DARPin-Based Inhibition of PARP1 for Muscular Dystrophy and Overtraining Syndrome

SRILASYA MALLAJOSYULA

Wilmington High School, Wilmington, MA

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Muscular dystrophy (MD) is a hereditary condition that results from the aging of muscles in the human body. Recently, hyperactivation of the Poly(ADP-ribose) polymerase 1 (PARP1) protein present in the fibroblast cells has been linked to MD and overtraining syndrome. Therefore, inhibiting these proteins could inhibit the overactivation of PARP1 and mitigate the disease. The AlphaFold software was used to create a 3D shape of the PARP1 protein to visualize the amino acid sequence. HDOCK was used to understand the binding interaction between two different biomolecules. The PARP1-DARPins binding energy was computed using the PRODIGY software. Designated Ankyrin Repeat Proteins (DARPins) are a class of engineered, non-antibody binding proteins used in protein diagnostics and therapeutics. The results show that the DARPins bind to the predicted binding site of the PARP1 protein as computed by the P2Rank webserver. The DARPins also bind to the DNA-binding site of the PARP1 protein. Therefore, DARPin binding could inhibit the overfunctioning of the PARP1 protein. Based on binding energy, DARPin D3 (PDB ID: 4K5C) formed the strongest interaction (-13.6 kcal/mol) with the PARP1 protein and was selected as the most appropriate candidate. In future studies, we will be performing DARPin mutational studies to enhance the binding affinity towards the PARP1 protein. These results highlight the potential of DARPin-based theragnostic as a novel approach for targeting PARP1-related muscle degradation and help in alleviating the global burden of the disease.

1. INTRODUCTION

Muscular dystrophy (MD) is a hereditary condition that results from the aging of muscles in the human body. [1] This type of genetic disease causes progressive weakness and loss of muscle mass. Common symptoms of disease are abnormality in walking, muscle weakness, loss of muscle, and delayed development. [2] In addition to this, people are constantly walking on their tiptoes, experiencing constipation, difficulty swallowing, fatigue, and/or shallow breathing. Detection of MD is found through physical exams, genetic tests, muscle biopsy, blood tests, imaging tests, exercise assessments, electromyography, and nerve conduction studies. Treatments of this disease include medication, physical and occupational therapy, assistive devices, and maybe surgery. [2]

2. METHOD

Uniprot enables researchers to find a specific amino acid. [3] We used this software to find the PARP1 protein's amino acid sequence in humans. In addition, we used the AlphaFold 3 software. [4] This is an AI-generated software that can predict the 3D shapes of proteins based on their amino acid sequences. This was used to visualize the 3D structure of the PARP1 amino acid sequences. PROTTER is a tool that is used to display protein features and enables interactive visualization. [5] We used it to locate where the PARP1 protein is generally located inside the cell. Based on this image, it can be seen that it is located past the cell membrane. The Human Protein Atlas is used to locate all human proteins in the cells, tissues, and organs using a variety of 'omics technologies. [6] We used this software to identify further where the PARP1 protein was located. Based on this, we were able to look at the cell in the image and find that the protein is also located in the nucleus. P2Rank is a software that predicts the binding site on the protein surface. [7] We used this software to differentiate the DNA-cleaving region (which is what we are studying) and the DNA-binding region. Molecular Docking Simulation is a computational method in which two different biomolecules are docked together to understand their binding interaction. We used the Molecular Docking Simulations (HDOCK) software to identify different PARP1-darpins interactions. [8] The PRODIGY software was used to find the binding energy between the protein-darping molecules. We used this to find the best protein-darpin interaction. In addition, the PLIP software enables us to detect non-covalent interactions between the protein and the ligand in the 3D structure. [9] This was used to find the types of bonds and the number of bonds

