

COMPUTATIONAL TOOL BASED IDENTIFICATION OF BINDING OF NANOSCALE MICRONUTRIENTS WITH VP28 PROTEIN AGAINST WHITE SPOT SYNDROME VIRUS (WSSV)

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Science degree in Biotechnology

by

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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This is to certify that this project report is the bonafide work of **GAYATHIRI M (40770012)** who carried out the project entitled "**COMPUTATIONAL TOOL BASED IDENTIFICATION OF BINDING OF NANOSCALE MICRONUTRIENTS WITH VP28 PROTEIN OF WHITE SPOT SYNDROME VIRUS (WSSV)**" under our supervision from November 2022 to April 2023

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ABSTRACT

White Spot Syndrome Virus (WSSV), a highly pathogenic virus is a major threat to the global shrimp farming industry, causing high mortality rates and significant economic losses. The VP28 protein is an important structural protein of WSSV and is known to play a critical role in viral infection and replication. Nanoscale micronutrients have been shown to have antiviral properties, but their binding mechanism with viral proteins is not well understood. In this project, computational tools (bioinformatics) are used to identify the binding mechanism of nanoscale micronutrients (Calcium carbonate, Zinc oxide and Copper) with the VP28 protein of WSSV. Molecular docking is performed to study the binding interactions and stability of the VP28 protein-nanoscale micronutrient complex. The molecular docking performed in this project provides a detailed understanding of the binding interactions between the nanoscale micronutrients and the VP28 protein, including the key amino acid residues involved in the binding. The results of this study will provide valuable insights into the mechanism of action of nanoscale micronutrients against WSSV and aid in the development of novel antiviral therapies for the aquaculture industry.

Keywords: WSSV, VP28, Nanoscale micronutrients, Molecular Docking, Binding Interaction, Antiviral therapy

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ABBREVIATIONS

ABBREVIATION	EXPANSION
WSSV	White Spot Syndrome Virus
RNAi	RNA Interference
siRNA	Small interfering RNA
mOsmol/kg	Milliosmoles per kilogram
CDQ	Carbon Quantum Dots
ISG	Interferon-stimulated genes
LOX	Lysyl Oxidase
PDB	Protein Data Bank
RCSB-PDB	Research Collaboratory for Structural Bioinformatics PDB
OPLS	Optimized Potentials for Liquid Simulations
Å	Armstrong
ZnO	Zinc oxide
CaCO ₃	Calcium carbonate
MET	Methionine
PHE	Phenylalanine
PRO	Proline
GLU	Glutamic acid
GLY	Glycine
TYR	Tyrosine
ASP	Aspartic Acid
SER	Serine
VAL	Valine
THR	Threonine
kcal/mol	kilocalorie per mole
kJ/mol	kilojoule per mole

CHAPTER 1

1. INTRODUCTION

1.1 AQUACULTURE

Aquaculture is the breeding, inflating, feeding, and harvesting of aquatic animals and plants. In layman's words, it is referred to as farming of aquatic plants and animals in the water. Aquaculture is an eco-friendly strategy of predominantly generating nutritionally important food and commercial products to engender healthier habits and enhance one's health as most of the aquatic products are rich in nutrition and have various medicinal properties. It also aids in the restoration of endangered species thereby diminishing the threat of species extinction (Max Troell et al., 2013).

Aquaculture production has been studied to have trebled worldwide in live-weight volume within 20 years ranging from 34 Mt in 1997 to 112 Mt in 2017. Seaweeds, tilapia, catfish, bivalves and carps contributed to almost 75 % production of aquaculture species in the year 2017. The production of crustaceans has also seen a massive inflation in a span of 20 years. The Asian continent accounts for about 92% live-weight volume of culturing aquatic animals making it the leading producer of aquaculture products and China is the leading country by a great margin (Naylor et al., 2021).

India is the second largest aquaculture nation just behind China. The aquaculture production in India has been constantly soaring high for almost 2 decades and during the period 2015-2016, the production amount was 5.77 million tonnes. In India, Andhra Pradesh was the largest producer of freshwater fish and generated approximately 15 lakh tonnes of fish (Jayasankar., 2019)

Globally, aquaculture contributes to excessive food production. Due to the aggressive growth of the aquaculture industry, a diverse range of alleviation strategies have been developed for their social and ecological impacts. Meanwhile, aquaculture is also seeing an increase in interest as a means to conserve species, restore habitats and balance out the impact of wild capture (Mirutza et al., 2022).

2. SHRIMP

Shrimps belong to the crustacean family and are characterized by semi-transparent body flattened from side to side, long antennae and legs, thin exoskeleton, and lamellar gills. Shrimp cultivation is an important source of protein, also low in saturated fat and energy and contributes to a majority portion of the economic and food sector in most countries. Shrimps are usually seen in both freshwater and brackish water. Shrimps are mainly produced in most Asian countries and South America.

1. CLASSIFICATION

Kingdom : Animalia

Phylum : Arthropoda

Sub-phylum : Crustacea

Class : Malacostraca

Order : Decapoda

Suborder : Pleocyemata

Infraorder : Caridea

3. NUTRITION REQUIREMENT OF SHRIMPS

1. Table: Tabulation of the nutrients required by shrimps and their role in the metabolism of shrimps

NUTRIENTS REQUIRED	FUNCTIONS
Proteins	➤ Tissue repair and maintenance ➤ Source of energy ➤ Helps in the formation of hormones, enzymes, antibodies and haemoglobin

Amino acids	➤ Aids in catalysis of various biochemical reactions ➤ Metabolic source of energy ➤ Necessary for carbohydrate and lipid metabolism
Lipids	➤ Biological carriers for absorption of fat-soluble vitamins ➤ Act as lubricants for the passage of feed ➤ Essential component of cellular membranes
Vitamins	➤ Acts as antioxidants and aids in immune function ➤ B complex vitamins are responsible for energy metabolism and the development of the nervous system ➤ Vitamin A is essential for vision and the growth of tissues ➤ Vitamin B12 is required for the development of embryos

1.3.2 Table: Tabulation of the list of micronutrients and their functions in the metabolism of shrimps along with the mode of absorption

MICRONUTRIENT	FUNCTIONS	MODE OF ABSORPTION
Calcium	➤ Formation and maintenance of shrimp's exoskeleton	➤ Gastro-intestinal tract

	➤ Activator for several key enzymes such as ATPases and Pancreatic lipases. ➤ Stimulates muscle contraction ➤ Regulates transmission of nerve impulses ➤ Regulates permeability of cell membranes ➤ Regulates uptake of nutrients	➤ Gills ➤ Fins ➤ Skin
Zinc	➤ Component of nearly 80 metalloenzymes ➤ Acts as a cofactor for enzymes involved in protein synthesis ➤ Required for the development and function of many immune cells ➤ Important for the structure and stability of proteins.	➤ Gastro-intestinal tract ➤ Gills ➤ Fins ➤ Skin
Copper	➤ Essential component of various oxidation-reduction enzyme systems ➤ Involved in the metabolism of melanin	➤ Gastro-intestinal tract ➤ Gills ➤ Fins ➤ Skin

1.4 WHITE SPOT SYNDROME VIRUS (WSSV)

White Spot Syndrome Virus (WSSV) is a highly infectious viral disease that affects many species of shrimp, including the Pacific white shrimp (*Litopenaeus*

vannamei) and black tiger shrimp (*Penaeus monodon*) and results in higher levels of mortality. WSSV is a large, double-stranded DNA virus that belongs to the Nimaviridae family (Leu., 2008).

1. WSSV MORPHOLOGY

White Spot Syndrome Virus (WSSV) is a large, enveloped virus that belongs to the Nimaviridae family. The virus has a complex morphology with an icosahedral shape and a diameter of about 100-200 nm. It consists of a nucleocapsid, which is composed of linear double-stranded DNA (dsDNA) that is about 300 kilobases long, surrounded by a protein shell called the capsid. The capsid is composed of 162 capsomeres arranged in a T=169 icosahedral lattice, and each capsomere is made up of three proteins: VP19, VP24, and VP26.

The nucleocapsid is surrounded by a lipid envelope that is derived from the host cell membrane during virus assembly. The envelope contains several viral glycoproteins, including VP28 and VP19, which are essential for virus entry and attachment to host cells. Additionally, the envelope contains several other structural and non-structural proteins, such as VP24, VP26, VP31, and VP36 (Escobedo-Bonilla et al., 2008).

