkowski, A. R., Craig, P. A., & Cui, F. (2019). BioVR: a platform for virtual reality assisted biological data integration and visualization. *BMC bioinformatics*, 20(1), 1-10.
- [34] Norrby, M., Grebner, C., Eriksson, J., & Bostrom, J. (2015). Molecular rift: virtual reality for drug designers. *Journal of chemical information and modeling*, 55(11), 2475-2484.

- [35] Legeth, O., Rodhe, J., Lang, S., Dhapola, P., Pålsson, J., Wallergård, M., & Soneji, S. (2020). CellexalVR: A virtual reality platform to visualise and analyse single-cell data. *BioRxiv*, 329102.
- [36] Müller, C., Krone, M., Huber, M., Biener, V., Herr, D., Koch, S., ... & Ertl, T. (2018). Interactive molecular graphics for augmented reality using HoloLens. *Journal of integrative bioinformatics*, 15(2).
- [37] Vive input utility. (2016). Unity Asset Store <https://assetstore.unity.com/packages/tools/integration/vive-input-utility-64219>
- [38] Fruchterman, T. M., & Reingold, E. M. (1991). Graph drawing by force-directed placement. *Software: Practice and experience*, 21(11), 1129-1164.
- [39] Jeong E, Kojima K, Nagasaki M: An efficient grid layout algorithm for biological networks utilizing various biological attributes. *BMC Bioinformatics*. 2007, 8: 76-10.1186/1471-2105-8-76.
- [40] Floyd, R. W. (1962). Algorithm 97: shortest path. *Communications of the ACM*, 5(6), 345.
- [41] Kou, L., Markowsky, G., & Berman, L. (1981). A fast algorithm for Steiner trees. *Acta informatica*, 15(2), 141-145.
- [42] Cowen, L., Ideker, T., Raphael, B. J., & Sharan, R. (2017). Network propagation: a universal amplifier of genetic associations. *Nature Reviews Genetics*, 18(9), 551.
- [43] Abran, A., Khelifi, A., Suryn, W., & Seffah, A. (2003). Usability meanings and interpretations in ISO standards. *Software quality journal*, 11(4), 325-338.
- [44] Mirel, B. (2007, October). Usability and usefulness in bioinformatics: evaluating a tool for querying and analyzing protein interactions based on scientists' actual research questions. In 2007 IEEE International Professional Communication Conference (pp. 1-8). IEEE.
- [45] Bolchini, D., Finkelstein, A., Perrone, V., & Nagl, S. (2009). Better bioinformatics through usability analysis. *Bioinformatics*, 25(3), 406-412.
- [46] Mannapperuma, C., Street, N., & Waterworth, J. (2019, February). Designing usable Bioinformatics Tools for specialized users. In International Conference on Information Technology & Systems (pp. 649-670). Springer, Cham.
- [47] Javahery, H., & Seffah, A. (2007, November). Refining the usability engineering toolbox: lessons learned from a user study on a visualization tool. In Symposium of the Austrian HCI and Usability Engineering Group (pp. 185-198). Springer, Berlin, Heidelberg.

- [48] Saraiya, P., North, C., & Duca, K. (2005). An insight-based methodology for evaluating bioinformatics visualizations. *IEEE transactions on visualization and computer graphics*, 11(4), 443-456.
- [49] Chen, S. J., Liao, D. L., Chen, C. H., Wang, T. Y., & Chen, K. C. (2019). Construction and analysis of protein-protein interaction network of heroin use disorder. *Scientific reports*, 9(1), 1-9.
- [50] Brooke, J. (1996). Sus: a “quick and dirty” usability. *Usability evaluation in industry*, 189.
- [51] Bangor, A., Kortum, P., & Miller, J. (2009). Determining what individual SUS scores mean: Adding an adjective rating scale. *Journal of usability studies*, 4(3), 114-123.
- [52] Davis, F. D. (1986). *A technology acceptance model for empirically testing new end-user information systems*. Cambridge, MA.
- [53] Marcus, A., Comorski, D., & Sergeyev, A. (2005, May). Supporting the evolution of a software visualization tool through usability studies. In *13th International Workshop on Program Comprehension (IWPC'05)* (pp. 307-316). IEEE.
- [54] Marcus, A., Comorski, D., & Sergeyev, A. (2005, May). Supporting the evolution of a software visualization tool through usability studies. In *13th International Workshop on Program Comprehension (IWPC'05)* (pp. 307-316). IEEE.
- [55] Goss-Sampson, M. (2019). *Statistical analysis in JASP: A guide for students*.
- [56] Cronbach, L. J. (1951). Coefficient alpha and the internal structure of tests. *psychometrika*, 16(3), 297-334.
- [57] Sampsel, L. J. (2018). Voyant tools. *Music Reference Services Quarterly*, 21(3), 153-157.
- [58] DeLano, W. L. (2002). Pymol: An open-source molecular graphics tool. *CCP4 Newsletter on protein crystallography*, 40(1), 82-92.
- [59] Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: visual molecular dynamics. *Journal of molecular graphics*, 14(1), 33-38.
- [60] Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, 25(13), 1605-1612.
- [61] Castrillo, J. I., Pir, P., & Oliver, S. G. (2013). Yeast Systems Biology: towards a systems understanding of regulation of eukaryotic networks in complex diseases and biotechnology. In *Handbook of Systems Biology* (pp. 343-365). Academic Press.