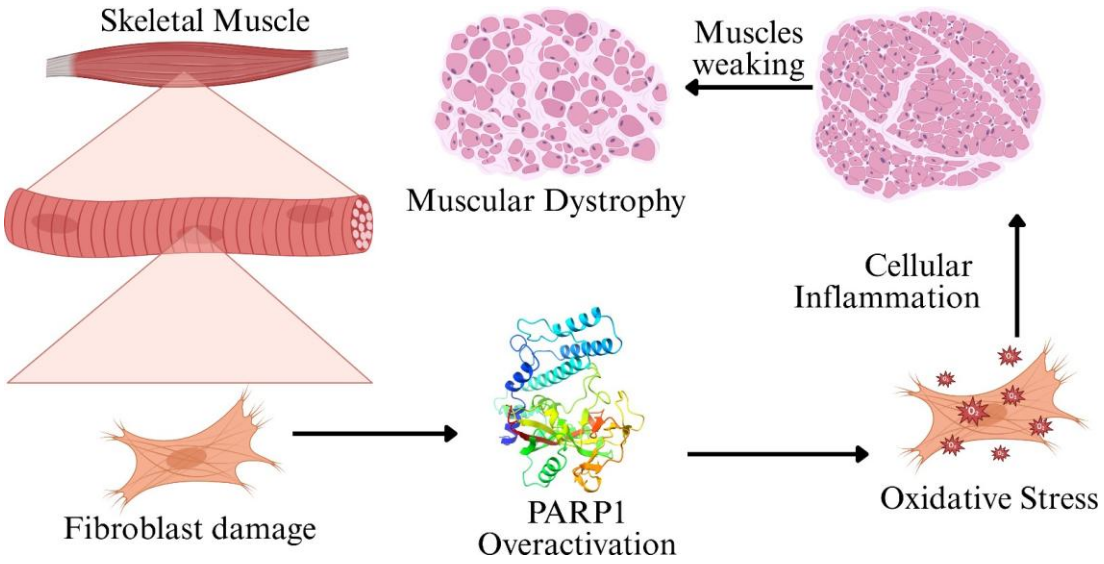

Fig. 1. This figure shows the formation of MD due to the formation of the PARP1 protein. The fibroblast cells in the skeletal muscle get damaged, resulting in the PARP1 overactivation. This leads to oxidative stress inside the fibroblast cells. This results in cellular inflammation, which causes the skeletal muscle to weaken. This eventually leads to MD.

for each category. All the categories of bonds were neatly organized in a Google Sheet. Next, we mutated the amino acid sequence by changing one amino acid. A protein mutation is a change to the amino acid sequence that results in a different shape and function of the protein. We did this to understand the different shapes and functions of the proteins when they are mutated. Once we mutated the amino acid sequence, we used AlphaFold 3 to get a 3D shape of the protein. Then, I used the ChimeraX software to save the 3D protein image, and then I used the Molecular Docking Simulations to find their binding interactions. Finally, molecular dynamics simulations were performed using the GROMACS software to understand the dynamics of the PARP1-DARPins interactions. [10]

3. RESULTS

In this research work, we utilized computational simulations to investigate the interactions between PARP1 and various DARPins that can be employed to inhibit the enzyme and prevent the disease. In this section, we discussed the surface properties, the protein-DARPin interaction, and the molecular docking simulation. The molecular dynamics simulations were used to validate the protein-DARPin binding further. Its interaction with DARPins has been discussed using molecular docking and molecular dynamics simulations.

Predicted binding site: We first needed to study the location and binding prediction site of the protein. As shown in Figure 2A, this is present within the cell, as identified using the PROTTER software. Figure 2B further confirms that it is present in the cell's nucleus. Figures 2C, 2D, and 2E show the active site of the protein, which needs to be blocked to prevent the enzymatic activity. All 3 of these figures found from P2-Rank and the Coach-D software show similar results, further validating the binding site. These sites were needed to be inhibited to prevent the enzymatic activity. Preventing this activity can prevent oxidative stress and MD.

Molecular Docking Simulations: Once the protein surface properties were obtained, we performed PARP1-DARPins analysis and showed it in Figure 3. We used Molecular Docking Simulations to understand the interactions. A total of 10 DARPins were used for analysis. DARPins that bind to the selected site were selected. From the visual inspection, DARPins 4j7w, 4k5c, 5fin, 7b4v, and 9glq were bound to the predicted binding site. We also performed chemical mutations on the selected DARPin (4k5c). We performed amino acid mutations and four mutants were obtained, shown in Figure 3: 4k5c_Y81R, 4k5c_F82E, 4k5c_H88L, and 4k5c_P84D.

