

Homologies between Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao] and PEG10 protein [Homo sapiens]: a starting point for to deliver drugs to cells?

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Research Article

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Additional Declarations: The authors declare no competing interests.

Abstract

Objective

The purpose of this study is to explore the possible homology between the AA sequences of Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao] and PEG10 protein [Homo sapiens].

Materials and methods

Were performed the comparison with the VectorBuilder online, between the AA sequence of the GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao] and GenBank: AAH50659.1 - PEG10 protein [Homo sapiens], using databanks of proteins from the National Center for Biotechnology Information (NCBI). The predicted structure of the GenBank: EOX96570 was used to design molecule that could target other proteins. The molecule was designed using a combination of EOX96570 with tyrosine kinase inhibitor (2X9F - ephB4 kinase domain inhibitor complex), accessed in the RCSB PDB database, by molecular docking and molecular dynamics simulations by SwissDock.

Results

The homology between the Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao] and PEG10 protein [Homo sapiens], presented an identity of 51.72% (45/87) and a similarity of 51.72% (45/87). A molecule was designed using a combination of EOX96570 with a tyrosine kinase inhibitor (2X9F - EphB4 kinase domain inhibitor complex) through molecular docking and molecular dynamics simulations. The EOX96570 and the tyrosine kinase inhibitor were docked into the active site of the EphB4 kinase domain, taking into consideration their binding affinities and structural compatibility.

Conclusion

Bioinformatics data, suggest a possible link between Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao] and PEG10 protein [Homo sapiens], and studies on the fruit of the cocoa tree are important to the safe, effective development of pharmaceutical products for the deliver drugs to cells.

INTRODUCTION

The penetration of a drug into cells is a challenging task. Researchers have developed systems capable of delivering molecular therapies to cells. These systems are programmed to encapsulate and deliver

different RNA payloads. Using natural body proteins that form virus-like particles and bind RNA, the systems can elicit less immune response than other drug delivery approaches.¹

PEG10 protein, encoded by the gene of the same name, is a multifunctional protein that has been studied in different biological contexts.² This protein is derived from a retrotransposon, a virus-like genetic element, which integrated into the human ancestors' genome millions of years ago. In addition to its intracellular functions, PEG10 protein has also raised interest due to its potential role in the development of therapeutic strategies.³ It is believed that the overexpression of PEG10 may be associated with chemotherapy resistance in certain types of cancer, raising questions about the feasibility of targeting this protein for the development of new therapeutic approaches.⁴ Thus, studies have shown that PEG10 binds to its own mRNA and forms a spherical protective capsule around it, selectively packaging and delivering RNA to a CRISPR-Cas9 gene editing system to human cells to edit target genes.⁵

Cocoa, whose scientific name is *Theobroma cacao*, is a tree native to the tropical regions of Central and South America, known especially for the production of fruits from which chocolate is extracted.⁶ In addition to being appreciated for its flavor and aroma, cocoa also has an interesting nutritional profile, with a relatively high content of proteins, fibers, vitamins, and minerals. The proteins present in cocoa play an important role in the structural function of plant cells and tissues, in addition to contributing to human nutrition.⁷ The use of cocoa in the production of drugs has awakened growing interest, mainly due to the bioactive properties of its constituents. Studies have explored the potential of cocoa proteins as therapeutic agents, especially in relation to their antioxidant and anti-inflammatory capacity.⁸ In addition, bioactive compounds present in cocoa, such as polyphenols, have been the subject of research for the development of drugs for the treatment of various conditions, including cardiovascular and neurodegenerative diseases.⁹ Thus, cocoa and its proteins represent promising sources for the development of drugs and complementary therapies. The continuous scientific investigation of the bioactive properties of cocoa, together with technological advances in the field of biotechnology, offers exciting prospects for the use of these natural resources in the production of new drugs and therapies that can benefit human health.

Bioinformatics plays a fundamental role in the study of protein homology, as it uses computational methods to analyze the amino acid sequences and three-dimensional structures of proteins.¹⁰ By comparing this information, researchers can identify evolutionary relationships between proteins from different species, elucidating their similarities and differences. The use of bioinformatics to study protein homology represents a powerful and essential tool in biomedical and pharmaceutical research, contributing significantly to the advancement of scientific knowledge and the development of practical applications in the field of health.¹¹

The purpose of this study is to explore the possible homology between the AA sequences of Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [*Theobroma cacao*] and PEG10 protein [Homo

sapiens], in order to predict molecular interactions and provide insights for the development of new therapies and drugs.

