Visualization of Point Mutations in Fibronectin Type-III Domain-Containing Protein 3 in Prostate Cancer

Samantha Vos
svos@vwu.edu
Virginia Wesleyan University
Virginia Beach, USA

Oyewole Oyekoya
oo700@hunter.cuny.edu
City University of New York
New York City, USA

Olorunseun Ogunwobi
oo158@hunter.cuny.edu
City University of New York
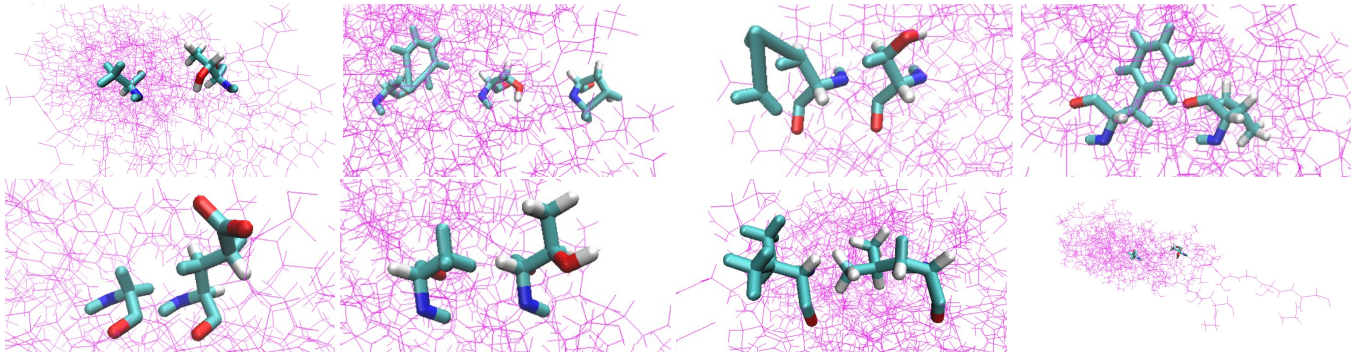New York City, USA

Figure 1: The 8 Mutations of FNDC3. The mutation is located on the left, while the original protein is located on the right. From left to right, the mutations are T11A, S19F, S19P, S27N, V34F, E102G, T109A, L119V, and a large scale.

ABSTRACT

MicroRNA, or miRNA, are small, single-stranded, noncoding RNAs that are involved in RNA silencing and post-transcriptional regulation of gene expression. MiRNA can silence mRNA that codes for cancerous proteins, or it can silence miRNA that codes for antitumor proteins. MiRNA-1207-3p can silence the mRNA that codes for FNDC1, a protein that is associated with aggressive prostate cancer. Fibronectin type III domain-containing (FNDC) proteins are an adipokine/myokine family that has many proteins involved in different functions in cells. A handful of the proteins from this family have been directly correlated to proliferation of either aggression or concentration of certain cancer cells. FNDC3 was the chosen protein from this family for visualization. It has been shown that an increase in FNDC3 concentration has been linked to more aggressive prostate cancer. The overexpression of another related protein, FNDC1, has been linked to a resistance in chemotherapy. The purpose of this project is to use visualization to see how mutations in the amino acid sequence of FNDC3 can change its structure, and in the process, change its functionality.

CCS CONCEPTS

- **Human-centered computing** → **Virtual reality**; • **Computing methodologies** → *Perception*.

KEYWORDS

scientific visualization, MicroRNA, FNDC3, mutations

1 INTRODUCTION

MicroRNA, or miRNA, are small, single-stranded, noncoding RNAs that are involved in RNA silencing and post-transcriptional regulation of gene expression. MiRNA has been shown to lead to an increase or decrease in proliferation of aggressive prostate cancer. MiRNA-1207-3p silences the mRNA that codes for Fibronectin type III domain-containing protein 1, which is correlated to an increase in the aggressivity of prostate cancer in men. Fibronectin type III domain-containing (FNDC) proteins are a family of adipokines and myokines. Adipokines and myokines are proteins that are secreted by adipose tissue and skeletal muscles, respectively, and function to provide communication between organs. This family of proteins includes FNDC1, FNDC3A, FNDC3B, FNDC4, FNDC5, FNDC6, FNDC7, FNDC8, FNDC9, FNDC10 and FNDC11. These members of the FNDC family are transmembrane proteins that may act as receptors for fibronectin, a protein found outside the cell that is used for communication between the intra and extracellular matrices,

ultimately controlling cell behavior in response to its environment. These proteins may regulate signal transduction, have a large array of functions, and participate in many different mechanisms and pathways within cells.