- [62] Wang Y. (2013) Gene Regulatory Networks. In: Dubitzky W., Wolkenhauer O., Cho KH., Yokota H. (eds) *Encyclopedia of Systems Biology*. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-9863-7_364
- [63] Boucher, B., & Jenna, S. (2013). Genetic interaction networks: better understand to better predict. *Frontiers in genetics*, 4, 290.
- [64] Pavlopoulos, G. A., Hooper, S. D., Sifrim, A., Schneider, R., & Aerts, J. (2011). Medusa: A tool for exploring and clustering biological networks. *BMC research notes*, 4(1), 1-6.
- [65] Breitkreutz, B. J., Stark, C., & Tyers, M. (2003). Osprey: a network visualization system. *Genome biology*, 4(3), 1-4.
- [66] Theocharidis, A., Van Dongen, S., Enright, A. J., & Freeman, T. C. (2009). Network visualization and analysis of gene expression data using BioLayout Express 3D. *Nature protocols*, 4(10), 1535-1550.
- [67] Demir, E., Babur, O., Dogrusoz, U., Gursoy, A., Nisanci, G., Cetin-Atalay, R., & Ozturk, M. (2002). PATIKA: an integrated visual environment for collaborative construction and analysis of cellular pathways. *Bioinformatics*, 18(7), 996-1003.
- [68] Han, K., Ju, B. H., & Park, J. H. (2002, August). InterViewer: Dynamic visualization of protein-protein interactions. In *International Symposium on Graph Drawing* (pp. 364-365). Springer, Berlin, Heidelberg.
- [69] Orlev, N., Shamir, R., & Shiloh, Y. (2004). PIVOT: protein interactions visualization tool. *Bioinformatics*, 20(3), 424-425.
- [70] Pavlopoulos, G. A., Wegener, A. L., & Schneider, R. (2008). A survey of visualization tools for biological network analysis. *Biodata mining*, 1(1), 1-11.
- [71] Suderman, M., & Hallett, M. (2007). Tools for visually exploring biological networks. *Bioinformatics*, 23(20), 2651-2659.
- [72] Zhou, G., & Xia, J. (2018). OmicsNet: a web-based tool for creation and visual analysis of biological networks in 3D space. *Nucleic acids research*, 46(W1), W514-W522.
- [73] Sommer, B., Baaden, M., Krone, M., & Woods, A. (2018). From virtual reality to immersive analytics in bioinformatics. *Journal of integrative bioinformatics*, 15(2).
- [74] Gonizzi Barsanti, S., Caruso, G., Micoli, L. L., Covarrubias Rodriguez, M., & Guidi, G. (2015). 3D visualization of cultural heritage artefacts with virtual reality devices. In *25th International CIPA Symposium 2015* (Vol. 40, No. 5W7, pp. 165-172). Copernicus Gesellschaft mbH.