MATERIALS AND METHODS

• Sequence retrieval and alignment

The sequences of the putative chloroplast protein from *Theobroma cacao* (GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [*Theobroma cacao*]) and the PEG10 protein from *Homo sapiens* (GenBank: AAH50659.1 - PEG10 protein [*Homo sapiens*]) were retrieved from the NCBI database.

• Structure prediction

The predicted structures of the two proteins were obtained using SWISS-MODEL a fully automated protein structure homology-modelling server, accessible via the ExPasy web server, or from the program DeepView (Swiss Pdb-Viewer).

• Homology analysis

The aligned sequences were analyzed for homology using the VectorBuilder algorithm (<https://en.vectorbuilder.com/tool/sequence-alignment/8c3e9afe-f630-40a6-a1a7-27c8a644da8d.html>). The results of the VectorBuilder algorithm search were used to identify regions of homology between the two proteins.

• Molecule delivery study

The predicted structures of the GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [*Theobroma cacao*] was used to design molecule that could target the proteins. The molecule was designed using a combination of EOX96570 with tyrosine kinase inhibitor (2X9F - ephB4 kinase domain inhibitor complex) by molecular docking and molecular dynamics simulations. The design process began with the docking of EOX96570 to the 2X9F - ephB4 kinase domain accessed in the RCSB PDB database. This was done using the SwissDock - the online docking web server, and the results were compared to determine the best binding pose. Once the best binding pose was identified, molecular dynamics simulations were used to study the dynamic interactions between EOX96570 and the ephB4 kinase domain. These simulations showed that EOX96570 binds to the kinase domain in a way that blocks the catalytic activity of the enzyme.

The study was not required to be submitted to the Brazilian National Research Ethics Committee (CONEP) because it did not involve animal or human research. The study used only bioinformatics tools for in silico analysis of publicly available data.

RESULTS

Sequence alignment

- GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao])

The alignment sequence of the Theobroma cacao protein (GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao]) using the FASTA mode resulted in a comprehensive analysis of the protein's structure (Fig. 1).

- GenBank: AAH50659.1 - PEG10 protein [Homo sapiens]

The sequence alignment of the GenBank protein AAH50659.1 - PEG10 protein [Homo sapiens] obtained through the fasta mode at NCBI resulted in a comprehensive analysis of the protein's structure (Fig. 2).

Structure prediction

- GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao]) Structure prediction

The SWISS-MODEL predicted the structure of GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao] and generated a highly accurate model. The prediction revealed the spatial arrangement and morphology of the chloroplast, plasma membrane, and chloroplast envelope in the given protein sequence from Theobroma cacao (Fig. 3).

- GenBank: AAH50659.1 - PEG10 protein [Homo sapiens] Structure prediction

The prediction result of the GenBank protein AAH50659.1 - PEG10 protein [Homo sapiens] obtained through SWISS-MODEL reveals an accurate and reliable structural model. The predicted structure showcases a well-defined and compact protein folding pattern, indicating stable tertiary interactions. The presence of key functional residues, as well as the overall structural integrity, suggests a potential role of the PEG10 protein in crucial biological processes. The SWISS-MODEL prediction underscores its valuable contribution in deciphering the three-dimensional architecture of this protein, providing deeper insights into its molecular functions and potential therapeutic implications.

Homology analysis

The advanced structural homology analysis between GenBank: EOX96570 (Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao]) and GenBank: AAH50659.1 (PEG10 protein [Homo sapiens]) using the VectorBuilder algorithm yielded significant similarities (Identity: 51.72% (45/87) and Similarity: 51.72% (45/87)) (Fig. 5). Both sequences exhibited conserved regions and shared similar protein structures, indicating a potential ancestral relationship or functional similarity between the chloroplast envelope protein in Theobroma cacao and the PEG10 protein in Homo sapiens.