Molecular d ocking Analysis: In the next step after the molecular docking simulation, the molecular docking analysis was performed, in which binding energy and protein-DARPins interactions were compared. Binding energy was computed using PRODIGY software, as shown in Figure 4. From this Binding Energy, DARPin 4k5c was selected as an appropriate candidate because it had the lowest binding energy of -13.5 J. These results were further validated by using the PLIP Software. The protein-DARPin analysis showed that 4k5c had the maximum number of hydrophobic interactions, further proving that 4k5c is the best DARPin.

The following DARPin selection criteria were used. First, the DARPin should bind to the predicted binding site as shown by the P2Rank and Coach-D Software from Figures 2c and 2e. Next,

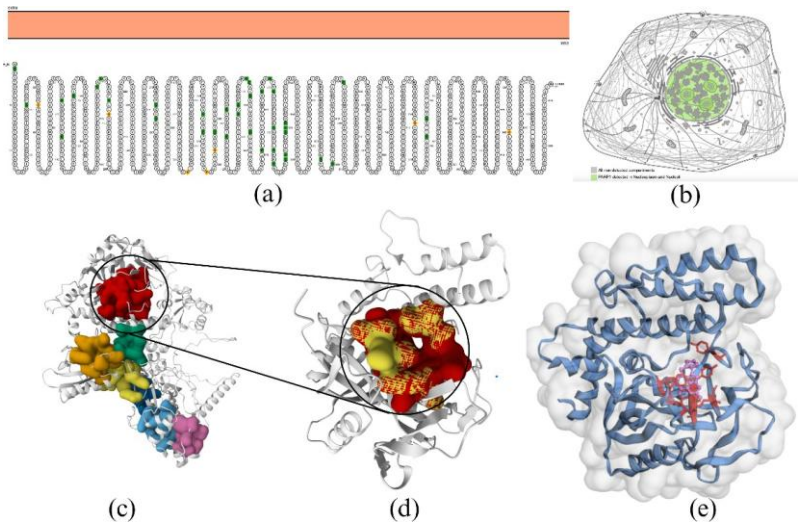

Figure 2: (a) Obtained from the PROTTER software, and shows that the PARP1 protein is present inside the myocyte; (b) The Human Protein Atlas shows that the PARP1 protein is located in the nucleus; (c) and (d), From the P2Rank software, show the binding location of the protein; (e) Obtained through the Coach-D software is used to be able to predict where the ligand/protein is likely to bind to the PARP1 protein. The image shows where the DARPin could bind to the PARP1 at the predicted binding site.

