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In Silico Docking Studies of Ag Nanoparticles and its Derivatives Against NS5B Protein (HCV): Integrating Nanobiotechnology and Nanoinformatics

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Abstract

The present study focused on the prediction of interactions of three ligands, i.e., silver nanoparticles, tyrosine-capped silver nanoparticles, and silver oxide nanoparticles, with the nonstructural protein 5B (NS5B) protein of the hepatitis-C virus (HCV). AutoDock 4, Discovery Studio, ChemDraw Ultra, OpenBabel, and Chimera software were used. Computational docking helps to evaluate the conformations of small ligands attached to macromolecular proteins. NS5B plays a crucial role in HCV replication. It is an RNA-dependent RNA membrane-associated polymerase, weighing approximately 66-kDa. The results were obtained from AutoDock 4 and visualized in Discovery Studio and Chimera. Silver nanoparticles showed interactions with Lysine-81 (LYS81), Lysine-172 (LYS172), Lysine-173 (LYS173), Tyrosine-176 (TYR176), and Aspartate-177 (ASP177). Tyrosine capped silver nanoparticles formed bonds with Serine-218 (SER218), Aspartate-220 (ASP220), Glutamate-357 (GLU357), and Leucine-362 (LEU362) in the palm region. Silver oxide nanoparticles interacted with Leucine-260 (LEU260), Tyrosine-261 (TYR261), Arginine-280 (ARG280) and Alanine-281 (ALA281) in the finger domain. All these three ligands showed promising results to inhibit the NS5B enzyme preventing HCV replication. The most effective ligand was tyrosine-capped silver nanoparticles. Its relative highest binding energy i.e. -5.29 kcal/mol showed its intense binding with the protein molecule causing more damage to the integral residues forming the active site.

Keywords: Silver nanoparticles, Nanoinformatics, Docking, NS5B, HCV Replication.

1. Introduction:

Nanotechnology refers to the synthesis of nanomaterials (1–100 nm) and their applications. From 1 nm to 100 nm, a particle exhibits new and different behaviour due to quantum effects. Currently, chemical, physical, and, most preferably, biological synthesis of nanoparticles is being carried out frequently. Owing to the dynamic

properties of nanomaterials, nanotechnology has played an important role in the field of medicine, incorporating subfields of nanomedicine, nanoengineering, nanobiotechnology, and nanoparticle-based vaccine development.[1-3].

Metal nanoparticles like gold (Au), silver (Ag), and platinum (Pt) have gained extensive consideration in the past few years because of their crucial and

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mechanical intrigue [4]. They can impressively change biological and physicochemical characteristics as they have high electrical properties, increased tolerance to mechanical and thermal pressure, a high surface area, and high physical and magnetic properties [5,6]. These unique characteristics have enabled nanomaterials to be used in different fields, including medical, magnetic, physical, and electronic devices. Investigations and diagnosis are facilitated by the use of simple and engineered nanomaterials in medical equipment and procedures such as diagnostic kits, imaging, magnetic resonance imaging (MRI), and drug delivery [7]. In the light of nanotechnology, the field of biomedical industry and nanomedicine has been a point of intense consideration for efficient and quick diagnosis and creating various methods of therapies utilising nanoparticles in various diagnostic gadgets [8]. Ag, Pt, and Au nanoparticles are considered noble nanoparticles. These nanoparticles exhibited nontoxic positive effects in biological systems, revealing a new dimension of exploration in biological research [9]. Some engineered nanoparticles, such as titanium dioxide (TiO_2), zinc oxide (ZnO), ferrous oxide (FeO), cupric oxide (CuO), silver oxide (Ag_2O) and aluminium oxide (Al_2O_3) also have antimicrobial properties and perform noteworthy activities in numerous medical applications. TiO_2 for instance, is used to inhibit the spread of various diseases [10-12]. Silver nanoparticles have exhibited great efficacy in combating microbial agents [13].