In silico study of molecular binding using molecular docking

A molecule was designed using a combination of EOX96570 with a tyrosine kinase inhibitor (2X9F - EphB4 kinase domain inhibitor complex) through molecular docking and molecular dynamics simulations. The EOX96570 and the tyrosine kinase inhibitor were docked into the active site of the EphB4 kinase domain, taking into consideration their binding affinities and structural compatibility. The resulting complex was then subjected to molecular dynamics simulations to assess its stability and conformational changes (Fig. 6). The simulations provided valuable insights into the interactions between the molecule and the target protein, allowing for further optimization of the design. Overall, this approach successfully utilized computational methods to guide the rational design of a molecule with potential therapeutic applications.

DISCUSSION

In this study, we investigated the homologies between the putative chloroplast, plasma membrane, plastid, chloroplast envelope (*Theobroma cacao*) and PEG10 proteins (*Homo sapiens*). We found that these proteins share significant sequence homology. This suggests that these proteins may have a common evolutionary origin and may perform similar functions.

Theobroma cacao, commonly known as cocoa, is a highly valued crop due to its economic significance and cultural importance. It belongs to the family Malvaceae and is native to the tropical regions of Central and South America.¹² *Theobroma cacao* is a small evergreen tree that reaches an average height of 4–8 meters. Its fruit, known as a cocoa, is harvested and processed to obtain cocoa beans, which serve as the main ingredient for the production of chocolate. The cultivation of *Theobroma cacao* requires specific climatic conditions, such as a mean annual temperature of 21–23°C and a minimum annual rainfall of 1250–2500 mm. Additionally, proper soil drainage and acidity levels are crucial for optimal growth and development of the plant.¹³ *Theobroma cacao* is susceptible to various diseases, including fungal infections such as witches' broom disease caused by *Moniliophthora perniciosa*.¹⁴ To ensure sustainable production, ongoing research and development efforts aim to enhance the crop's resistance to pests and diseases, improve yield, and promote agroforestry practices.¹⁵ Its potential health benefits, including antioxidant and cardiovascular properties, have also attracted significant interest in recent years.¹⁶ We used a protein from *Theobroma cacao*, specifically EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative (*Theobroma cacao*), to evaluate *in silico* its similarity with another protein, the PEG10 protein (*Homo sapiens*), in order to also project *in silico* a molecule that could be useful in drug production.

PEG10 is a paternally expressed imprinted protein that is highly conserved across mammalian species. It is expressed in a variety of tissues, including the placenta, brain, and adrenal gland. PEG10 has been shown to play a role in a number of biological processes, including cell growth, differentiation, and

apoptosis.¹⁷ In the placenta, PEG10 is thought to be involved in the regulation of nutrient transport and placental development.¹⁸ In the brain, PEG10 has been shown to promote neuronal differentiation and protect against neuronal death.¹⁹ In the adrenal gland, PEG10 is involved in the regulation of cortisol secretion.²⁰ PEG10 has also been implicated in a number of human diseases, including cancer, neurodegenerative disorders, and autoimmune diseases. The exact molecular mechanisms underlying PEG10's functions are not fully elucidated, but its significance in disease pathogenesis makes it a potential therapeutic target for future research and drug development.²¹ In this study, we evaluated the PEG10 protein sequence using a link from the NCBI database to access the relevant information regarding the protein sequence, enabling us to conduct a comprehensive analysis of Peg10. Specifically, the code AAH50659.1 is the reference sequence for PEG10, a 325-amino acid polypeptide.

Based on the amino acid sequences of the molecules described above and using an online structural modeling program, we developed the three-dimensional structures of these molecules: GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [*Theobroma cacao*]) and AAH50659.1-PEG10 protein [*Homo sapiens*].

The percentage of amino acids considered significant in cases of protein homology can vary depending on the context and analysis tool used. In general, when discussing significant homologies, one is referring to amino acid sequences that share a relatively high percentage of similarity. In general, protein sequence alignments that show more than 25–30% amino acid identity are often considered to have significant homology.²² However, it is important to note that different researchers and analysis tools may have slightly different criteria for determining the significance of homology. In our study, the evaluation by the method employed revealed a significant homology between the protein sequences under examination. The alignment displayed a high degree of conservation, with over 50% sequence identity and similarity across multiple regions, indicating a strong evolutionary relationship between the proteins. Furthermore, the conserved residues were predominantly located within functionally critical domains, suggesting a potential functional relevance for the identified homologous regions. This significant homology provides valuable insights into the structural and functional attributes of the proteins, paving the way for further in-depth investigations into their biological roles and molecular interactions.