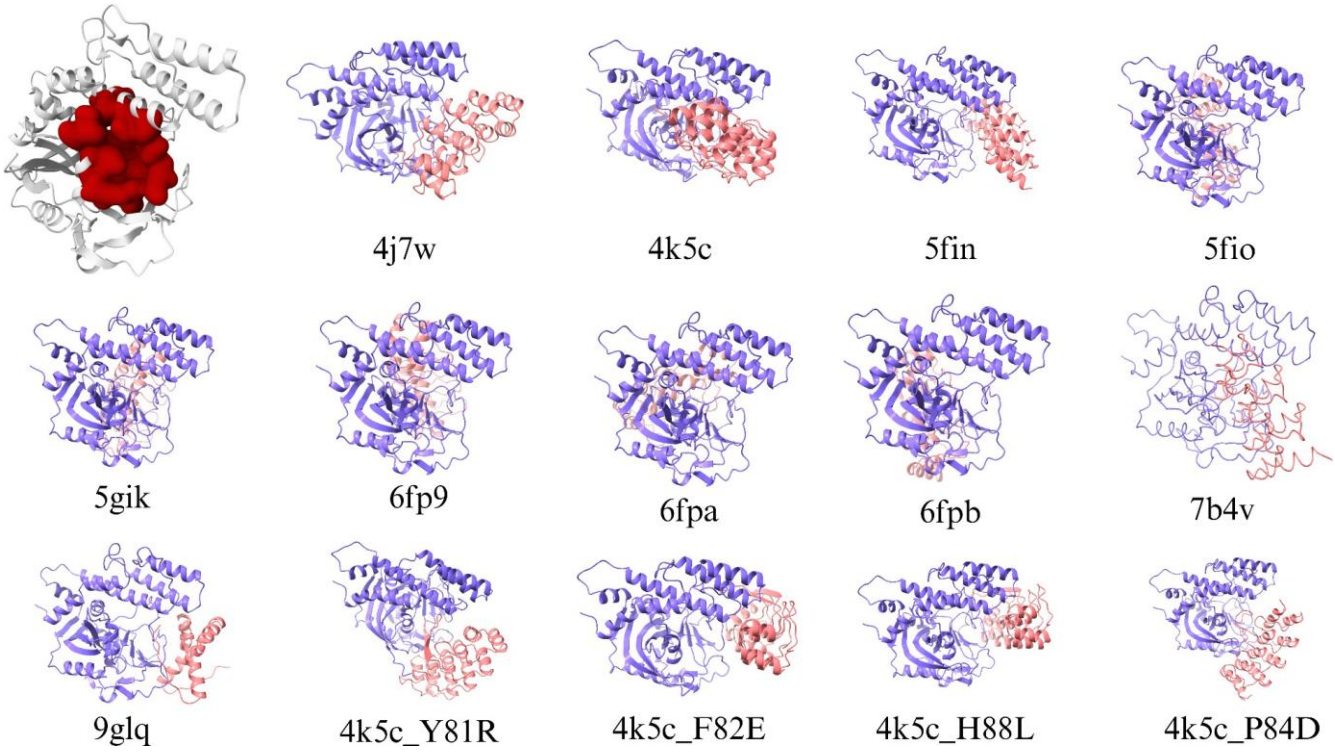

Fig. 3. Molecular docking simulations: Molecular docked structure of the PARP11-DARPins complexes. The purple portion is the PARP1 protein and the pink binding region is the DARPins. These structures were found by using the HDOCK software.

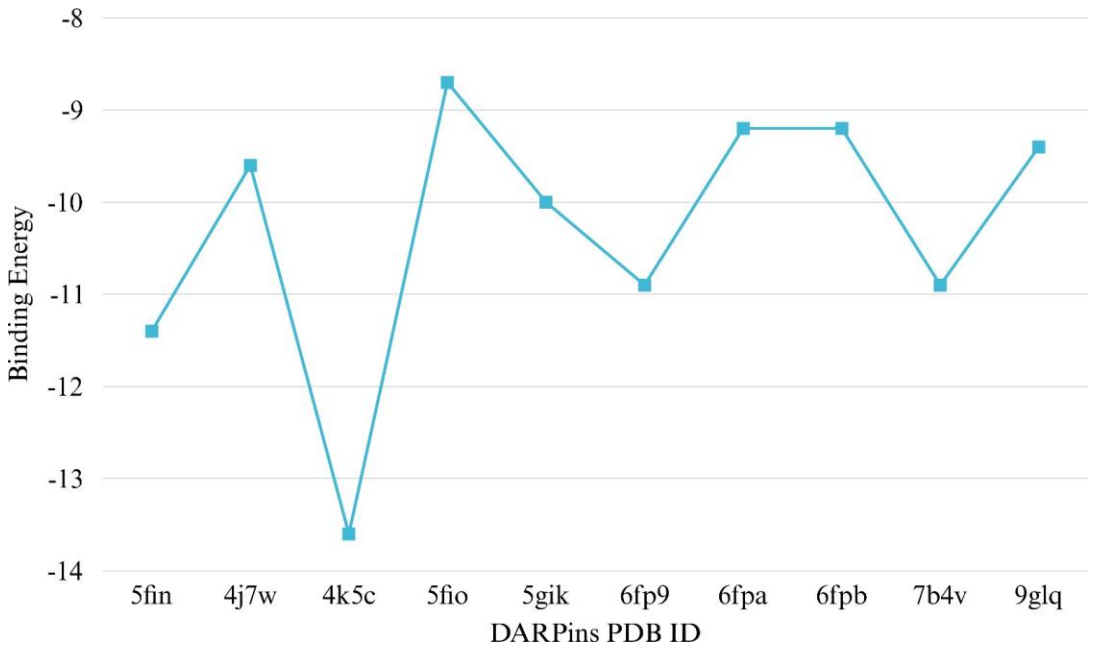

Fig. 4. PARP1-DARPins binding energy: prodigy software. The most negative binding energy is the best DARPin. The PRODIGY software obtained this. This means that the 4k5c is the best DARPin for the PARP1 protein, since it has the strongest binding affinity.