These proteins are still being researched, and it is not yet known how the proteins perform their various functions. The structure of a protein is crucial to its functionality. Binding sites are where ligands and other molecules can bind to proteins to perform various reactions. Changes in the structure of the binding site of a protein can change its ability to bind to a specific molecule, and can even render it useless. Unfortunately, the binding site of FNDC3 has not been located, so making mutations to this site is not possible. However, there has been various experimentation with mutating the amino acid structure of this protein that has led to some structural changes that may affect the functionality of FNDC3.

Understanding how mutations in the amino acid can change not only the structure but also the functionality of the protein can be confusing, which is why virtual reality is helpful. VMD has the ability to take molecular models of proteins, DNA, and other cellular components and make mutations to them. This research will utilize VMD to visualize the mutations in the protein FNDC3.

2 RELATED WORK

Das and Ogunwobi [2] investigated if miR-1207-3p operates as a regulator in prostate cancer cells. They discovered that miR-1207-3p was extremely underexpressed in PCa cells and miR-1207-3p significantly inhibits proliferation, migration, and induces apoptosis in these cells. The miR-1207-3p targets fibronectin type III domain containing 1, which is positively correlated, among other compounds, to aggressive prostate cancer (PCa).

Das et al. [3] explored the possibility of using miR-1207-3p to identify aggressive PCa and if its expressivity in cells could explain the disproportionate aggressiveness of PCa in men of African ancestry. They used a total of 404 patients between 1988 and 2003 who had primary adenocarcinoma of the prostate and identified their race. RNA samples were collected from all patients and used real-time qRT-PCR to analyze them. The researchers found that miR-1207-3p expression was significantly higher in PCa that results in death than in PCa that did not. MiR-1207-3p expression was also much lower in moAA with PCa than in Caucasian men. They reasoned that miR-1207-3p is a prognostic biomarker for PCa, and it may explain the extreme aggressiveness in moAA.

Zhou et al. [9] built a 3D model of Toxoplasma gondii ROP20 protein which can infect almost all the warm-blooded animals. They used VMD, among other SMART software, to construct and analyze the protein. This allowed the researchers to provide positive information on treatment and vaccine for toxoplasmosis, justifying the use of VMD and other SMART software for medical field exploration and education.

Naidoo et al. [6] investigated how miR-1205 is underexpressed in PCa cells and how it suppresses CRPC tumors in vivo. The 8q24 chromosomal locus has high PCa susceptibility and also has a region carrying a PVT1 gene that encodes miR-1205. This microRNA targets FRYL, which is overexpressed in neuroendocrine PCa (NEPC). A treatment of NEPC is neuroendocrine differentiation (NED). The researchers discovered a negative correlation between miR-1205

and NED in normal cells, but in cells with FRYL, miR-1205 induced the production of NED independently of FRYL

Syed et al. [7] explained that FNDC3 is a protein with a large array of functions, including maintenance of spermatid intercellular bridges and spermatid-Sertoli cell adhesion during spermatogenesis, craniofacial, skeletal and lung development, and adipogenesis. However, how exactly FNDC3 functions in each of these mechanisms is not yet known.

Daudon et al. [4] elaborated on the FNDC family and their functionality. The researchers explained how little it known about the exactly mechanisms in which the proteins use, or how they impact other pathways within cells. This research was to find a connection, if any, between the FNDC family and fertility in women.

Chung and Choi [1] concluded research about adipokines and myokines. Adipokines and myokines are protein base hormones that facilitate communication between organs. Adipokines are produced by adipose tissue, while myokines are produced by skeletal muscles.