2. WSSV CLASSIFICATION

Family: Nimaviridae

Genus: Whispovirus

The complex morphology of WSSV is a significant factor in its ability to infect and cause disease in a wide range of crustaceans, including shrimp and crayfish. The virus has a high mutation rate, which allows it to evolve rapidly and adapt to new host species, making it a significant threat to the global shrimp aquaculture industry. (Han-Ching Wang et al., 2019).

1.5 VP28 PROTEIN

VP28 is a major envelope protein of the White Spot Syndrome Virus (WSSV), which is a highly pathogenic virus that infects and causes mortality in various shrimp

species. VP28 is a highly conserved glycoprotein that plays a crucial role in the pathogenesis of the virus and the development of host immunity against WSSV. (Guohua Yi., 2004).

6. BIOINFORMATICS

Bioinformatics is a field that combines biology, computer science, and statistics to analyze and interpret biological data, particularly large and complex datasets such as those generated by genomics, proteomics, and metabolomics studies. The field of bioinformatics involves the development and application of computational methods and tools for organizing, analyzing, and interpreting biological data. This includes the development of algorithms and databases for storing and retrieving biological information, as well as the use of statistical models and machine learning techniques to extract insights from biological data (Luscombe et al., 2001).

7. MOLECULAR DOCKING

Molecular docking is a computational method used to predict the preferred orientation and conformation of a ligand molecule (such as a drug or small molecule) when it binds to a receptor molecule (such as a protein). It is a valuable tool in drug discovery and development, as it can be used to identify potential drug candidates and optimize their binding to the target protein.

Molecular docking typically involves two steps: the generation of the conformations of the ligand molecules and the calculation of the binding affinity between the ligand and the receptor. The binding affinity is calculated using energy scoring functions, which evaluates the interaction between the ligand and receptor. (Garrett M. Morris and Marguerita Lim-Wilby., 2008).

8. NANOPARTICLES

Nanoparticles are particles that have a size range of 1-100 nanometers in at least one dimension. They can be made from a variety of materials such as metals, semiconductors, polymers, and ceramics. Due to their small size, nanoparticles often exhibit unique properties and behaviors that are different from their bulk counterparts (Hongjun You et al., 2013).

CHAPTER 2

2. REVIEW OF LITERATURE

1. SHRIMP PRODUCTION

While shrimp production is a significant contributor to global food security and economic growth, it is also associated with significant environmental challenges. These challenges include deforestation, destruction of mangrove forests, water pollution, and the introduction of non-native species that can adversely affect local ecosystems. It is to be noted that high-density farming of shrimp often leads to the overuse of antibiotics and other chemicals, resulting in the development of antibiotic-resistant bacteria and other health issues. Therefore, it is important to implement sustainable shrimp farming practices that minimize environmental damage and maintain the long-term viability of shrimp farming. The various mitigation strategies include the use of alternative feeds and the implementation of closed recirculating systems, which can reduce the environmental impact of shrimp farming (Páez-Osuna, 2001).

2. PROBLEMS IN SHRIMP AQUACULTURE AND THE WAYS TO COMBAT

The rapid growth of shrimp farming has led to a variety of environmental problems, including water pollution, habitat destruction, and disease outbreaks. The most effective way to address these issues is through the development and implementation of sustainable practices that minimize the environmental footprint of shrimp farming. The improvements such as the use of more efficient feed, the reduction of chemical usage, and the implementation of best management practices to minimize the impact of waste discharge on local ecosystems can be made. (Boyd and Clay, 1998).

The rapid expansion of shrimp farming in India has led to several negative impacts on the environment, including the destruction of mangrove forests, pollution of water bodies, and the degradation of soil quality. It causes economic and social impacts of shrimp farming on local communities, including land-use conflicts and displacement of traditional fishermen. Adoption of more sustainable aquaculture practices, such as

integrated multi-trophic aquaculture contributes to minimizing the environmental impact of shrimp farming (Jayanthi et al., 2018).

Shrimp farming has contributed significantly to the economic development of coastal communities, improving the income and livelihoods of many individuals. The industry has also created job opportunities for people with different levels of education and skills, reducing unemployment and poverty in the region. Shrimp farming has improved the social status of farmers and workers, providing them with a sense of pride and professional satisfaction. Moreover, it has enhanced gender equality, empowering women to engage in shrimp farming activities and earn an income. There are also several challenges faced by the shrimp farming industry, including environmental degradation, land conflicts, and the use of child labor (Ray et al., 2021)

3. NUTRITION REQUIREMENT OF SHRIMPS

Shrimps require a balanced diet that provides essential nutrients for their growth and development. The key nutrients that are required for shrimp growth include proteins, lipids, carbohydrates, vitamins, and minerals. Proteins are particularly important for muscle development and repair, while lipids are needed for energy storage and cellular membrane structure. Carbohydrates are the primary source of energy, while vitamins and minerals are required for various physiological functions (Molina-Poveda., 2016).

4. VIRAL PATHOGENS

Viral pathogens are infectious agents that cause diseases in a wide range of organisms, including humans, animals, plants, and even bacteria. They are microscopic in size and consist of a small piece of genetic material (DNA or RNA) surrounded by a protein coat. Viral pathogens require a host cell to replicate and spread, and they can cause a range of diseases from mild infections like the common cold to severe illnesses like HIV, Ebola, and COVID-19. Some common examples of viral pathogens that affect humans include influenza, herpes, hepatitis, measles, mumps, rubella, and Zika. The study of viral pathogens and their effects on organisms is known as virology, and research in this field is critical for the development of effective

vaccines, antiviral therapies, and public health strategies to prevent the spread of viral diseases (Knipe and Howley., 2013)

While many viruses have evolved mechanisms to evade or suppress the host immune response, the immune system is still capable of mounting effective responses against viral infections. The development and implementation of vaccines are critical tools in generating immunity to viral pathogens (Hickman and Suthar., 2018).

1. VIRAL PATHOGENS AFFECTING SHRIMPS

Viral diseases are a significant threat to the shrimp aquaculture industry, which is a major source of seafood worldwide. Over the past few decades, the industry has experienced several outbreaks of viral diseases that have caused significant economic losses. The most notable viral diseases that affect shrimp culture include:

- a. White Spot Syndrome Virus (WSSV): This virus causes white spots on the carapace, lethargy, and ultimately death. WSSV has been responsible for massive mortalities in shrimp farms worldwide and is considered the most significant viral disease affecting the industry.
- b. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV): This virus causes damage to the shrimp's cuticle, resulting in necrosis and discoloration. While IHHNV does not typically cause high mortality rates, it can weaken shrimp and make them more susceptible to other infections.
- c. Taura Syndrome Virus (TSV): This virus causes lethargy, anorexia, and a reduction in growth rates. TSV has been responsible for significant economic losses in shrimp farming operations in the Americas.
- d. Yellow Head Virus (YHV): This virus causes yellowing of the cephalothorax, lethargy, and mortality rates up to 100 % in infected populations (Tandel et al., 2017)

5. WHITE SPOT SYNDROME VIRUS (WSSV)

White spot syndrome virus (WSSV) is a highly infectious pathogen that affects crustaceans, particularly shrimp and prawns. WSSV belongs to the family Nimaviridae and is a large, double-stranded DNA virus with a genome size of approximately 300 kilobases (Escobedo-Bonilla et al., 2008).

WSSV infects and replicates in a variety of cells, including those in the lymphoid organ, hepatopancreas, and gills of the host. Infected animals show a range of symptoms, including lethargy, loss of appetite, and the formation of white spots on the exoskeleton. Mortality rates can be very high, and in some cases, entire populations of shrimp and prawns have been wiped out by the virus (Sanchez-Paz., 2010).

1. ENVELOPE PROTEINS OF WSSV

WSSV is a major pathogen of shrimp that causes significant economic losses in the aquaculture industry. The envelope proteins of WSSV play important roles in virus entry and assembly, making them attractive targets for antiviral strategies. Antisera against six WSSV envelope proteins used in neutralization assays showed that the virus infection could be significantly delayed or neutralized by antibodies against three WSSV envelope proteins (VP68, VP281 and VP466) (Wu et al., 2005)

White Spot Syndrome Virus (WSSV) has several envelope proteins, but the major ones are VP24, VP26, VP28, VP31, VP37, and VP466. Among these, VP28 is the most abundant and important envelope protein in terms of its role in the pathogenesis of the virus and the development of host immunity against WSSV. It has been shown to be an essential component of the virus and plays a crucial role in the initial attachment and penetration of the virus into shrimp cells. VP28 has also been extensively studied as a target for antiviral strategies to control WSSV infection in shrimp (Huang., 2002; Zhou et al., 2009).