Because of their exceptional properties, silver nanoparticles have been utilised for many applications, including as anti-viral and antibacterial agents. They are being used in healthcare products, beauty care products, the food industry, pharmaceutical industries, and medical and electronic devices [14]. The biological characteristics of silver nanoparticles depend on various parameters [15]. In systematic and local administration, the bioavailability of therapeutic agents gets improved because of the physicochemical properties of the nanoparticles

[16]. Silver nanoparticles produced by using the berry extract of *Sea Buckthorn*, display a broad range of antioxidant, anti-inflammatory and anticancer activities [17]. AgNPs are proven to be safe antibacterial and antibiofilm compounds against MDR *K. pneumonia* [18]. Silver nanoparticles produced by *Sphingobium* sp. MAH-11 may act as an intense antimicrobial agent in many treatments [19]. Hence, the synthesis of silver nanoparticles in a controlled manner is useful in several biomedical applications [20].

Nanoparticles, especially silver, have antiviral activities against many viruses that are ruining lives worldwide. Biological data are being produced at an extraordinary rate [21]. Because of this exponential growth of information, computers have turned out to be irreplaceable for biological research. Such an approach is perfect due to the simplicity with which computers can deal with a huge amount of information [22]. The utilization of computational systems to comprehend and sort out the data related to biological macromolecules is known as bioinformatics. In recent times, an agreement has started to rise about the informatics foundation expected to accumulate, curate, and share data among every one of the partners in nanotechnology [23]. The inconstancy of nanomaterials makes risk assessment unrealistic. Easily accessible data and artificial intelligence approaches are necessary to guarantee consumer wellbeing [24]. A more effective way is required by utilizing nanobiotechnology, nanoengineering and nanoinformatics for efficient and broad sharing of data related to nanotechnology.

Nanobiotechnology involves designing, fabricating, modulating, and using nanomaterials, such as nanoparticles and nanocarriers, for drug delivery systems. It combines techniques from nanotechnology, biology, pharmacology, and physics to develop novel nanomaterials and devices for improved efficiency and applications. Nanoparticles, nanotubes, and nanofibers are used in drug delivery systems, imaging, antimicrobial and anticancer therapies, and in-vitro diagnostics [25]. Silver (Ag) has also been used in various

applications of nanobiotechnology. It is a valuable element in drug development and biological assays due to its affinity for microbial cells and antimicrobial activities. Its nanomaterials have been used in catheters, wound dressings, orthopaedic devices, and dental implants[26].

Nanoengineering uses nanomaterial interactions to create functional nanostructures, relying on precise organization to achieve unique functionality. Nanoengineering has significantly contributed to recent biomedicine advancements, as it provides access to unique properties in biomaterials that traditional techniques cannot. This approach significantly improves the performance and functionalities of biomaterials, enriching their overall functionality [26,17]. Nanoengineering has been used in silver (Ag) nanoparticles. Researchers have explored the use of silver-based engineered nanomaterials for innovative point-of-use disinfection systems for drinking water purification, which have proven to be highly effective in treating contaminated water. The scientific community has also used nanoengineering for environmentally friendly silver nanoparticles (AgNPs) due to their potential applications in environmental pollution detection and water quality monitoring[28,29].

Nanoinformatics is depicted as "the science regarding figuring out which data is important to the nanoscale science and, after that, creating and implementing systems for gathering, approving, sharing, analyzing, modeling, and applying that information." It is an emerging science that encompasses databases and tools. Some of them are Nanomaterial Biological Interactions Knowledge Base, InterNano, Nanoparticle Information Library, etc., and nowadays they are being used as nanomedicines and found to have high efficiency in molecular docking. In drug discovery, docking is a critical computational technique for predicting protein-ligand interactions. The two fundamental characteristics of docking programmes are docking precision and scoring reliability [30]. Docking accuracy demonstrates how similar the predicted ligand is to the experimental data, whereas scoring

reliability positions ligands because of their affinities. Docking accuracy evaluates the searching algorithm and scoring reliability assesses scoring functions. In the docking program, the numerous searching algorithms work differently for randomness, speed, and the area covered. Many searching algorithms show good performance when used against the known structure. Presently, numerous sorts of docking programs are easily accessible, among which, AutoDock is frequently used and openly accessible [31]. As protein-nanoparticle interactions are not easy to examine utilizing experimental techniques, molecular docking tools facilitate ease of this difficulty.