MacFarlane, Leigh-Ann, and Murphy [5] expressed the importance of miRNA in cancer. They found that miRNA may be involved in the development of cancer due to the expression profiles of miRNA being altered in specific tumors.

Venkatesan et al. [8] looked at how digital extended reality (XR) technologies could be used for educational purposes in the medical field. XR allows 3D visualization of content without the constraints of 2D displays. They used figures of different case studies and applications to provide a perspective on XR in the medical field. They discovered that XR will be beneficial for multiple aspects including biomedical education, medical training, surgical guidance, and molecular data visualization.

3 METHODOLOGY

The visualization of the FNDC3 protein was created using VMD. The protein was uploaded as a PBD file and was used to create a PSF using the Automatic PSF Builder. A PSF is a protein structure file, which is used along with the PBD file to create mutations in the primary structure of the protein. The Sequence Viewer shows the amino acid sequence so the user can see which amino acids can be mutated to change the structure of the protein. Different mutations, including point mutations and frame-shift mutations, can change the structure of the protein, deactivating or otherwise affecting its functionality.

After the user has identified the type of amino acid and the position at which the mutation takes place, they can use the Mutate Residue tool to change the protein. By entering the three-letter code of the desired amino acid and the residue number, the user can change which amino acid is located at that specific residue, effectively changing the protein structure. VMD will then produce the edited molecule in the display. The user can return to the Sequence Viewer and confirm the primary sequence has been changed.

Succeeding the mutation of the selected amino acid, the user can change the representation of the protein in order to make it appear in a certain color and shape. The Representations tool can change the coloring and drawing method the protein is represented in. For this project, the selected mutated amino acid was colored to its elements, meaning that oxygen was red, nitrogen was blue,

carbon was cyan, and hydrogen was white. The drawing style was Licorice, which allowed the structure of the amino acid to be clearly distinguished from the rest of the non-mutated protein. The remainder of the protein was colored magenta (ColorID 27), to create a stark contrast not only to the black background but also to the other colors associated with the selected amino acid. The drawing method for the remaining protein was Lines, which are a thinner and sharper drawing method than the Licorice to ensure the selected amino acids individuality.

After the selected representations have been established, the user can save the visualization state using the Save Visualization tool. The user can upload the same protein in the exact same orientation and representations that they had previously saved. This also allows the user to upload multiple molecules into the same visualization state to create scenes and interactive simulations. To upload multiple saved visualizations, the user can utilize the Load Visualization tool and select multiple visualizations. In this project, I uploaded all 8 mutations and their originals into the same visualization state, including their assigned representations and orientations. Once all 8 mutations and originals were uploaded, I used various features to orient them into a visually pleasing state. When the user uploads multiple proteins, they all load directly on top of each other. There are multiple features that allow the user to move all of the proteins at the same time or just one. Toggle Fixed is a tool that when turned on, allows you to move the selected proteins. However, one can turn off Toggle Fixed for some proteins and then only move the ones that have Toggle Fixed on. The user can orient multiple proteins on a visualization state without having them overlap. Toggle Display, when turned on, will remove the selected protein from view. This allows the user to hide certain proteins so they can work on a select few or one without being distracted or overstimulated from the other proteins. This comes in handy when there are more than just a few proteins.

Once the user has orientated the visualization state exactly how they'd like it, they can save the entire state. I then used this to create a video of all of the proteins and the 10 amino acids involved in the mutations separately so that the testers would be able to have a base-line for the amino acids they would be viewing. I then used Lightworks, a video editing software, to edit and compile the screen recorded videos I made of the visualization states. Each mutation and its original was recorded from multiple perspective, zoomed into the selected amino acids that had been mutated. The testers can then see the mutations from different angles to be able to identify the original amino acid and the mutated amino acid.

4   USER STUDY

The video will be administered to participants to test its teaching capabilities. A survey will be administered afterwards to receive feedback as to the effectiveness of using virtual reality to teach others about biological processes. The survey contained 8 questions asking if the participants could identify the amino acids from the originals and the mutations. They were to refer back to the video as many times as necessary, as it was designed to test VMD's illustrative properties, not the user's memory. The 10 amino acids that previewed the proteins were labeled with their full name, and the questions referred to these full names when asking for the amino

acid identification. At the end of the survey, there was a question asking to rate the effectiveness of VMD's illustrations on a scale of 1 to 5. The results from this survey and video viewing was collected and analyzed.