2.6 VP28 PROTEIN

WSSV is a serious and highly infectious disease that affects penaeid shrimps, causing significant economic losses in the aquaculture industry. VP28 is a protein that is found on the surface of the WSSV virus and is crucial for the virus's ability to infect

shrimp cells. VP28 is considered a vital envelope protein indispensable for triumphing viral infection. As such, it has been proposed as a potential target for developing control strategies to combat WSSV (Sivakumar et al., 2020).

7. STRATEGIES TO COMBAT WSSV

The emergence of WSSV as a significant pathogen has had a significant impact on the global shrimp farming industry, which is worth billions of dollars annually. In response, the industry has implemented a range of measures to try to control the spread of the virus, including improved biosecurity protocols, genetic selection for disease resistance, and the use of antiviral treatments (Sanchez-Paz., 2010)

Shrimp acclimated to salinity levels of 15 and 28 psu had significantly longer survival times compared to those maintained in low (5 psu) or high (54 psu) salinity. Shrimp survival can be split into two groups based on salinity, with some dying faster in extreme salinities and others exhibiting a significantly longer survival time in water with salinity close to the iso-osmotic point (777.5 mOsmol/kg). The results suggest that maintaining water salinity close to the iso-osmotic point may be beneficial for the survival of shrimp infected with WSSV (Ramos-Carren˜o et al., 2014).

1. ANTI-VIRAL STRATEGIES TO COMBAT WSSV

siRNA targeting the WSSV envelope protein gene reduced viral replication and mortality rates in infected shrimp. RNA interference (RNAi) has the ability to specifically silence genes of the White Spot Syndrome Virus (WSSV) and could be a potential new therapy for controlling WSSV infection in shrimp (Xu et al., 2007).

Polyamine CQDs (Carbon Quantum Dots) were synthesized using a hydrothermal method. The *Litopenaeus vannamei* shrimps were divided into three groups: a control group treated with PBS, a group treated with the CQDs, a group infected with WSSV, and a group infected with WSSV and treated with the CQDs. The survival rate, viral load, and immune response of the shrimp in each group was monitored. The results showed that the CQDs had a significant antiviral effect against WSSV in *Litopenaeus vannamei*. The group treated with the CQDs had a higher survival rate and a lower viral load compared to the group infected with WSSV but not

treated with the CQDs. Additionally, the CQDs were found to stimulate many innate downstream immune responses, including haemolymph clotting mechanism, antioxidant defence mechanism, antimicrobial peptide system, and antimicrobial immune responses (Huang., 2020).

2.7.2 ANTI-VIRAL STRATEGIES TO COMBAT WSSV BY FOCUSING VP28 PROTEIN

The study aimed to investigate the role of the envelope protein VP28 in WSSV infection of shrimp by examining the inactivation of WSSV by normal rabbit serum. The study used a combination of in vitro and in vivo experiments to examine the effect of normal rabbit serum on WSSV infection of shrimp. The results showed that normal rabbit serum can effectively inactivate WSSV and prevent its infection of shrimp. Further analysis showed that the inactivation of WSSV by normal rabbit serum was due to the presence of antibodies that recognize the envelope protein VP28. This suggests that VP28 plays an important role in the WSSV infection of shrimp. The study also demonstrated that pre-treating shrimp with normal rabbit serum can significantly reduce WSSV infection, providing a potential therapeutic strategy for controlling WSSV infections in shrimp (Robalino., 2006)

The development of an antiviral strategy against white spot syndrome virus (WSSV) by expressing VP28-specific small interfering RNA (siRNA) in bacteria is discussed. The study aimed to evaluate the protective effects of VP28-siRNA against WSSV infection in shrimp. The researchers first designed and synthesized VP28-specific siRNA and then cloned it into an expression vector. The recombinant plasmid was then transformed into *E. coli* BL21 (DE3) for protein expression. The expressed VP28-siRNA was purified and tested for its antiviral activity against WSSV in shrimp. Results showed that the expressed VP28-siRNA in bacteria exhibited significant antiviral activity against WSSV in shrimp. The shrimp injected with VP28-siRNA exhibited lower mortality rates and higher survival rates compared to the control group. The study suggests that the expression of VP28-specific siRNA in bacteria can effectively protect shrimp against WSSV infection (Zhu and Zhang., 2011).

VP28 is a major structural protein of WSSV and a potential target for vaccine development. The protective efficacy of a multimeric recombinant VP28 (mrVP28) as a vaccine against WSSV in shrimp was evaluated. It was expressed that mrVP28 in *E. coli* and purified it using a nickel-chelating column. The purified mrVP28 was used to immunize shrimp via injection or oral administration. The immunized shrimp were then challenged with WSSV and the protective efficacy was evaluated by measuring the survival rate and viral load in the shrimp. Both injection and oral administration of mrVP28 induced high levels of protection against WSSV infection in shrimp. Specifically, the survival rate of the immunized shrimp was significantly higher than that of the control group, and the viral load was significantly lower. The protective efficacy was dose-dependent, and the highest dose of mrVP28 resulted in 100% survival of the shrimp. mrVP28 could be developed as a practical and cost-effective vaccine for preventing WSSV infection in shrimp farming (Taengchaiyaphum et al., 2017).

A hemocyanin-derived peptide named LvHcL48 was identified and characterized by *L. vannamei* hemocyanin. The peptide was shown to have antiviral properties against WSSV in vitro by inhibiting virus replication in *L. vannamei* primary hemocytes. The in vivo experiment was conducted by injecting *L. vannamei* with the hemocyanin-derived peptide before challenging them with WSSV. The results showed that pre-treatment with the peptide significantly reduced WSSV replication in the shrimp, indicating that the peptide has the potential as a means of controlling WSSV. The transcription of WSSV genes was inhibited by LvHcL48 which also decreased the copy number of WSSV. Through experimental analysis, LvHcL48 was studied to attenuate WSSV replication due to its interaction with VP28. Results, therefore, indicate that hemocyanin does not only generate AMPs but could also produce different peptides upon different microbial infections, suggesting some specificity in the innate immune response of shrimp (Shixiong Zhan., 2018).

2.7.3 *IN-SILICO* STUDIES FOCUSING ON THE CONTROL OF WSSV BY TARGETTING VP28 PROTEIN

The study aimed to identify and localize a WSSV gene that encodes an envelope protein, which could help in developing diagnostic and therapeutic methods for WSSV infections. Immuno-electron microscopy with gold-labelled antibody revealed that the gold particles were dispensed in the outer envelope of WSSV virions and showed that vp28 encodes a virus envelope protein. The results showed that VP28 is a major envelope protein of WSSV and is highly expressed during viral infection. It was also found that VP28 is localized in the outer envelope of WSSV virions, which suggests its importance in viral entry and infection. The study further demonstrated that VP28 is highly conserved among WSSV isolates, which could make it a potential target for the development of vaccines and antiviral agents (Xiaobo Zhang., 2002).

The study aimed to investigate the interaction between two major envelope proteins of WSSV, VP24 and VP28, and their role in viral entry and infection. Co-immunoprecipitation was used to identify and characterize the interaction between VP24 and VP28. The results showed that VP24 interacts with VP28 to form a stable complex that is critical for viral entry and infection. The disruption of the VP24-VP28 interaction using specific antibodies or RNA interference may significantly reduce WSSV infection, indicating that the interaction is a potential target for the development of antiviral agents (Xie and Yang., 2006).

VP26 and VP28 make up a significant portion of the WSSV envelope and play a crucial role in viral infection. The localization and crystal structure of VP26 and VP28 represent the first two structural envelope proteins of WSSV. Both VP26 and VP28 were found to adopt a β -barrel architecture with a protruding N-terminal region. The localization of these proteins was investigated using immunoelectron microscopy and observed them as surface protrusions on the outer surface of the virus. It was suggested that the predicted N-terminal transmembrane region of these proteins may anchor on the viral envelope membrane, allowing the core β -barrel to protrude outside the envelope and interact with the host receptor or fuse with the host cell membrane for effective viral infection. The structural comparison of VP26 and VP28 with other viral

proteins revealed an evolutionary relationship between WSSV envelope proteins and structural proteins from other viruses. The findings have the potential to extend further toward drug and vaccine development against WSSV and other viruses with similar structural viral proteins (Tang et al., 2007).

Four envelope proteins (VP28, VP26, VP24, and VP19) were found to interact with each other to form a stable complex that is critical for viral entry and infection. Upon analyzing the interactions among the four proteins, five pairwise protein interactions and two self-associations were identified. It was also found that the complex is localized in the outer envelope of WSSV virions, suggesting its importance in the early stages of viral infection. The multi-protein complex is also expected to shed light on the structural organization of WSSV (Qing Zhou., 2009).