Currently, medication and immunisation advancements for the evacuation of different viral ailments are under critical consideration, and various viral strains have been developed that are no more sensitive to drugs and vaccines. So, it is imperative to present multidisciplinary approaches with established epidemiology alongside the clinical phases to present a new drug or vaccine that possesses great effectiveness against the resistant strain.

Viral hepatitis, a global health issue, has gained recognition due to WHO elimination strategies. Hepatitis C virus (HCV) is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, classified as a distinct entity in 1989 [32]. Around 71 million people worldwide suffer from HCV, a bloodborne viral infection, with no vaccine available, and chronic infection occurs in 80% of those exposed [33]. The hepatitis C virus is included in the family Flaviviridae. It has a +RNA single strand (Choo 1989). The HCV genome contains roughly 9,600 nucleotides, which encode 3,000 amino acid residues for polyprotein precursors. About 170 million people are carriers of HCV around the world. A significant number of these people are waiting to suffer from critical HCV-related liver diseases. NS5B stands for the nonstructural 5B protein present in HCV. It weighs about 66 kDa. It is an RNA-dependent, membrane-associated polymerase. [34]. It takes part in RNA replication; however, the exact molecular

mechanism is not completely known yet. The RNA replication process is comprised of two phases. In the first phase, the formation of a new RNA strand starts at the 3' end of the RNA template. This initiation phase does not need a primer to start; therefore, it can be called a primer-independent or de novo mechanism. The hydroxyl group at the 3' position of the first NTP forms a bond with the new-coming NTPs. In the second phase, elongation occurs by adding more complementary NTPs. The repetitive cases of hepatitis C virus (HCV) infection cause more than 71 million people to face chronic stages that result in different liver diseases. A deeper understanding of HCV revealed vital proteins that are important for HCV survival and enabled the scientists to target them to make HCV therapy more efficient [35,36]. Antiviral drugs are designed to directly act on three important HCV functional proteins, i.e., NS5B polymerase, NS5A, and NS3 [30]. The present HCV therapy using ledipasvir, ombitasvir, and sofosbuvir has some adverse effects like anemia, rash, bilirubin, nausea,

pruritus, and photosensitivity [37].

The increase in liver diseases, along with their adverse effects, demand improved treatment. This article focuses on the docking of different derivatives of silver nanoparticles to look into alternative, safe, and highly efficient HCV therapy methods.

2. Material & Methods:

2.1 NS5B Structure:

NS5B has three structural domains denoted as fingers (residues 1 to 187 and 228 to 286), palm (residues 188 to 227 and 287 to 370), and thumb (residues 371 to 563). Its catalytic site contains residues ranging from 214 to 332. At the enzymatic molecular surface, there is a site in a pocket specific for the binding of the rGTP molecule. This specific site is at a distance of 30 Å from the catalytic site. It is situated at the junction of the fingers and thumb domains, regulating enzymatic activity allosterically. (Table 1)

Table 1: Motifs and functional regions of NS5B proteins.

Conserved Elements	Residues		Location	Function
Motifs	A	216-227	Palm	Binds Magnesium and choose nucleic acid type.
	B	287-306		Differentiate between rNTP or dNTP
	C	312-325		Coordinates Magnesium
	D	332-353		Helps accommodate active site NTPs
	E	354-372		Retains rigidness of secondary structure important for spatial arrangement of thumb and palm domains
	F	132-162	Fingers	Binds incoming NTPs and RNA
	G	95-99		Binds primer and template
Functional Regions	I	91-94		Binds template
	II	168-183	Thumb	Binds template
	III	401-14		Binds nascent RNA duplex

2.2 Retrieval of NS5B 3D Structure from Protein Data Bank:

Protein Data Bank (PDB) was built up as the first freely available repository for biological molecules in 1971. It was a single worldwide library for 3-

dimensional structures of biomolecules and their complexes with other small molecules. Presently, the PDB archive contains ~167518 entries (as of August 2020). The PDB repository contains data obtained through three techniques: X-ray crystallography, nuclear magnetic resonance

spectroscopy (NMR), and electron microscopy [38]. The 3D structure of the HCV-encoded nonstructural 5B (NS5B) protein with the 2HWH

identity number was taken from the PDB website. Figure 1 shows the structure of the NS5B protein of HCV in ribbon form.