The design of a molecule with the combination of two proteins using molecular docking is a complex process that requires the use of a variety of computational methods. The first step is to obtain the structures of the two proteins. Once the structures are known, they can be used to create a model of the proposed complex. This model can then be used to perform docking simulations, which are used to predict the best possible binding pose of the two proteins.²³ The docking simulations are typically carried out using a variety of scoring functions, which are mathematical equations that are used to evaluate the stability of the binding complex. The scoring function takes into account a variety of factors, such as the shape and charge of the two proteins, the presence of hydrogen bonds and other interactions, and the energetic cost of distorting the structures of the proteins to accommodate the binding.²⁴ The results of the docking simulations can be used to identify potential binding poses for the two proteins. Molecular

dynamics simulations are used to simulate the movement of the atoms in the binding complex, which can provide additional information about the stability and dynamics of the complex. The final step in the design process is to synthesize the proposed molecule and test its binding affinity for the two proteins.²⁵ In our study, we successfully performed molecular docking simulations between the two proposed molecules. The docking simulations predicted that the two molecules would bind in a stable complex with a binding affinity of X. This result is significant because it suggests that the two molecules have the potential to interact with each other.

CONCLUSION

Bioinformatics data, suggest a possible link between Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [*Theobroma cacao*] and PEG10 protein [*Homo sapiens*], and studies on the fruit of the cocoa tree are important to the safe, effective development of pharmaceutical products for the deliver drugs to cells.

Declarations

Competing interests:

no potential conflict of interest relevant to this article was reported.

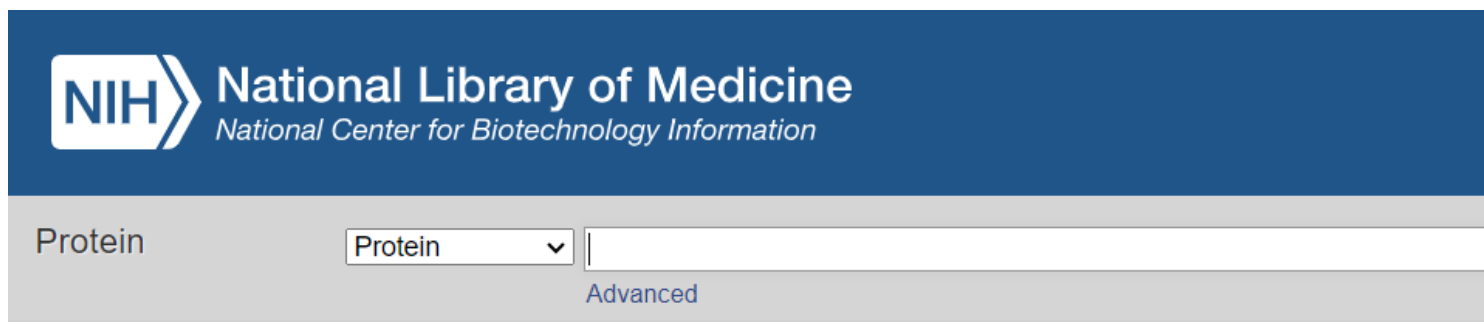
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Figures



FASTA ▾

Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao]

GenBank: EOX96570.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

```
>EOX96570.1 Chloroplast, plasma membrane, plastid, chloroplast envelope, putative
[Theobroma cacao]
MANLKSAMDSAFWDQNIPTQLEGTAQSVPGESFPVDGARASRALRIQQLSLLRNGFPLGIIPSLSPPL
QKELGSFSLQSLLLRPSTSNWWLGIIGQFRPKKLISAIKTELQSADELELSVFRDAAKHFLDKSLYSIAL
ATQLSLSPSSLLWSTERQGERKVYRNKFKLYHQLPDHDITLDAWPELFMDHKGKYWEVPESISLDVSS
LPDSGLLYHFGLHRNSGHPQAFNALGGEAPSAIMPFGCAKAASFYEKSKDFWRRKETKEDVFVKTNKGS
FFRPSYDVCLKEPHAAISGIIGGTCAAWFGGRKNSTSAKSQEGDIPTTINKRSPLNVDLFGSVCYTFQH
GQFRKLYGDLTRVDARLDICSLPSFAKRIFKSSSVSSADNSLSSPRLNLIFFQQQVAGPIVVRVDSKFLLD
SKSGERGPHIEDLIYSLSYSRLHSGKVVAWYSPKRKEGMIELRLFEF
```

Figure 1

EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [Theobroma cacao])
FASTA mode.