5   RESULTS AND ANALYSIS

There were 10 participants in the user study. The average of the scores was a 57.5 out of 100 percent, and the mean was 75. This shows that the illustrative prowess of VMD is not as high as expected, and that this software can be improved upon to show a greater illustrative and hopefully educative power. The results from the survey were evenly distributed, with a positive correlation between the score received and the rating of VMD's illustrative properties.

Feedback I received consisted of the video's structure. Some participants concluded that they found it difficult to continuously going back to the video to check their answers to the quiz. They expressed that they believed the proteins should've been oriented differently for the user friendly capabilities of the video. Most participants agreed that the Representation Tools were useful in illustrating the proteins structure and mutations.
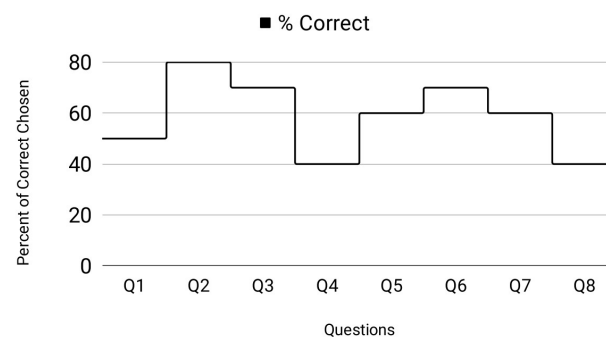

Figure 2: Percent of Questions Answered Correctly by the Number of Respondents.

6   DISCUSSIONS

Through this study, it had been shown that VMD needs to be improved in its illustrative and educational abilities. I have faced a few important setbacks in this project. First, I had a lot of trouble trying to upload DNA into VMD. VMD accepts multiple different kinds of files, most commonly PBD files. Proteins can easily be found in PBD files, however DNA and RNA were increasing difficult to find, especially the specific miRNA and mRNA that I needed to utilize for my original project plan. This problem was what lead to changing the project itself so that I could continue with the project.

When I decided to focus instead one just one protein that was instead affected by miRNA, I needed to find the binding site so that I could change this structurally and change the binding capabilities of the protein. However, not enough research has been done into FNDC3 or the rest of the FNDC family proteins, so the binding site has not be located for their protein. After I discovered this, I settled
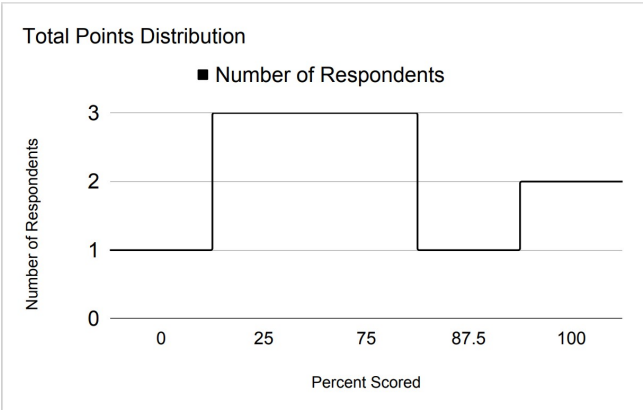
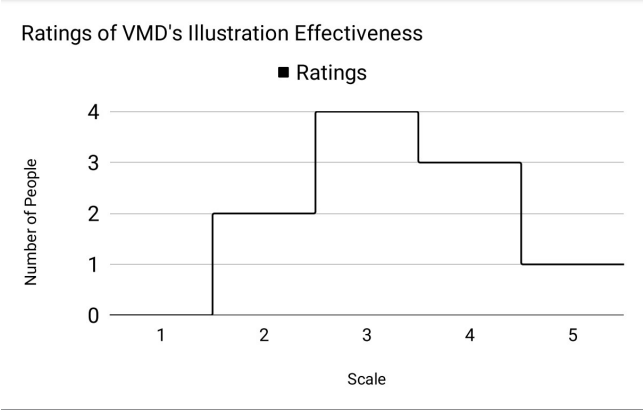**Figure 3: Percent Scored the Number of Respondents.**