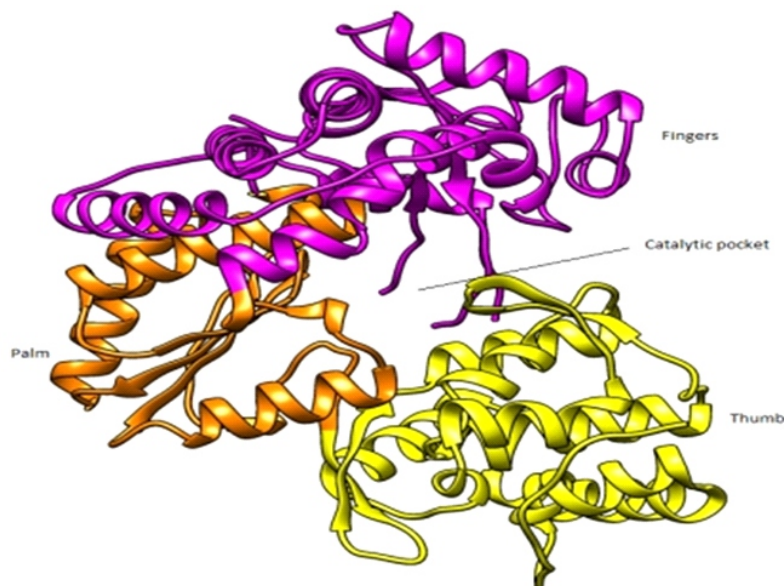


Figure 1: Presentation in ribbon form of NS5B protein of HCV.

2.3 Deleting Water Molecules:

Water molecules present in the NS5B structure were deleted using AutoDock tools because many of the water molecules present in the protein structure are either loosely bound and easily displaced by a ligand or not necessarily quite in the positions they appear to be. Fitting water molecules to the residual electron density after the protein structure is fitted is an inexact science (Figure 2). In most cases, water molecules are not involved in the

binding. That's why they are preferably removed to ease computations and clear water molecules present in a catalytic pocket so that the pose searching process would not be disturbed. In docking, there is a search for molecules that can create multiple favourable contacts with the protein; water molecules might confound this procedure. As a result, the wrong conformation pose is obtained as the ligand forms more solvent-assisted salt bridge interactions.

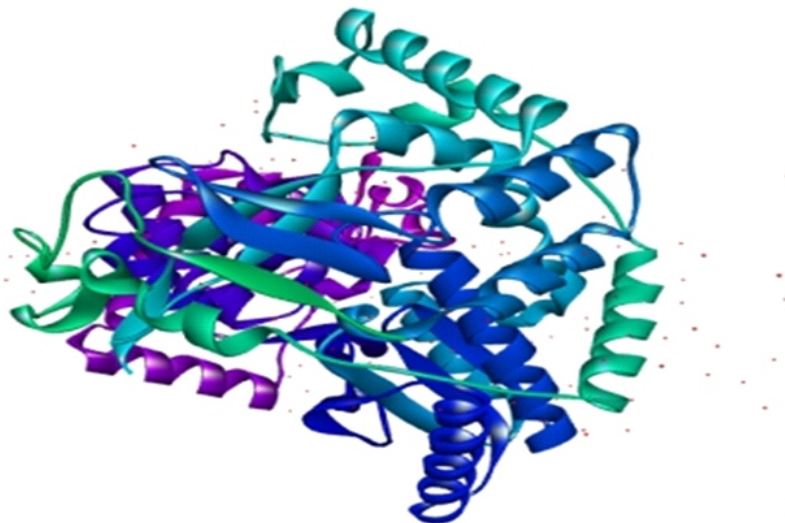


Figure 2: Water molecules are represented as red dots.