White spot syndrome virus has a complex architecture with numerous structural proteins forming a membrane protein complex (MPC) that is essential for viral replication and assembly. Cryo-electron microscopy and X-ray crystallography are used to obtain high-resolution structures of the individual structural proteins of the MPC. Computational methods were used to construct a 3D model of the entire MPC. It was found that the WSSV MPC is composed of seven structural proteins, including VP24, VP26, VP28, VP32, VP33, VP36, and VP39. The MPC was found to consist of three distinct layers, with VP24, VP28, and VP36 forming the outer layer, VP26 and VP32 forming the middle layer, and VP33 and VP39 forming the inner layer. The findings provide insights into the mechanisms of viral replication and may lead to the development of new strategies for controlling WSSV infection in shrimp aquaculture (Chang et al., 2010).

A shrimp receptor protein PmRab7 interacts with viral envelop protein VP28 to form the PmRab7–VP28 complex, which causes the instigation of WSSV. Computer simulation and docking studies were used to gain insight into the interaction and its underlying molecular mechanisms. The study found that the PmRab7-VP28 complex plays an important role in the infection of shrimp by WSSV. Specific amino acid residues crucial for the interaction between PmRab7 and VP28 were identified and disrupting this interaction has been recognized to be a potential therapeutic target for

controlling WSSV infection in shrimp. The results also suggested that the binding affinity between PmRab7 and VP28 is higher than that between PmRab7 and other viral proteins, which indicates the importance of the PmRab7-VP28 complex in the WSSV infection process (Verma et al., 2012).

Protein-protein interactions (PPIs) play a key role in viral replication and pathogenesis, and therefore, identifying the potential WSSV-host PPIs is an important area of research. The study aimed to predict the potential PPIs between WSSV and its host by using bioinformatics approaches. The study predicted 99 potential WSSV-host PPIs, involving 38 viral proteins and 45 host proteins. The predicted PPIs included several host proteins that are known to be involved in immune response, signal transduction, and cytoskeletal organization, indicating the potential role of WSSV in manipulating the host cellular processes. The predicted PPIs also included several viral proteins that are known to be involved in viral replication and pathogenesis. Additionally, the study identified several host proteins that were predicted to interact with viral proteins that are essential for viral replication and pathogenesis, suggesting their potential as targets for developing antiviral therapies (Sun et al., 2014).

388 marine bioactive compounds were screened against the envelope protein VP28 by employing flexible molecular docking, and MD simulations and the lead molecules were taken based on the MM/PBSA binding free energy (DG_b). Their results proposed that two compounds, Astropectenol B and Lindgomycin showed the highest binding free energies with -40.453 KJ/mol and -31.031 KJ/mol, respectively (Sivakumar et al., 2016).

Since WSSV is a major threat to shrimp farming and effective treatments are still unavailable, inhibitory peptides targeting envelope proteins are a promising strategy for antiviral therapy. The Wimley-White interfacial hydrophobicity scale (WWIHS) was used in combination with structural data to identify potential inhibitory peptides that could target VP28. Two potential inhibitory peptides were found, one of which exhibited not only antiviral activity against WSSV but also broad-spectrum antimicrobial activity. The identified inhibitory peptide can serve as a lead compound for the development of anti-WSSV strategies. The study highlights the potential of using computational

approaches to identify potential inhibitory peptides that can be used as therapeutic targets against viral infections like WSSV (Lu et al., 2019). Identification of small molecule inhibitors of VP28 through molecular docking and molecular dynamics simulation studies is considered as an impending approach in drug development and design against WSSV (Sivakumar et al., 2020).

2.8 ROLE OF TRANSITION METAL IONS

Metal ions play a crucial role in many biological processes, including the structure and function of viruses. The several metal ions that interact with viral proteins include zinc, iron, copper, and magnesium. These metal ions can affect the stability and activity of viral proteins. These metal ions can interact with viral proteins through specific coordination bonds, such as histidine coordination, as well as through nonspecific electrostatic interactions. Metal ions can be used to inhibit viral replication by disrupting metal-dependent viral enzymes or by competing with viral proteins for metal ion binding sites (Chaturvedi and Shrivastava., 2004).

Metal ions play important roles in the physiology of bacterial cells, including the regulation of gene expression, enzymatic activity, and structural stability. In bacterial pathogens, metal ions have been shown to play key roles in virulence and survival in host environments. On the roles of metal ions in bacterial virulence and viability, focusing on the major metal ions involved in bacterial physiology, including iron, zinc, manganese, copper, and nickel. It was found that metal ions are essential for the growth and survival of bacterial pathogens and that their acquisition and utilization are tightly regulated by the bacteria. Metal ions could contribute to bacterial virulence by facilitating adhesion to host cells, evasion of the host immune system, and the production of virulence factors such as toxins and proteases (Begg., 2018).

Zinc ions (Zn^{2+}) play an important role in the replication and pathogenesis of many viruses. Zn^{2+} ions have been shown to have antiviral effects against some viruses, while other viruses have developed mechanisms to evade the antiviral effects of Zn^{2+} . Zn^{2+} ions can inhibit viral replication by blocking viral entry, genome replication, and protein processing. Zn^{2+} ions have been shown to bind to viral proteins and enzymes, inhibiting their activity and disrupting the viral replication cycle. Additionally,

Zn_2^+ ions can induce the expression of interferon-stimulated genes (ISGs) in host cells, which can inhibit viral replication and stimulate the immune response. The role of Zn_2^+ ions in viral pathogenesis is complex and depends on the specific virus and host cell. Zn_2^+ ions have both antiviral and proviral effects, and the development of effective antiviral therapies targeting Zn_2^+ ions require a better understanding of the mechanisms underlying their interactions with viruses (Tsuneo Ishida., 2019).

The effect of dietary organic zinc on the expression of zinc signaling-related genes and proteins, as well as the growth, immune response, and antioxidant capacity in juvenile Pacific white shrimp was studied. Results showed that dietary organic zinc significantly increased the expression of zinc signaling-related genes and proteins in the shrimp. It also promoted growth, immune response, and antioxidant capacity in the shrimp. The expression of genes related to growth and immune response, as well as antioxidant enzymes, were significantly up-regulated in the shrimp fed with organic zinc. The dietary organic zinc can modulate zinc signaling and promote growth, immune response, and antioxidant capacity in juvenile Pacific white shrimp. The use of dietary organic zinc in the aquaculture industry improves shrimp health and productivity (Shi et al., 2021).

Copper is involved in the shrimp's immune response. Copper-containing enzymes, such as lysyl oxidase (LOX), are involved in the synthesis and maintenance of connective tissue, which is important for wound healing and tissue repair. The mRNA level of LoxL was upregulated in the hemocytes and intestines of shrimp challenged with white spot syndrome virus (WSSV) or bacteria. Further, the knockdown of LoxL via RNA interference led to an increase in WSSV replication and bacterial loads, and a decrease in shrimp survival rate, suggesting that LOXL functions against pathogen infection in shrimp. LOXL can be a potential target for the development of therapies to enhance the immune response of shrimp and reduce the impact of pathogen infections in shrimp farming (Lu PY., 2022).

2.9 METAL ION-PROTEIN INTERACTION

Metal ions play crucial roles in structural stabilization, catalysis, electron transfer, and signaling in proteins. The various structural motifs that facilitate metal ion binding in proteins include metal-binding loops, metal-binding sites, and metal-binding domains. Metal ions can be coordinated by amino acid side chains, backbone atoms, and water molecules. The factors that influence metal ion binding specificity and affinity, include the number and identity of metal-binding ligands, the pH and ionic strength of the environment, and the presence of competing ligands. The role of electrostatics in metal ion binding includes the effect of charge distribution and dipole moments. The diverse functions of metal ions in proteins include enzymatic catalysis, electron transfer and the importance of metal ion binding in disease and drug design (Yamashita et al., 1990).

Protein-metal affinity has a central role in a variety of applications such as protein partner identification, protein characterization, phosphoprotein analysis and fractionation, protein purity assessment, protein folding evaluation, and site-specific protein immobilization (Ueda et al., 2002).

Proteins often bind to metal ions to carry out specific biological functions. Magnesium (Mg), calcium (Ca), and zinc (Zn) are three important metal ions that play crucial roles in biological systems. The binding and selectivity of Mg, Ca, and Zn in proteins are determined by a complex interplay between the coordination number and geometry of the metal ion, the nature and number of ligands, and the local environment of the metal ion in the protein binding site. Therefore, it guides in designing metal-binding proteins with specific functions and selectivities, and in the development of novel therapeutics targeting metal-dependent biological processes (Dudev and Lim., 2003).