- [75] Bostanci, E., & Clark, A. (2011). Living the Past in the Future. *Intelligent Environments*.
- [76] Jung, T., tom Dieck, M. C., Lee, H., & Chung, N. (2016). Effects of virtual reality and augmented reality on visitor experiences in museum. In *Information and communication technologies in tourism 2016* (pp. 621-635). Springer, Cham.
- [77] Carrozzino, M., & Bergamasco, M. (2010). Beyond virtual museums: Experiencing immersive virtual reality in real museums. *Journal of Cultural Heritage*, 11(4), 452-458.
- [78] Schofield, G., Beale, G., Beale, N., Fell, M., Hadley, D., Hook, J., ... & Thresh, L. (2018, June). Viking VR: Designing a virtual reality experience for a museum. In *Proceedings of the 2018 Designing Interactive Systems Conference* (pp. 805-815).
- [79] Degli Innocenti, E., Geronazzo, M., Vescovi, D., Nordahl, R., Serafin, S., Ludovico, L. A., & Avanzini, F. (2019). Mobile virtual reality for musical genre learning in primary education. *Computers & Education*, 139, 102-117.
- [80] Mosher, M. A., Carreon, A. C., Craig, S. L., & Ruhter, L. C. (2021). Immersive Technology to Teach Social Skills to Students with Autism Spectrum Disorder: a Literature Review. *Review Journal of Autism and Developmental Disorders*, 1-17.
- [81] Moreira, P., de Oliveira, E. C., & Tori, R. (2016, November). Impact of immersive technology applied in computer graphics learning. In *Brazilian Symposium on Computers in Education (Simpósio Brasileiro de Informática na Educação-SBIE)* (Vol. 27, No. 1, p. 410).
- [82] Cakmak, Y. O., Daniel, B. K., Hammer, N., Yilmaz, O., Irmak, E. C., & Khwaounjoo, P. (2020). The Human Muscular Arm Avatar as an Interactive Visualization Tool in Learning Anatomy: Medical Students' Perspectives. *IEEE Transactions on Learning Technologies*, 13(3), 593-603.
- [83] Rozmi, M. D. A. B., Thirunavukkarasu, G. S., Jamei, E., Seyedmahmoudian, M., Mekhilef, S., Stojcevski, A., & Horan, B. (2019). Role of immersive visualization tools in renewable energy system development. *Renewable and Sustainable Energy Reviews*, 115, 109363.
- [84] Abdinejad, M., Talaie, B., Qorbani, H. S., & Dalili, S. (2021). Student perceptions using augmented reality and 3d visualization technologies in chemistry education. *Journal of Science Education and Technology*, 30(1), 87-96.
- [85] Pirch, S., Müller, F., Iofinova, E., Pazmandi, J., Hütter, C. V., Chiettini, M., ... & Menche, J. (2021). The VRNetzer platform enables interactive network analysis in Virtual Reality. *Nature communications*, 12(1), 1-14.
- [86] Aouaa, N., Gherbi, R., Meziane, A., Hadjar, H., & Setitra, I. (2018, October). Visual data mining by virtual reality for protein-protein interaction networks.

In 2018 IEEE/ACS 15th International Conference on Computer Systems and Applications (AICCSA) (pp. 1-8). IEEE.

- [87] Cordeil, M., Dwyer, T., Klein, K., Laha, B., Marriott, K., & Thomas, B. H. (2016). Immersive collaborative analysis of network connectivity: CAVE-style or head-mounted display?. *IEEE transactions on visualization and computer graphics*, 23(1), 441-450.
- [88] Zheng, M., & Waller, M. P. (2017). ChemPreview: an augmented reality-based molecular interface. *Journal of Molecular Graphics and Modelling*, 73, 18-23.
- [89] Brandes, U. (2001). A faster algorithm for betweenness centrality. *Journal of mathematical sociology*, 25(2), 163-177.
- [90] Zhang, J., & Luo, Y. (2017, March). Degree centrality, betweenness centrality, and closeness centrality in social network. In *Proceedings of the 2017 2nd International Conference on Modelling, Simulation and Applied Mathematics (MSAM2017)* (Vol. 132, pp. 300-303).
- [91] Chuah, S. H. W. (2018). Why and who will adopt extended reality technology? Literature review, synthesis, and future research agenda. *Literature Review, Synthesis, and Future Research Agenda* (December 13, 2018).
- [92] Costanza, E., Kunz, A., & Fjeld, M. (2009). Mixed reality: A survey. In *Human machine interaction* (pp. 47-68). Springer, Berlin, Heidelberg.
- [93] Gasques Rodrigues, D., Jain, A., Rick, S. R., Shangley, L., Suresh, P., & Weibel, N. (2017, May). Exploring mixed reality in specialized surgical environments. In *Proceedings of the 2017 CHI Conference Extended Abstracts on Human Factors in Computing Systems* (pp. 2591-2598).
- [94] Hoenig, W., Milanes, C., Scaria, L., Phan, T., Bolas, M., & Ayanian, N. (2015, September). Mixed reality for robotics. In *2015 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS)* (pp. 5382-5387). IEEE.
- [95] Brigham, T. J. (2017). Reality check: basics of augmented, virtual, and mixed reality. *Medical reference services quarterly*, 36(2), 171-178.
- [96] Tolentino, L., Birchfield, D., Megowan-Romanowicz, C., Johnson-Glenberg, M. C., Kelliher, A., & Martinez, C. (2009). Teaching and learning in the mixed-reality science classroom. *Journal of Science Education and Technology*, 18(6), 501-517.
- [97] Brown, B., MacColl, I., Chalmers, M., Galani, A., Randell, C., & Steed, A. (2003, April). Lessons from the lighthouse: collaboration in a shared mixed reality system. In *Proceedings of the SIGCHI conference on Human factors in computing systems* (pp. 577-584).
- [98] Benko, H., Ishak, E. W., & Feiner, S. (2004, November). Collaborative mixed reality visualization of an archaeological excavation. In *Third IEEE and ACM*