2.4 Adding Hydrogen Atoms:

Protein-ligand or protein-protein complexes are virtually observed in molecular docking simulations. Macromolecules are present in a charged form with no atom missing in the human or animal body. So, it is necessary and sensible to add charges and missing atoms (hydrogen and, in some cases, non-hydrogen) to proteins before proceeding with a docking experiment. Most macromolecular structure data do not contain hydrogen atoms in their corresponding PDB files, and docking software requires the hydrogen atoms to be in place to compute algorithmic calculations. So, the addition of hydrogen atoms is necessary for docking. The polar hydrogen atoms allow the establishment of hydrogen bonds that may be present between the macromolecule and the ligands tested. Hence, the missing hydrogen atoms were added to the NS5B protein.

2.5 Compute Gasteiger Charge:

To get important and useful outcomes from any electrostatic calculations, designating suitable atomic partial charges for ligands and macromolecules is necessary. Marsili-Gasteiger partial charges are appointed employing a two-phase algorithm. First, each atom in a molecule is designated with seed charges. Then, some of these initial charges are transferred from one atom to the other bonded atom. The direction of the movement of partial charges depends on the electronegativity difference between two bonded atoms. With each cycle of the repeating algorithm, attenuation of charges occurs. Gasteiger partial charges of 17.0062 were added to the NS5B protein in the AutoDock tool.

2.6 Energy Minimization:

Computational chemistry depicts energy minimization as the process of searching for a pattern in space where atoms are gathered, the total inter-atomic force on every atom is near zero, and the potential energy surface (PES) is a static point. This searching mechanism during the energy minimization process is based on some computational models of chemical bonding. Energy minimization is essentially about "settling" the mind in a relatively energetically favourable state.

Protein structures often have errors of various magnitudes, such as atoms partially spanning, side chains in the long proteins, etc. Energy minimization lies in the pathway that gives the most reduction in the overall energy of the system, relaxing lengths, angles, non-binned interactions, etc. in most preferred states.

2.7 Retrieval of Ligand 3D Structures:

Silver nanoparticles, tyrosine-capped silver nanoparticles, and silver oxide nanoparticles were used as ligands in molecular docking. The structure of silver, tyrosine-capped silver, and silver oxide nanoparticles was drawn in Chemdraw Ultra 12.0 software. Chemdraw Ultra software is used to draw a nearly unlimited variety of biological and chemical drawings.

2.8 Preparing Grid Parameter File (GPF):

The grid box centre was assigned to an active site cavity in NS5B protein with 1 angstrom spacing at each grid point. The x, y, and z coordinates for grid points were 1.874, -0.603, and 27.509, respectively. In this way, the whole protein molecule was covered within a grid box to allow free rotation of the ligand molecule within the protein. (Figure 3). All the grid-related information was saved as a grid parameter file (gpf). The gpf determines the search space in the receptor.

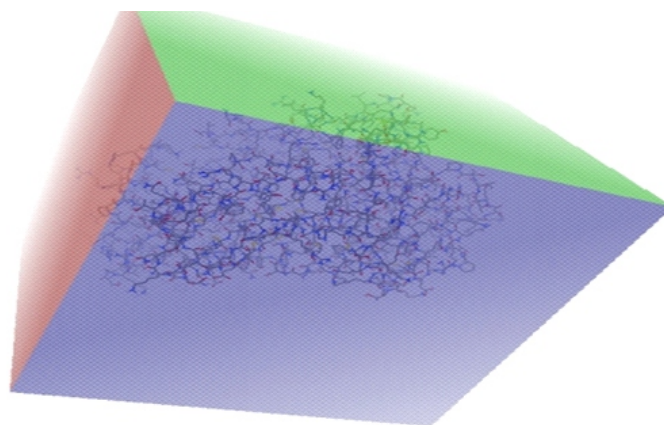


Figure 3: Protein embedded in the Grid box.

2.9 Running Autogrid4:

The autogrid was run by using a grid parameter file (gpf) (Figure 4). Autogrid4 gets information about the receptor around which potential is to be computed, map types to be figured out, and the extent and location of those maps from the grid

parameter file –[39]. The probe atom is employed in 3D space at regular points to pre-calculate the energy in the receptor. These pre-calculated energies get stored as grid maps. Each type of atom contains its grid map. Besides, electrostatic and desolvation maps are also generated. In this way, in a ligand molecule, every type of atom is subjected to a rapid evaluation of energy to precalculate its affinity potentials[40].