Metal ions play crucial roles in many biological processes, including enzyme catalysis, signal transduction, and DNA replication. Proteins bind metal ions with high specificity, and the selectivity of metal ion binding is critical for proper protein function. A combination of computational and experimental methods is used to analyze the binding of different metal ions to several proteins. The study found that the competition

among metal ions for protein binding sites is influenced by several factors, including the metal ion size, charge, and coordination geometry. Thus, metal ions with similar size and coordination geometry tend to compete strongly for binding sites, while metal ions with different sizes and geometries can bind to the same site without competition. It showed that different metal ions can have different effects on enzyme activity, depending on their ability to coordinate with specific amino acid residues in the protein active site (Todor Dudev et al., 2013).

The prion protein (PrP) is a cell surface glycoprotein that is involved in several biological functions. In its misfolded form, PrP is responsible for the development of prion diseases, which are fatal neurodegenerative disorders. Copper ions are known to interact with PrP and influence its physiological and pathological functions. The binding of copper to PrP has been shown to induce conformational changes in the protein structure that can affect its stability, aggregation propensity, and neurotoxicity. Copper ions can induce structural changes in PrP that are necessary for its physiological functions, such as binding to other proteins and lipid membranes (Giulia Salzano., 2019).

Protein-ligand interactions play a crucial role in many biological processes, including drug discovery and development (Schoning-Stierand et al., 2020). The binding of transition metal ions to proteins plays a crucial role in various biological processes, such as catalysis, signal transduction, and gene regulation. The thermodynamics of transition metal ion binding to proteins is highly dependent on both the metal ion and the protein. Cu (II) and Ni (II) ions showed the strongest binding to both BSA and cytochrome c, while Co (II) and Zn (II) showed weaker binding. It was also found that the binding of transition metal ions to proteins can be influenced by the presence of other metal ions. The findings suggest that the thermodynamics of these binding events are highly dependent on both the metal ion and the protein, and can be influenced by a range of environmental factors. It can therefore aid in the design of new therapeutic strategies (Song et al., 2020).

10. MOLECULAR DOCKING

Molecular docking is a computational method used to predict the binding modes of ligands to proteins, and it can be used to predict the interactions between metal ions immobilized ligands and proteins in affinity chromatography. Molecular docking was used to predict the binding modes of metal ion immobilized ligands to proteins in affinity chromatography. Nickel, cobalt, and zinc were the metal ions used for immobilizing the ligands. The study presented the results of molecular docking simulations of metal ion immobilized ligands to several proteins, including lysozyme, human serum albumin, and bovine serum albumin. It was also shown that the binding modes of the ligands to the proteins were dependent on the metal ion used for immobilization and the specific amino acid residues involved in coordination chemistry. The study also demonstrated the usefulness of molecular docking in the design and optimization of metal ion immobilized ligands for affinity chromatography (Salha et al., 2020).

11. BIOINFORMATICS TOOLS

Bioinformatic tools are software programs, algorithms, or web-based applications that are specifically designed to analyze, process, and interpret biological data. These tools are used to manage, analyze, and visualize large biological datasets, such as DNA and protein sequences, gene expression data, protein structures, and metabolic pathways.

1. PDB (PROTEIN DATA BANK)

The Protein Data Bank (PDB) is a database that provides access to three-dimensional structures of biological macromolecules, such as proteins, nucleic acids, and complex assemblies. The PDB contains over 170,000 structures of biological macromolecules, with new structures being added on a regular basis. These structures are determined experimentally using techniques such as X-ray crystallography, NMR spectroscopy, and electron microscopy (Burley., 2017).

2. RCSB-PDB

The RCSB Protein Data Bank (RCSB PDB) is one of the three organizations that maintain the Protein Data Bank (PDB) and is in the United States. The RCSB PDB

provides a range of tools and resources for accessing and analyzing three-dimensional structures of biological macromolecules, including proteins, nucleic acids, and complexes. In addition to searching for structures, the RCSB PDB website also provides a range of analysis and visualization tools (Kouranav., 2006).

3. UNIPROT DATABASE

UniProt (Universal Protein Resource) is a comprehensive database of protein sequences and functional information. UniProt collects and curates information from multiple sources, including scientific literature, genome sequencing projects, and other protein databases. The database contains information on proteins from all types of organisms, from bacteria to humans. UniProt provides a range of tools for searching and analyzing protein data, including keyword and sequence searches, BLAST searches, and advanced query features (The UniProt Consortium., 2014).

4. AVOGADRO

Avogadro is a free, open-source molecular visualization and modelling software designed for computational chemistry, molecular modelling, and simulations. It is used to build, visualize, and analyze molecules using a variety of computational tools and methods. Avogadro provides a user-friendly interface that allows users to build molecules using a simple drag-and-drop interface. It also includes a wide range of features, such as optimization of molecular geometries, calculation of molecular properties, and visualization of molecular orbitals and electron densities (Hanwell., 2012).

5. AUTODOCK

AutoDock is a widely used software tool for molecular docking, a computational method used to predict the binding affinity and orientation of small molecules (ligands) to a receptor. It can be used to model the interaction between ligands and protein targets, enabling the discovery of potential drug candidates. AutoDock uses a combination of genetic algorithms and empirical scoring functions to predict the binding energies and conformations of ligands within a binding pocket of a protein. AutoDock is widely used in drug discovery and design, as it enables researchers to model the

binding of small molecules to proteins, providing insights into the molecular basis of drug-receptor interactions. It is also used in other fields, such as protein engineering and the study of protein-protein interactions (Sandro Cosconati., 2010).

6. CHIMERA

Chimera is a widely used software tool for the interactive visualization and analysis of molecular structures. Chimera allows users to visualize and analyze molecular structures in 3D, including proteins, nucleic acids, and other biomolecules. It includes a range of tools for manipulating and analyzing structures, such as measuring distances and angles, calculating electrostatic potentials, and generating molecular surfaces. One of the key features of Chimera is its ability to display large molecular complexes and to create high-quality images and animations for presentations and publications (Pettersen., 2004).

7. LIGPLOT+

LIGPLOT+ is a software package used for generating schematic diagrams of protein-ligand interactions. LIGPLOT+ is widely used in the field of structural biology to visualize and analyze the interactions between proteins and ligands. The software generates 2D diagrams that show the interactions between the protein and the ligand in a clear and informative way. The diagrams include information on hydrogen bonds, hydrophobic interactions, salt bridges, and other types of interactions between the protein and ligand (Laskowski and Swindells., 2011).

2.12 APPLICATIONS OF BIOINFORMATICS

Bioinformatics plays a key role in genomics research, which involves the study of the complete set of DNA sequences (genome) of an organism. It helps in the analysis of genome sequences, identifying genes, predicting their functions, and understanding the genetic basis of diseases (David Stephen Horner., 2009).

Bioinformatics is also used in proteomics research, which involves the study of the complete set of proteins (proteomes) in an organism. It helps in the identification and characterization of proteins, predicting their structures and functions, and understanding their interactions (Blueggel., 2004).

Bioinformatics is used in drug discovery research to identify potential drug targets, design new drugs, and optimize drug efficacy and safety. It helps in the analysis of large-scale genomic and proteomic data to identify disease-related targets and design drugs that specifically target these targets (Xia., 2017).

Personalized medicine involves tailoring medical treatment to individual patients based on their genetic and environmental information. Bioinformatics is used in personalized medicine to develop personalized treatment plans based on an individual's genetic and molecular information. It helps in the analysis of genomic and proteomic data to identify disease-related biomarkers and design personalized treatment strategies (Fernald et al., 2011).

13. METAL NANOPARTICLES

Metal nanoparticles are a type of nanoparticle that is composed of metals, such as gold, silver, platinum, copper, and others. They typically have a size range of 1-100 nanometers in at least one dimension and exhibit unique properties and behaviors due to their small size and high surface area-to-volume ratio. Metal nanoparticles have been extensively studied and applied in various fields, including biomedical, environmental, and materials sciences (Jhamkande et al., 2019).

1. APPLICATIONS OF METAL NANOPARTICLES

Metal nanoparticles can be used as carriers for drug delivery to specific target sites. They can be functionalized with targeting ligands or therapeutic agents to improve their selectivity and efficacy. Metal nanoparticles can also help protect the drugs from degradation and improve their solubility and bioavailability (Sánchez-López et al., 2020)

Metal nanoparticles can be used as biosensors for the detection of biomolecules or pathogens. They can be functionalized with specific ligands or antibodies to bind to the target molecules, and their optical or electrical properties can be used to detect the binding events (Tavakkoli Yaraki et al., 2020).