International Symposium on Mixed and Augmented Reality (pp. 132-140). IEEE.

- [99] Kantonen, T., Woodward, C., & Katz, N. (2010, March). Mixed reality in virtual world teleconferencing. In *2010 IEEE Virtual Reality Conference (VR)* (pp. 179-182). IEEE.
- [100] Kwok, A. O., & Koh, S. G. (2021). COVID-19 and extended reality (XR). *Current Issues in Tourism*, 24(14), 1935-1940.
- [101] Fujiuchi, K., & Riggie, J. (2019). Academic library collections in the age of extended reality (XR). *Collection Management*, 44(2-4), 296-303.
- [102] Ergün, O., Akin, Ş., Dino, İ. G., & Surer, E. (2019, March). Architectural design in virtual reality and mixed reality environments: a comparative analysis. In *2019 IEEE Conference on Virtual Reality and 3D User Interfaces (VR)* (pp. 914-915). IEEE.
- [103] Akin, S., Ergun, O., Surer, E., & Dino, I. G. (2020). An immersive performative architectural design tool with daylighting simulations: a building information modeling-based approach. *Engineering, Construction and Architectural Management*.
- [104] Genç, B., & Dogrusoz, U. (2006). A layout algorithm for signaling pathways. *Information Sciences*, 176(2), 135-149.
- [105] McGuffin, M. J. (2012). Simple algorithms for network visualization: A tutorial. *Tsinghua Science and Technology*, 17(4), 383-398.
- [106] Sauro, J. (2011). A practical guide to the system usability scale: Background, benchmarks & best practices. Measuring Usability LLC.
- [107] Nacu, Ş., Critchley-Thorne, R., Lee, P., & Holmes, S. (2007). Gene expression network analysis and applications to immunology. *Bioinformatics*, 23(7), 850-858.
- [108] Zhang, Q., & Zhang, Z. D. (2013). SubNet: a Java application for subnetwork extraction. *Bioinformatics*, 29(19), 2509-2511.
- [109] Sohler, F., Hanisch, D., & Zimmer, R. (2004). New methods for joint analysis of biological networks and expression data. *Bioinformatics*, 20(10), 1517-1521.
- [110] Chowdhury, S. A., Nibbe, R. K., Chance, M. R., & Koyutürk, M. (2011). Subnetwork state functions define dysregulated subnetworks in cancer. *Journal of Computational Biology*, 18(3), 263-281.
- [111] Nibbe, R. K., Chowdhury, S. A., Koyutürk, M., Ewing, R., & Chance, M. R. (2011). Protein-protein interaction networks and subnetworks in the biology

of disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 3(3), 357-367.

- [112] Nibbe, R. K., Koyutürk, M., & Chance, M. R. (2010). An integrative-omics approach to identify functional sub-networks in human colorectal cancer. *PLoS computational biology*, 6(1), e1000639.