2.10 Preparing Docking Parameter File (DPF):

In docking parameters, a genetic algorithm with default settings was selected to be used during the docking process. The docking parameter file reveals AutoDock about the utilisation of map files, movement of the ligand molecule, center, torsions, beginning of the ligand, movement of the flexible residues in the receptor if modelling of the side chain motion is required, type of algorithm to employ, and its iteration (Figure 5). It contains the file extension ".dpf." –[41].

2.11 Running AutoDock4:

AutoDock runs search algorithms using grid maps

to determine ligand-protein binding at each point and find suitable conformations. Subsequently, numerous docked conformations are acquired. AutoDock needs grid maps for each type of ligand atom evaluated by AutoGrid, a ligand PDBQT file, and a docking parameter file that determines the parameters for the docking[42].

Presently, AutoDock contains four distinct algorithms, i.e., the original MonteCarlo simulated annealing (SA), a traditional Darwinian genetic algorithm (GA), local search (LS) and a hybrid genetic algorithm with local search (GALS). The Lamarckian Genetic Algorithm performs a highly effective search –[43].

2.12 Result Analysis:

The results obtained from AutoDock were observed and analyzed in Chimera and Discovery Studio Visualizer version 17.2.0.16349.

3. Results:

NS5B contains three domains named palm, fingers, and thumb (Figure 4). The palm includes residues from 188 to 227 and 287 to 370; the finger includes residues from 1 to 187 and 228 to 286, and the thumb contains residues from 371 to 563 [35].

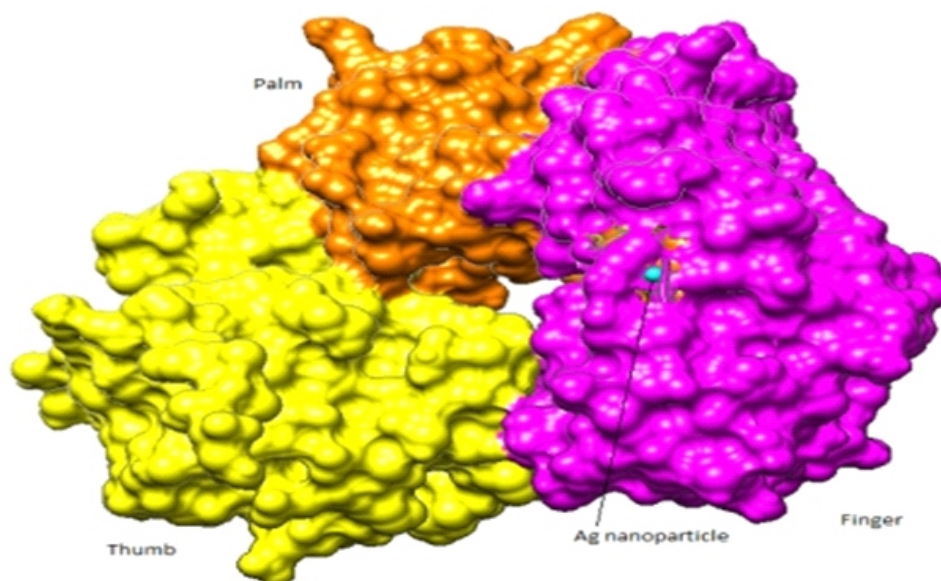


Figure 4: Silver nanoparticle is embedded in the finger domain.

3.1 Ag NP-NS5B Protein Interaction:

Silver nanoparticles interacted with the finger domain of the NS5B protein. Five amino acid residues, i.e., LYS81, LYS172, LYS173, TYR176, and ASP177, showed interaction with Ag nanoparticles. LYS81 was involved in metallic bonding with Ag nanoparticles. LYS81 is one of the amino acids to which RNA binds. Hence, Ag nanoparticles can cause hindrances in the attachment of RNA to LYS81. Amino acid residues from 168–183 in the finger domain also help to bind template RNA [41]. Interaction of Ag nanoparticles with LYS172, Methionine-173 (MET173), TYR176, and ASP177 can inhibit the template RNA from binding with the finger domain (Figure 5).