Figure 4: Ratings of VMD's Illustrative and Educational Capabilities Based off the Video of Mutated Proteins.

for making any mutations at all. Another issue I ran into was that RSCB PBD recorded that no mutations have been located in this protein, so I needed to do my own independent research into FNDC3 in order to find any mutations that existed. Eventually, I found a scientifically backed source that had located some mutations in FNDC3, but it was not known what kind of affect these mutations had on the protein other than structural change. These obstacles I ran into were extremely demoralizing, but I decided to stick to it and push through these challenges to produce a project that I can be proud of.

7 CONCLUSION

The visualization of amino acids in mutated FNDC3 using VMD was moderately successful based off of the results that were analyzed from the video I produced. I believe that future work into this project should include increasing the user friendly capabilities by adjusting the orientation of the project. I also believe that work should be done to add miRNA and mRNA into the visualization so that the educational side, which would show the process of miRNA deactivating mRNA to affect the production of the proteins, could be utilized in addition to VMD's illustrative capabilities.

REFERENCES

[1] Hye S Chung and Kyung Mook Choi. 2018. Adipokines and myokines: a pivotal role in metabolic and cardiovascular disorders. *Current medicinal chemistry* 25, 20 (2018), 2401–2415.

[2] Dibash K Das and Olorunseun O Ogunwobi. 2017. A novel microRNA-1207-3p/FNDC1/FN1/AR regulatory pathway in prostate cancer. *RNA & disease (Houston, Tex.)* 4, 1 (2017).

[3] Dibash K Das, Joseph R Osborne, Hui-Yi Lin, Jong Y Park, and Olorunseun O Ogunwobi. 2016. miR-1207-3p is a novel prognostic biomarker of prostate cancer. *Translational oncology* 9, 3 (2016), 236–241.

[4] Mathilde Daudon, Yves Bigot, Joëlle Dupont, and Christopher A Price. 2022. Irisin and the fibronectin type III domain-containing family: structure, signaling and role in female reproduction. *Reproduction* 164, 1 (2022), R1–R9.

[5] Leigh-Ann MacFarlane and Paul R Murphy. 2010. MicroRNA: biogenesis, function and role in cancer. *Current genomics* 11, 7 (2010), 537–561.

[6] Michelle Naidoo, Fayola Levine, Tamara Gillot, Akintunde T Orunmuyi, E Oluwabunmi Olapade-Olaopa, Thahmina Ali, Konstantinos Krampis, Chun Pan, Princesca Dorsaint, Andrea Sboner, et al. 2021. MicroRNA-1205 Regulation of FRYL in Prostate Cancer. *Frontiers in Cell and Developmental Biology* 9 (2021), 647485.

[7] Adeela Syed, Tamás Lukacsovich, Miles Pomeroy, A Jane Bardwell, Gentry Thomas Decker, Katrina G Waymire, Judith Purcell, Weijian Huang, James Gui, Emily M Padilla, et al. 2019. Miles to go (mtgo) encodes FNDC3 proteins that interact with the chaperonin subunit CCT3 and are required for NMJ branching and growth in Drosophila. *Developmental biology* 445, 1 (2019), 37–53.

[8] Mythreye Venkatesan, Harini Mohan, Justin R Ryan, Christian M Schürch, Garry P Nolan, David H Frakes, and Ahmet F Coskun. 2021. Virtual and augmented reality for biomedical applications. *Cell reports medicine* 2, 7 (2021), 100348.

[9] Jian Zhou, Wanchun Wang, Pengxia Song, Lin Wang, Yali Han, Jingjing Guo, Zhen Hao, Xi Zhu, Qiong Zhou, Xiadong Du, et al. 2018. Structural predication and antigenic analysis of Toxoplasma gondii ROP20. *Acta Parasitologica* 63, 2 (2018), 244–251.