APPENDICES

APPENDIX A

DATA COLLECTION INSTRUMENTS

System Usability Scale: 10 Items, Answers from 1 (Strongly Disagree) to 5 (Strongly Agree)
I think that I would like to use this application frequently.
I found this application unnecessarily complex.
I thought this application was easy to use.
I think that I would need assistance to be able to use this application.
I found the various functions in this application were well integrated.
I thought there was too much inconsistency in this application.
I would imagine that most people would learn to use this application very quickly.
I found this application very cumbersome/awkward to use.
I felt very confident using this application.
I needed to learn a lot of things before I could get going with this application.

Technology Acceptance Model Scale: 14 items, Answers from 0 (Strongly Disagree) to 10 (Strongly Agree)
I liked the training with the application.
Was using the application fun?
During the test, I felt pain and/or discomfort.
It is easy to learn to use the application.
The application reacts readily to my movements.
I did not find it hard to interact with the protein interaction network using the application.

I found the interface clear and explanatory.
The interface is simple to use.
I liked the interface design.
The instructions of the application are clear. I understood what to do in the application and how.
The application helps me to understand if and when I am acting incorrectly.
I found the feedback useful.
I found it easy to reach targets.
I would like to keep training with this system in the future.

Open-Ended Questions: 5 Subsection and Total 18 Questions (except demographic questions)

Demografik Sorular

Ad - Soyad
Yaş
Cinsiyet
Eğitim Durumu
Lisans Eğitiminiz Hakkında (Üniversite - Bölüm)
Yüksek Lisans Eğitiminiz Hakkında (Üniversite Bölüm)

Genel Sorular

Daha önce herhangi bir görselleştirme yazılımı kullandınız mı? Kullandıysanız neler, ne sıklıkla?
BioNet ile ilgili ilk izlenimleriniz neler?
BioNet'in protein-protein ağlarını incelemeyi kolaylaştırdığını düşünüyor musunuz? Üç boyutlu olarak incelemenin iki boyutlu incelemeye göre avantaj sağladığını düşünüyor musunuz?
BioNet'in en güçlü ve en zayıf yönü sizce neydi?
İşlevsellik yönünden neler geliştirilebilir/geliştirilmeli?
BioNet'i iş veya okul araştırmalarınızda kullanmayı düşünür müsünüz?

Görünüm ile İlgili Sorular

BioNet'i iş veya okul araştırmalarınızda kullanmayı düşünür müsünüz?
Renk teması ile ilgili bir problem gözlemlediniz mi?
Yazı stili ve boyutu anlaşılır ve okunaklı mıydı?

	Görünüm yönünden neler geliştirilebilir/geliştirilmeli?
Alıştırma Hakkında Sorular	
	Alıştırmalara başlamadan önce gönderilmiş olan Kullanıcı Rehberi’ni okumuş muydunuz? Kullanıcı Rehberi olmaksızın BioNet’i nasıl kullanacağınızı öğrenmeniz ne kadar zor olurdu?
	BioNet’in, verilen sürede alıştırmadaki adımları tamamlamanız için size yeterli bilgi sağladığını düşünüyor musunuz? Eğer düşünmüyorsanız başka ne tür bilgiye ihtiyaç duydunuz?
	Alıştırmadaki adımları takip ederken sizi en zorlayan şey neydi?
	(Multimedia Informatics öğrencileri için bir soru) Alıştırma farklı disiplininden uzak biri için ne kadar zordu?
	(Bioinformatics öğrencileri için bir soru) Alıştırma sağlık bilişimi disiplininden biri için ne kadar zordu? Gerçek kullanım durumlarına ne kadar yakındı?
VR/MR Hakkında Sorular	
	Daha önce VR/MXR teknolojisini deneyimlediniz mi? Deneyimlediyseniz izlenimleriniz nelerdi?
	İzlediğiniz videodan yol çıkarak protein etkileşim ağlarını BioNet ile sanal gerçeklik kullanarak incelemenin faydalı olabileceğini düşünüyor musunuz?
	İzlediğiniz videodan yol çıkarak protein etkileşim ağlarını BioNet ile karma gerçeklik kullanarak incelemenin faydalı olabileceğini düşünüyor musunuz?

APPENDIX B

THE USABILITY TEST EXERCISE

This exercise is based on the Construction and Analysis of Protein-Protein Interaction Network of Heroin Use Disorder [1]. Heroin use disorder (HUD) is a disease in which genetic and environmental factors affect. The study aims to better understand HUD's mechanism with the help of topology analysis by constructing a protein interaction network for HUD.