the binding energy is the amount of energy required to pull two molecules apart, and the DARPins that have the lowest binding energy (most negative) were selected (PDB ID = 4k5c). This means that the DARPin has a high binding affinity, or that it binds strongly together. Lastly, the protein-DAPRin interaction was computed, and the highest number was selected, which happened to be the 4k5c DARPin. The molecular dynamics simulation was performed to validate that the protein and DARPin bind strongly. In the entire MD simulation, the DARPins remained bound to the PARP1 protein, further confirming that the DARPin binds strongly to the protein.

Molecular Dynamics Simulations:

4. DISCUSSION

Muscular Dystrophy is a hereditary condition that results from the aging of muscles in the human body. [1] Overtraining syndrome is the response to excessive exercise, leading to decline in performance. It also leads to muscle pain, fatigue, and mood changes. The application of Muscular Dystrophy and Overtraining Syndrome is that it is a therapeutic Role in Muscular Dystrophy and a preventive role in Overtraining Syndrome. Since the PARP1 protein is linked to various cancers and neurodegenerative diseases, it can be used in the early detection of these diseases. DARPins' molecular weight (size) is smaller than that of conventional antibodies used for detection purposes. Therefore, it easily penetrates the cell membrane thus increasing the bioavailability. In addition, DARPins provide an alternate detection and treatment strategy that can be used in these diseases.

Limitations and Future Work: Some of the limitations of the current research are as follows: Since the research involves computational simulations it needs further experimental validation in which surface plasmon resonance (SPR) and/or isothermal calorimetry (ITC) can be used. In addition, we have used a limited number of DARPins for the analysis; however, in future studies we will be performing amino acid mutations to generate DARPins derivatives to compare with the parent compound.

5. CONCLUSION

In this research project, we investigated the usage of DARPin-based inhibition of PARP1 found in muscular dystrophy and overtraining syndrome. Using computational tools such as AlphaFold, HDOCK, PRODIGY, and PLIP, we identified and analyzed PARP1–DARPin interactions. These tools, together, showed that the PDB ID = 4k5c DARPin was the appropriate candidate, as it had the strongest binding affinity. These results pave the way towards the treatment of muscle disorders, cancer research, and neurodegenerative diseases.

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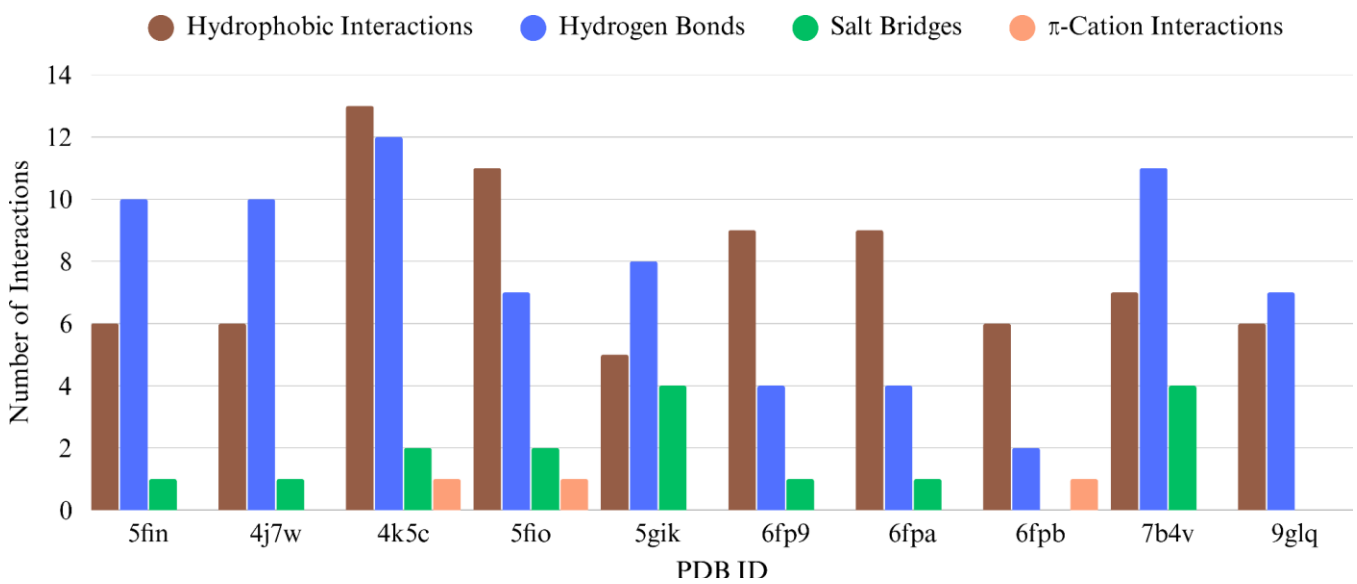