CHAPTER 3

3. AIM AND OBJECTIVES

1. AIM OF THE STUDY

- To identify the binding of nanoscale micronutrients (zinc, calcium and copper) with VP28 protein against WSSV.

2. OBJECTIVES OF THE STUDY

- To perform molecular docking of nanoscale micronutrients (Zn, Ca, Cu) with the structural VP28 protein.
- To examine and identify the binding energy of nanoscale micronutrients with VP28 protein in controlling the viral activity of WSSV.

CHAPTER 4

4. MATERIALS AND METHODS

MOLECULAR DOCKING STUDIES

1. LIGAND PREPARATION

To study the binding affinities of Calcium carbonate, Zinc oxide and Copper nanoparticles to the target protein VP28, Autodock 4.0 was chosen. Their 2D coordinates were retrieved from PubChem in SDF/Mol format.

Using OpenBabel GUI, the metal compounds were converted to Autodock standard ligand format (pdbqt) and their 3D coordinates were generated for docking analysis (O'Boyle et al., 2011).

The Calcium carbonate, Zinc oxide and Copper (crystal structure) were built from Avogadro open-source software (<http://avogadro.cc/>). Both the forms (single molecule of Calcium carbonate, Zinc oxide and Copper) were compared for their binding with the VP28 protein.

2. PROTEIN PREPARATION

The three-dimensional X-ray crystal structure of VP28 was retrieved from Protein Data Bank [PDB ID:2ED6 (<http://www.rcsb.org/pdb/home/home.do>)].

The crystal structure with a reasonable resolution was taken for molecular docking studies.

The protein structure was prepared by removing the water molecules and heteroatoms initially.

The A chain containing the active site was then chosen by deleting the remaining chains in the protein structure obtained from PDB (<https://pubchem.ncbi.nlm.nih.gov/>).

The OPLS force field energy minimization step was used to refine the loop structure of the protein.

The Protein was then converted to pdbqt format by adding polar Hydrogen, Kollman Charge, and by computing Gasteiger.

By setting map types as the metal ions, a grid box was generated in the protein active site for each iteration using AutoGrid4.

The number of grid points in the xyz coordinates of the grid box was set to 60x60x60, and the spacing in Armstrong(Å) was set to 0.375Å.

The AD4 parameter file was used for setting the default parameters for running AutoGrid.

The grid file is saved in gpf format that contains the parameters for running AutoGrid. After launching AutoGrid, it generated the output in glg format along with the maps.

4.3 LIGAND-RECEPTOR INTERACTION

The interaction between the target protein and the ligands was analyzed with the molecular docking method using AutoDock tools (Morris et al., 2009).

The AutoDock application is launched after generating the grid map by setting the macromolecule as the rigid filename and choosing the ligands.

The Lamarckian Genetic Algorithm was used for the docking analysis. The number of eval was set to 250000 with the default docking parameter and the output file is saved with the ligand in dpf format.

The AutoDock application was run with the dpf file and generated the output in dlg format. The output was analyzed based on the conformation ranking by the least binding energy value.

CHAPTER 5

5. RESULTS AND DISCUSSION

5.1 DOCKING ANALYSIS

Initially, the residues in the binding site were defined in the grid box. The Calcium carbonate, Zinc oxide and Copper nanoparticles were docked to the binding pocket of VP28 by a flexible sampling method ensuring not more than 50 rotatable bonds and 300 atoms. The docked complex which satisfies the minimum energy criterion was picked for further analysis. The metal-protein interaction profile was depicted using Chimera (Pettersen et al., 2004) given in Figures 5.2.1, 5.3.1 and 5.4.1. The molecular docking of the target protein VP28 with the Calcium carbonate, Zinc oxide and Copper nanoparticles shows a best binding energy of -6.2 kcal/mol, -8.2kcal/mol and -8.1kcal/mol respectively. The ligand Calcium carbonate nanoparticles formed two interactions with the amino acid residues SER74 and ASP75. The ligand Zinc oxide nanoparticles formed three interactions with the amino acid residues THR201, HIS195 and SER199. The ligand Copper nanoparticles formed three interactions with amino acid residues ASN154, MET139 and MET137. The results are shown as a 3-d cartoon representation of the ligand and the target. Binding energy values and the non-bond interactions are elaborated on in Tables 5.2.1, 5.3.1 and 5.4.1. The binding mode of Calcium carbonate, Zinc oxide and Copper nanoparticles with protein complex implies the effective sharing of hydrogen bonds and pi-stacking with the binding site residues. The Calcium carbonate nanoparticles exhibit a strong h-bond interaction with VP28 amino acid residue ASP75 with a contact distance of 2.70Å°. The Zinc oxide nanoparticles exhibit a strong h-bond interaction with VP28 amino acid residue HIS195 with a contact distance of 3.34Å°. The Copper nanoparticles exhibit a strong h-bond interaction with VP28 amino acid residue MET139 with a contact distance of 3.34Å°. The Calcium carbonate and Zinc oxide nanoparticles form a hydrophobic interaction with GLU50 and THR 197 & THR135 residues respectively. The detailed interactions are represented in Figures 5.2.2, 5.3.2 and 5.4.2.