Helpful Information: What is a protein interaction network? Protein interaction networks are graphs in which proteins are shown as nodes and interactions as edges. In this way, visualizing protein interaction networks allows you to observe many details that would not be possible on paper or raw data. Protein interaction networks are frequently used in studies about many topics ranging from diseases to drug development for these diseases.

1. Create the protein interaction network for the HUD by uploading the hud pin.txt data file with the configuration options you want. This document is a data file that describes the interaction between the susceptibility genes associated with HUD and their neighbor interactors.

Suggestion: You can warm up the controls and interface by manipulating the network and using different configuration options.

2. Observe how much information you can learn about which network parameters belonging to proteins over this network. Repeat this step, loading the network with different configuration settings.

Helpful Information: What are network parameters?

- **Node Degree:** The number of nodes that a node is directly interacting with. Nodes with high node degrees are called hub nodes. In our case, these hub nodes are the nodes that have the potential to cause disease.
- **Betweenness Centrality:** The ratio of how often a node is in shortcuts between other nodes. It is significant as it acts as a bridge for the flow of information on the network. Nodes with high betweenness degrees are called bottlenecks.
- **Closeness Centrality:** The total length of the shortest paths between a node and other nodes is calculated. Closeness centrality is the inverse of the mean of the calculated sum. Nodes with higher closeness centrality degrees are closer to other nodes and are more central than others.

3. Examine the shortest paths between the nodes you observed in the previous step and thought hub nodes or bottleneck nodes.

4. Examine subnetworks that contain proteins that are also effective in other diseases. For this, three seed node files are related to alcohol, cocaine addiction, and amphetamine addiction. Detail your review by reloading the same interaction network with a different layout option.

About seed node files:

- The document named `alcoholism_seed_nodes.txt` contains nodes related to the alcoholism pathway.
- The document named `amphetamine_addiction_seed_nodes.txt` contains nodes related to the amphetamine addiction pathway.
- The document named `cocain_addiction_seed_nodes.txt` contains nodes related to the cocaine addiction pathway.
- The document named `hud_pin_seeds.txt` contains the susceptibility genes associated with HUD.

REFERENCES

- [1] Chen, S. J., Liao, D. L., Chen, C. H., Wang, T. Y., & Chen, K. C. (2019). Construction and analysis of protein-protein interaction network of heroin use disorder. *Scientific reports*, 9(1), 1-9.

APPENDIX C

USER GUIDE AND VIDEOS

The user guide of BioNet-XR can be accessed from the link below:

<https://drive.google.com/file/d/1jTvhof2bRVd5CctFMXV9lwHNJkSR9k-V/view?usp=sharing>

The usage videos watched by the users during the tests can be accessed from the link below:

https://drive.google.com/drive/folders/1AM0Vu5A_4pvcULvdHyJw0IU2bnOcYxc9?usp=sharing

TEZ İZİN FORMU / THESIS PERMISSION FORM

ENSTİTÜ / INSTITUTE

Fen Bilimleri Enstitüsü / Graduate School of Natural and Applied Sciences

Sosyal Bilimler Enstitüsü / Graduate School of Social Sciences

Uygulamalı Matematik Enstitüsü / Graduate School of Applied Mathematics

Enformatik Enstitüsü / Graduate School of Informatics

Deniz Bilimleri Enstitüsü / Graduate School of Marine Sciences

YAZARIN / AUTHOR

Soyadı / Surname :

Adı / Name :

Bölümü / Department :

TEZİN ADI / TITLE OF THE THESIS (İngilizce / English) :

.....

.....

.....

.....

TEZİN TÜRÜ / DEGREE: Yüksek Lisans / Master

Doktora / PhD

1. Tezin tamamı dünya çapında erişime açılacaktır. / Release the entire work immediately for access worldwide.
2. Tez iki yıl süreyle erişime kapalı olacaktır. / Secure the entire work for patent and/or proprietary purposes for a period of two year. *
3. Tez altı ay süreyle erişime kapalı olacaktır. / Secure the entire work for period of six months. *

* Enstitü Yönetim Kurulu Kararının basılı kopyası tezle birlikte kütüphaneye teslim edilecektir.
A copy of the Decision of the Institute Administrative Committee will be delivered to the library together with the printed thesis.

Yazarın imzası / Signature

Tarih / Date