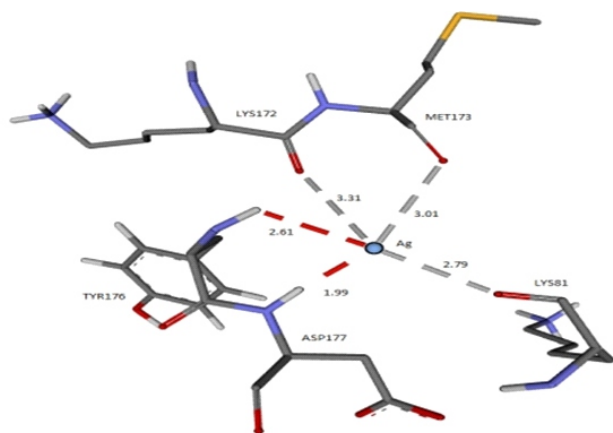


Figure 5: Ag nanoparticle interaction with NS5B residues.

3.2 Tyrosine Capped Ag NP-NS5B Protein Interaction:

Tyrosine-capped silver nanoparticles were found to have hydrogen bonding with SER218, ASP220, GLU357, and LEU362. The ligand molecule has three hydrogen bonds (green) with SER218 with bond lengths of 2.14 Å, 2.36 Å, and 2.51 Å. While ASP220 and GLU357 are interacting with the ligand through a single hydrogen bond with 2.23 Å and 1.80 Å distances, respectively. Furthermore, there is a metallic interaction of 3.06 Å with LEU362 (Figure 6).

Tyrosine-capped silver nanoparticles interacted with the palm region of the NS5B protein (Figure 7). The site where the ligand is attached to four amino

acid residues is a catalytic site. ASP220 and SER218 are part of motif A, and GLU357 and LEU362 are included in the motif E region [] (Figure 6). ASP220 plays a role in coordinating magnesium ions to assist in nucleotide addition during the RNA elongation process. While motif E helps to maintain the relative positioning of the thumb and palm domains []. The ligand molecule can inhibit ASP220 from binding with magnesium ions, thereby stopping the addition of new nucleotides during RNA synthesis. It may also distort the ability of motif A to select the type of nucleic acid that needs to undergo the polymerization process. Furthermore, it may deform the motif E, and hence the spatial arrangement of the thumb and palm domains can be disturbed. As the ligand molecule is present within the catalytic pocket, it may cause a hindrance for the RNA molecule that is being synthesized. In this way, tyrosine-capped silver nanoparticles can act as an inhibitor for the NS5B protein to stop the replication of HCV.

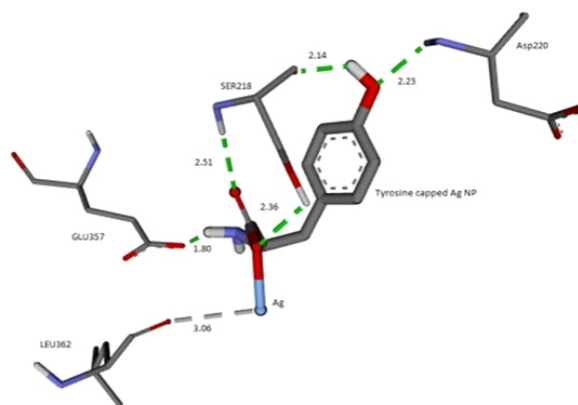


Figure 6: Residual interaction of NS5B protein with Tyrosine capped Ag NP.



Figure 7: Molecular docking of Tyrosine capped Ag NP to palm domain of NS5B protein.

3.3 Silver Oxide NP-NS5B Protein Interaction:

The oxygen atom of the ligand molecule showed three hydrogen bonds with TYR261, ARG280, and ALA281 with distances of 3.15 Å, 3.64 Å, and 1.98 Å, respectively. Additionally, one silver atom of the ligand molecule was involved in charge repulsion with ARG280 at 2.33 Å distance, and the other silver atom was involved in metallic interaction with LEU260 at 3.33 Å distance (Figure 8).