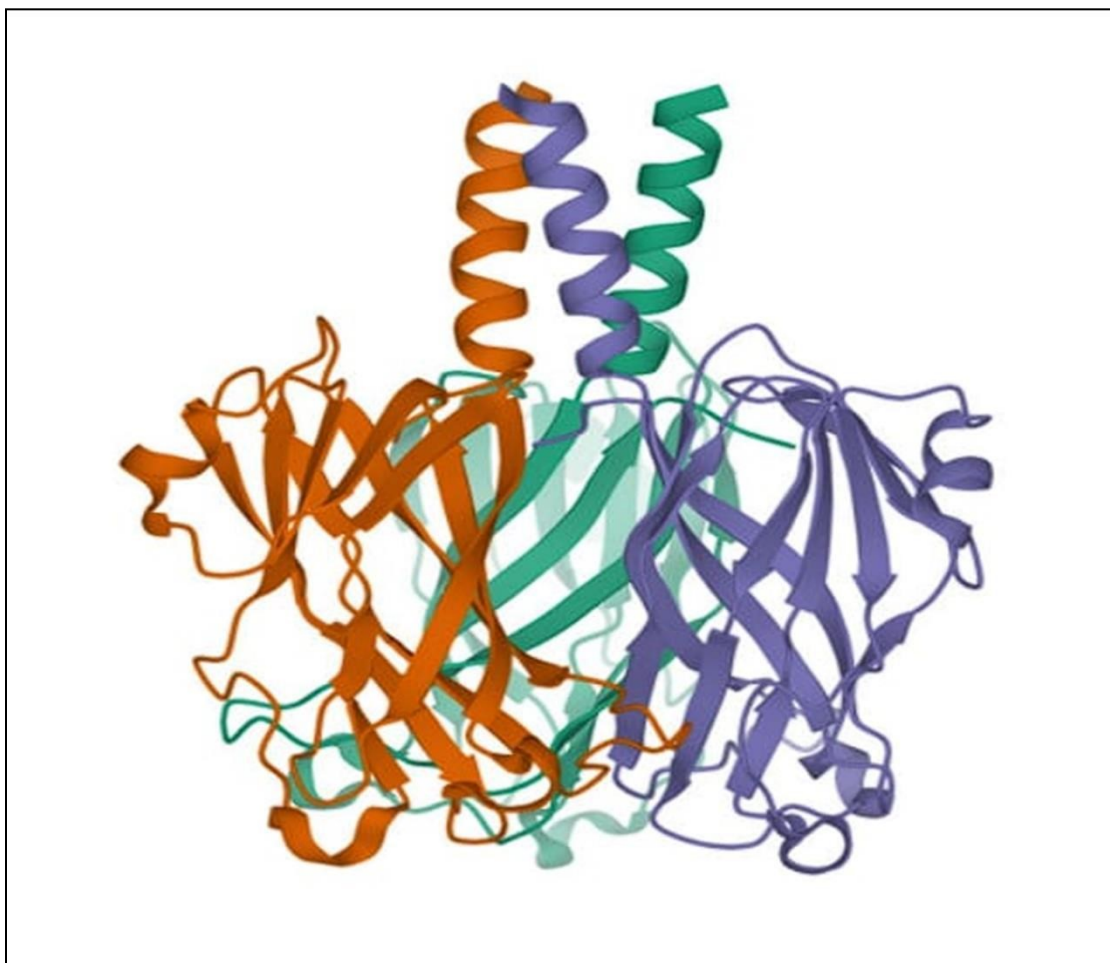


Fig 5.1 Crystal structure of VP28 protein

5.2 DOCKING CALCIUM CARBONATE WITH VP28 PROTEIN

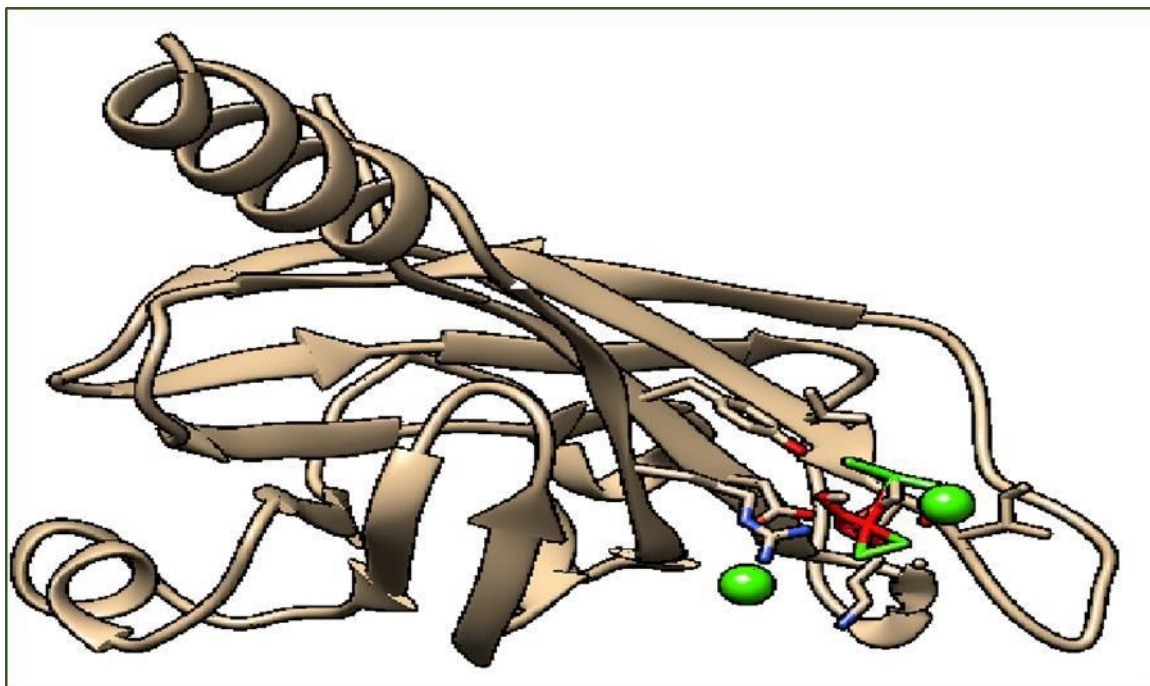


Fig 5.2.1 Docked complex of VP28 Protein with Calcium carbonate nanoparticles

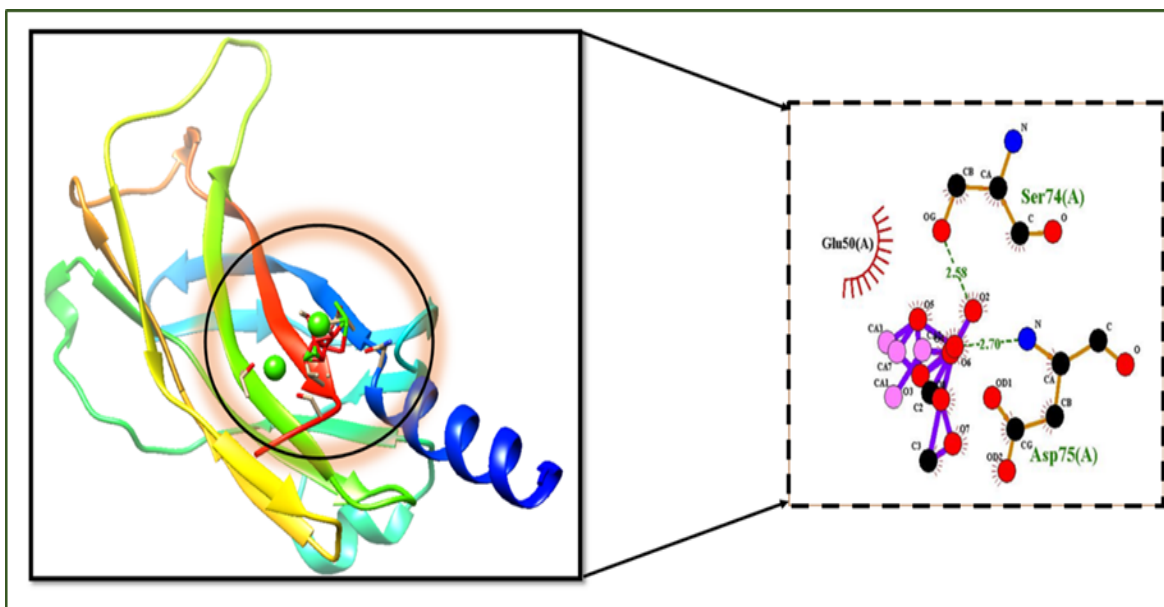


Fig 5.2.2 Hydrogen bond interaction of Calcium carbonate nanoparticles with VP28 protein

5.2.1 Table: Docking result of Calcium carbonate nanoparticles with the VP28 protein

DOCKED COMPLEX	BINDING ENERGY (kcal/mol)
<i>Complex 1</i>	-6.2
<i>Complex 2</i>	-6.0
<i>Complex 3</i>	-5.5
<i>Complex 4</i>	-4.2
<i>Complex 5</i>	-3.1