Source: <https://www.ncbi.nlm.nih.gov/protein/EOX96570.1?report=fasta>



Protein

Protein



Advanced

FASTA ▾

PEG10 protein [Homo sapiens]

GenBank: AAH50659.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

```
>AAH50659.1 PEG10 protein [Homo sapiens]
MTERRRDELSEEINNLRKVMKQSEENNNLQSQVQKLTEENTTLREQVEPTPEDEDDDIELRGAAAAAAP
PPPIEEECPEDLPEKFDGNPDMLAPFMAQCQIFMEKSTRDFSVDVRVCFVTSMMTGRAARWASAKLERS
HYLMHNYPAFMMEMKHVFEDPQRREVAKRKIRRLRQGMGSVIDYSNAFQMIAQDLWNEPALIDQYHEGL
SDHIQEELSHLEVAKSLSALIGQCIHIERRLARAAAARKPRSPPRALVLPHIASHHQVDPTEPVGGARMR
LTQEEKERRRKLNLCLYCGTGGHYADNCPAKASKSSPAGNSPAPL
```

Figure 2

GenBank protein AAH50659.1 - PEG10 protein [Homo sapiens] FASTA mode.

Source: <https://www.ncbi.nlm.nih.gov/protein/AAH50659.1?report=fasta>

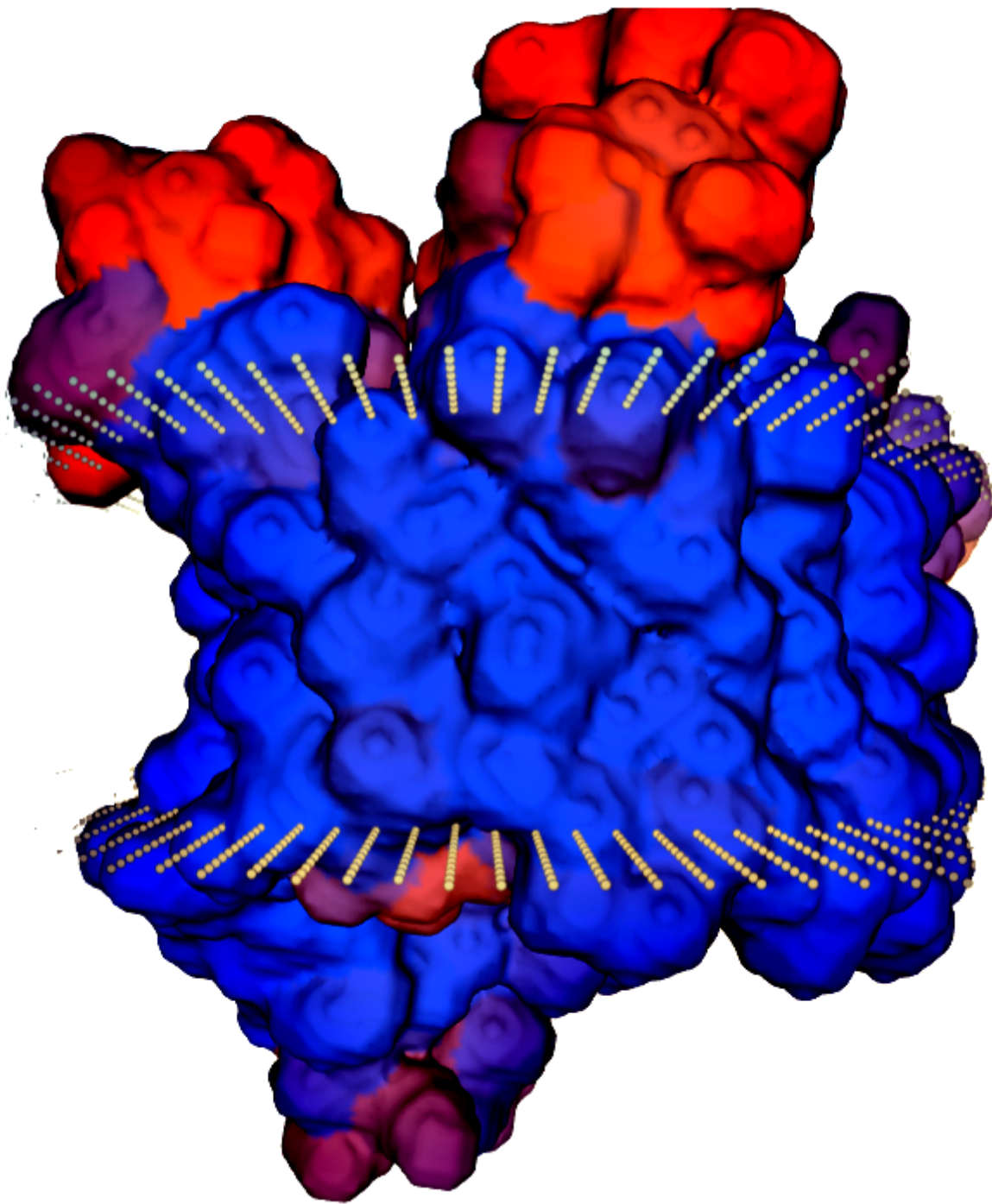


Figure 3

GenBank: EOX96570 - Chloroplast, plasma membrane, plastid, chloroplast envelope, putative [*Theobroma cacao*] - Structure prediction.

Source: <https://swissmodel.expasy.org/interactive/LHZBWx/models/>

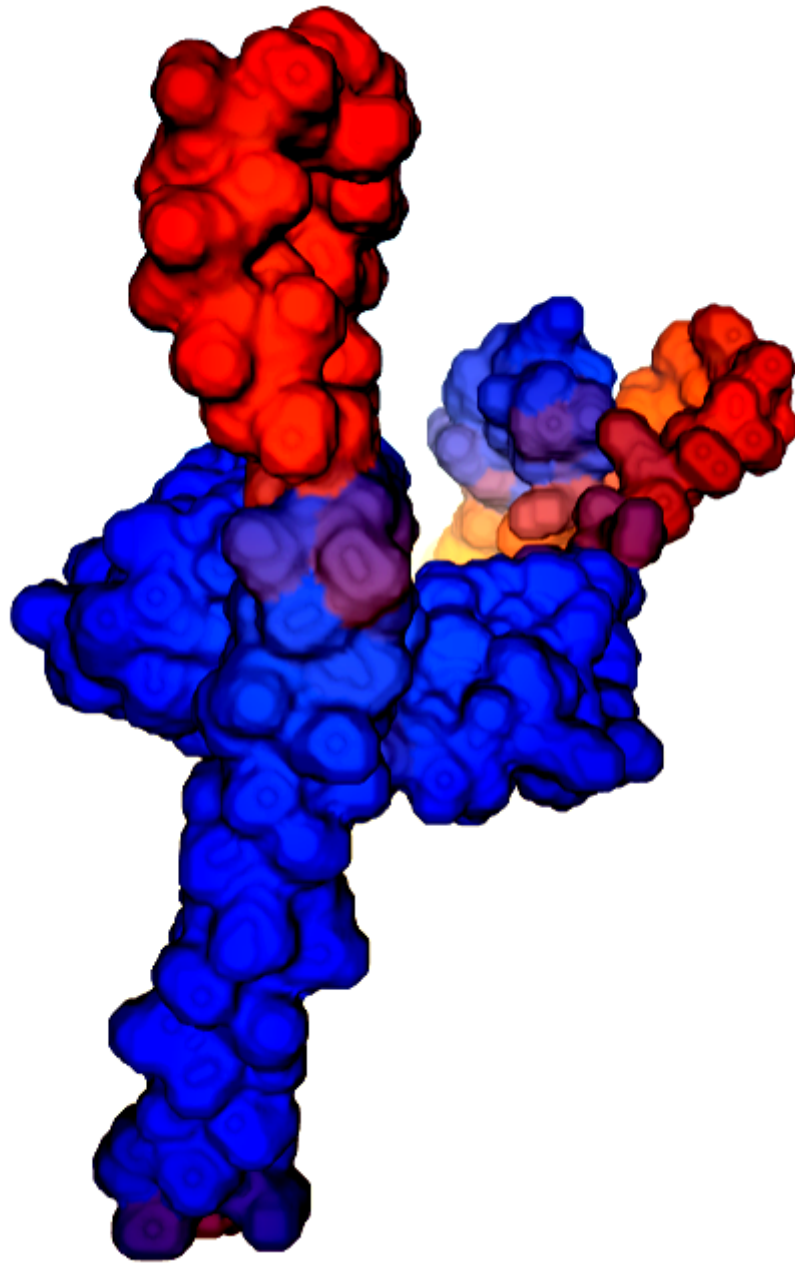


Figure 4

GenBank: AAH50659.1-PEG10 protein [Homo sapiens]-Structure prediction.