Fig. 5. The number of interactions formed between the PARP1 and DARPins. The 4k5c DARPins are the best in this scenario because the hydrophobic interactions are the highest, and the salt bridge interactions are near the lowest.

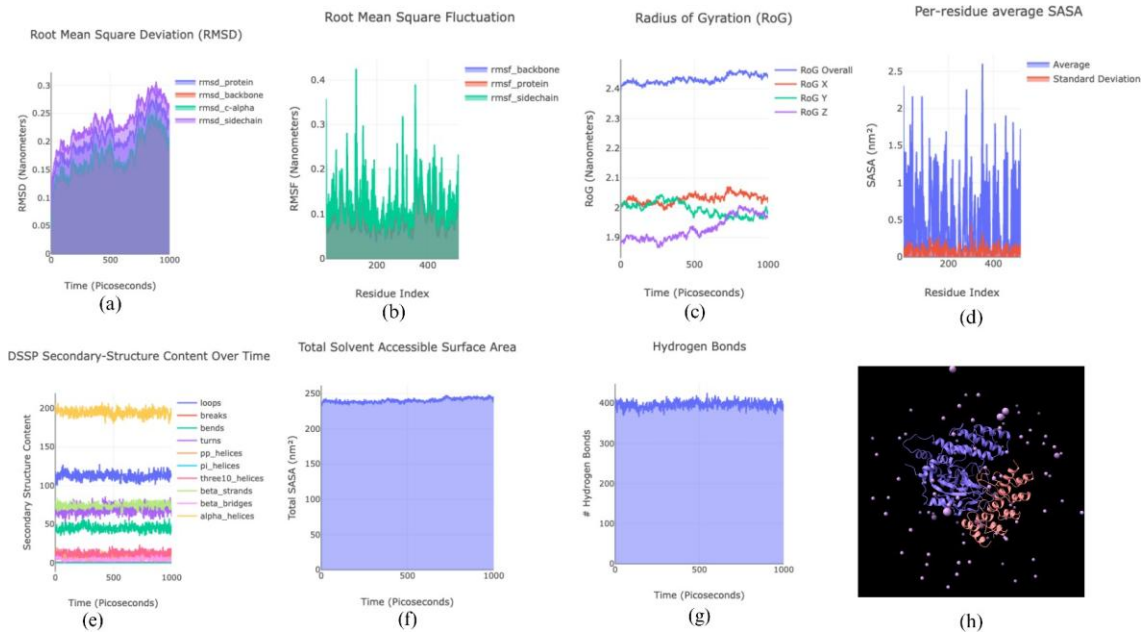

Fig. 6. Molecular Dynamics simulations by GROMACS. This shows the Molecular Dynamics simulation between the PARP1 protein and the DARPins, which was obtained by the GROMACS software. In this entire simulation, the DARPins remained bound to the PARP1 protein.

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