5.3 DOCKING ZINC OXIDE WITH VP28 PROTEIN

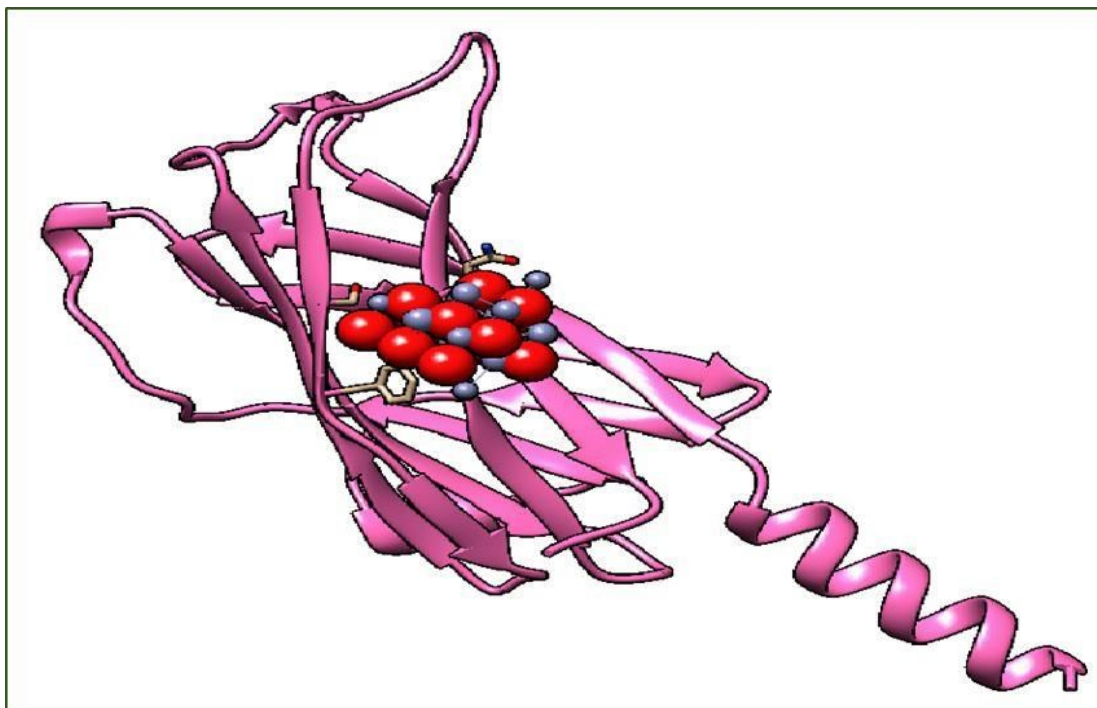


Fig 5.3.1 Docked complex of VP28 Protein with Zinc oxide nanoparticles

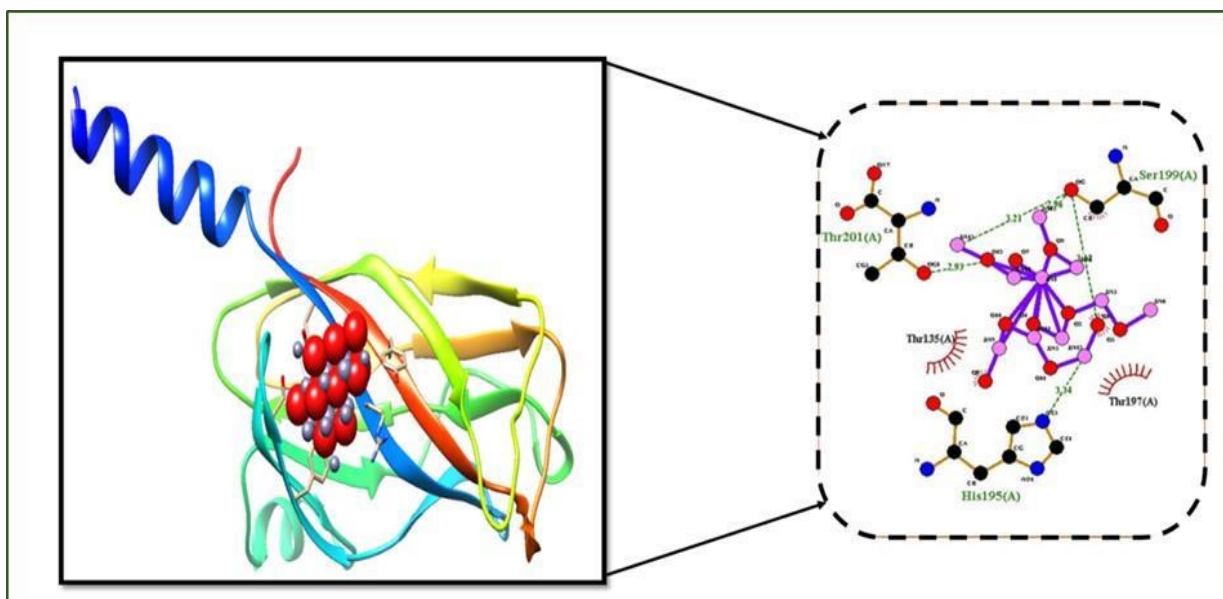


Fig 5.3.2 Hydrogen bond interaction of Zinc oxide nanoparticles with VP28 protein

5.3.1 Table: Docking result of Zinc oxide nanoparticles with the VP28 protein

DOCKED COMPLEX	BINDING ENERGY (kcal/mol)
<i>Complex 1</i>	-8.2
<i>Complex 2</i>	-8.0
<i>Complex 3</i>	-7.5
<i>Complex 4</i>	-6.2
<i>Complex 5</i>	-4.1

5.4 DOCKING COPPER WITH VP28 PROTEIN



Fig 5.4.1 Docked complex of VP28 Protein with Copper nanoparticles

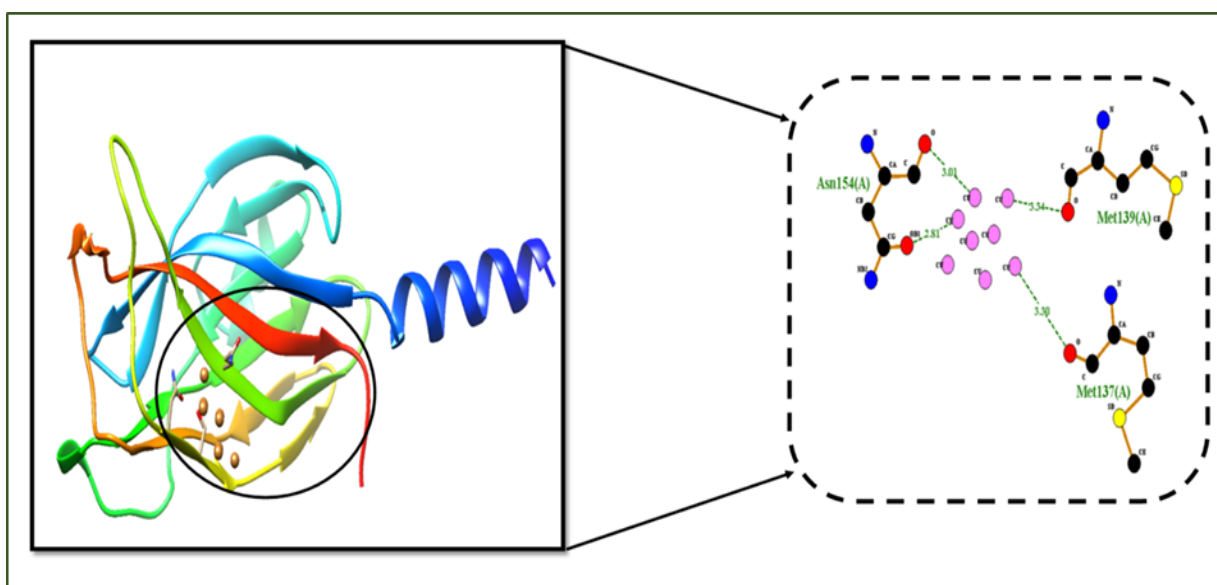


Fig 5.3.2 Hydrogen bond interaction of Copper nanoparticles with VP28 protein

5.4.1 Table: Docking result of Copper nanoparticles with the VP28 protein

DOCKED COMPLEX	BINDING ENERGY (kcal/mol)
<i>Complex 1</i>	-8.1
<i>Complex 2</i>	-7.9
<i>Complex 3</i>	-7.5
<i>Complex 4</i>	-7.2
<i>Complex 5</i>	-6.8

Zinc oxide nanoparticles (ZnO NPs) have been extensively studied for their potential applications in various fields, including antimicrobial agents, rubber materials, food additives, cosmetics products, and photocatalysts. In terms of their interaction with biological macromolecules, ZnO NPs have been shown to bind to subdomain IIIA of BSA and human insulin with binding energies of -5.44 and -1.56 kcal/mol, respectively (Giannousi et al., 2020). However, other studies have suggested that IB and IIA subdomains may also be potential binding regions for ZnO NPs (Ledesma et al., 2017).

Additionally, ZnO NPs can alter the expression of ICAM-1, IL-8 (interleukin 8), IL-1B (interleukin 1 beta), P-38, NF-kB, CCL18, and CD35 upon binding with chemokine and certain cellular proteins. Of these, P38 has the highest binding affinity with ZnO NPs with an energy of -8.81 kcal/mol, while IL-1B has the lowest binding energy of -3.23 kcal/mol (A. S. Abdelsattar et al., 2020). These findings suggest that ZnO NPs have the potential for a wide range of biomedical applications due to their binding interactions with biological macromolecules.

CHAPTER 6

6. SUMMARY AND CONCLUSION

The study aimed to investigate the binding affinities of three metal nanoparticles (CaCO₃, ZnO, and Copper) to the VP28 protein using molecular docking analysis. The ligands were prepared using Autodock standard ligand format (pdbqt) and their 3D coordinates were generated using OpenBabel GUI for docking analysis. The crystal structures of the metal nanoparticles were built from Avogadro software. The protein structure of the target protein VP28 was obtained from the Protein Data Bank (PDB ID: 2ED6) and prepared by removing water molecules and heteroatoms. The OPLS force field energy minimization step was used to refine the loop structure of the protein. The protein was then converted to pdbqt format by adding polar Hydrogen, Kollman Charge, and by computing Gasteiger. Grid maps were generated for each iteration in the protein active site using AutoGrid4, and the Lamarckian Genetic Algorithm was used for the docking analysis. The docking results were analyzed based on the conformation ranking by the least binding energy value. The lower the binding energy value, the stronger the binding affinity between the ligand and protein. Typically, the binding energy of a docked ligand is compared with the binding energy of other docked ligands, with the goal of identifying the ligand with the highest binding affinity for the protein target. In addition to the binding energy value, factors such as the location and orientation of the docked ligand within the protein's active site, the presence of hydrogen bonds and other interactions between the ligand and protein, and the physical and chemical properties of the ligand that may affect its ability to bind to the protein are also considered.

CONCLUSION

The results of this study will provide valuable insights into the mechanism of action of nanoscale micronutrients against WSSV and potentially aid in the development of designing new antiviral agents that target the VP28 protein of WSSV and could be used to control WSSV infections in aquaculture. This project represents a significant contribution to the field of aquaculture and could have important

implications for the development of sustainable and environmentally responsible practices in the industry.

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