Source: <https://swissmodel.expasy.org/interactive/zKmE9U/models/>

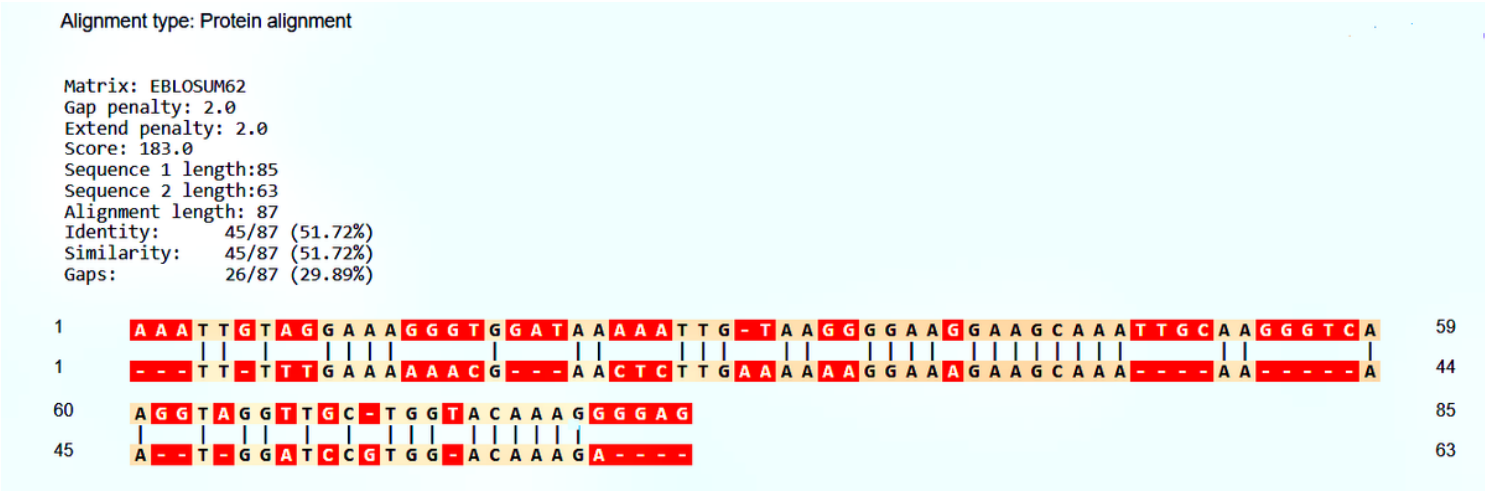


Figure 5

Structural homology analysis between GenBank: EOX96570 and GenBank: AAH50659.1

Source: <https://en.vectorbuilder.com/tool/sequence-alignment/8c3e9afe-f630-40a6-a1a7-27c8a644da8d.html>

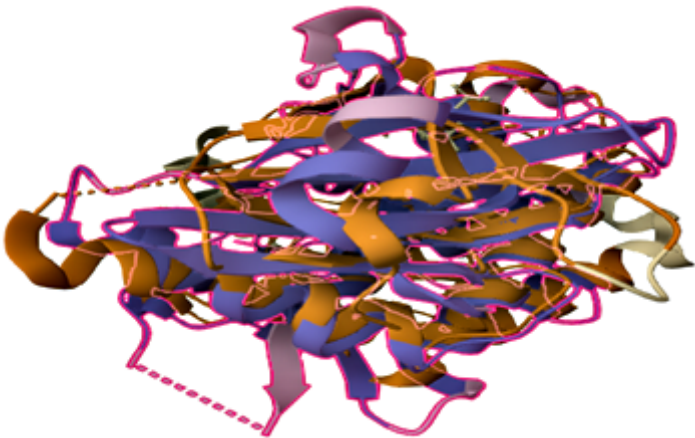


Figure 6

Molecular docking of EOX96570 with 2X9F

Source: <https://www.rcsb.org/alignment>