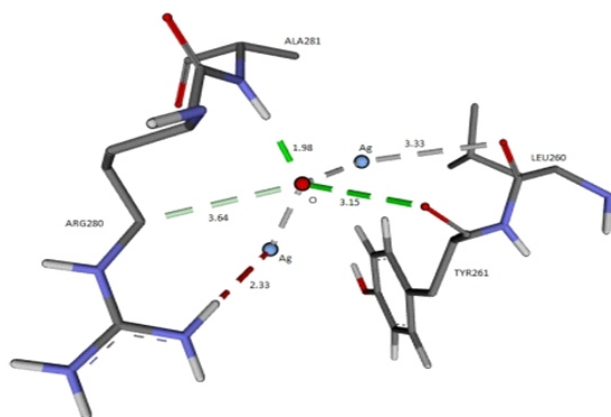


Figure 8: Residual interaction of NS5B protein with silver oxide NP.

NS5B encircles the active site due to the extensive interaction between the finger and thumb domains, and that's why it's not allowed to change their spatial arrangement freely with each other (Figure 9).

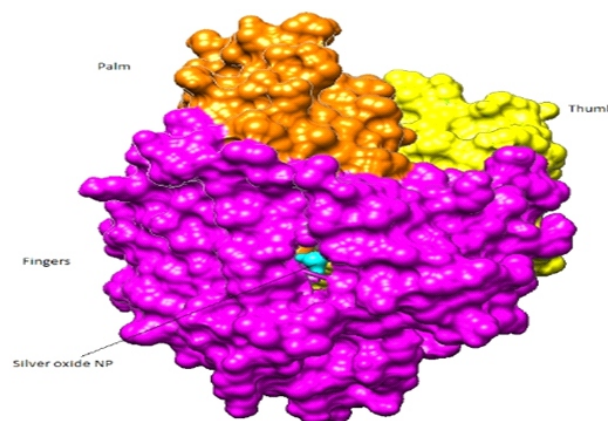


Figure 9: Molecular docking of AgO_2 NP to the Finger domain of NS5B protein.

Binding energy is emitted as a result of ligand-target binding, causing a decrease in overall complex potential energy. The release of binding energy facilitates the ligand's transformation from its maximum energy state to a bound conformation with minimum energy. Hence, the greater the released binding energy, the greater will be the binding affinity of the ligand to the protein. The ligand-binding process will be spontaneous if the binding energy is negative. The binding process will be nonspontaneous and require energy if the binding energy is positive. The binding energy of silver nanoparticles is -0.17 kcal/mol, indicating their low affinity with the protein. Hydrogen bonding dramatically affects the binding energies. Tyrosine-capped AgNP comparatively showed the highest binding energy, i.e., -5.29 Kcal/mol, due to the presence of multiple atoms in it forming multiple H-bonds, hence increasing binding energy (Table 2).

Table 2: Parameters obtained as a result of computational docking.

Ligands□	Ag Nanoparticles□	Tyrosine capped Ag nanoparticles□	Silver oxide nanoparticles□
Binding energies (kcal/mol)□	-0.17□	-5.29□	-2.06□
Ligand efficiency□	-0.17□	-0.38□	-0.69□
Inhibition constant (μM)□	744.78□	133.42□	31.07□
Intermol. Energy□	-0.45□	-6.93□	-2.06□
Vdw hb desolv energy□	-0.45□	-5.38□	-2.06□
Electrostatic energy□	0.00□	-1.55□	0.00□
Torsional energy□	0.27□	1.65□	0.00□
Ref RMS□	43.48□	35.43□	37.54□

4. Discussion:

Because fingers and thumbs are associated through two flexible finger loops (1 and 2), a conformational disturbance in one domain causes a change in the other domain [42]. The subdomains of the fingers and thumb determine the shape of the binding channel of the enzyme that binds the nucleic acid [43,44]. At the front and back of the enzyme, fingertips are involved in the formation of template and NTP channels respectively [45]. The NS5B protein in complex with GTP has been crystallized. The crystallised structure of NS5B complexed with the GTP molecule shows that GTP not only binds to the active site but also to the thumb domain near the delta-1 loop of the fingertip, which lies between the thumb and finger domains. Even though this GTP binding site is situated 30 Å far from the active site, it has a regulatory role in the dynamic interactions of the subdomains of fingers and thumb [43].

Silver oxide nanoparticles bind with four amino acid residues in the finger domain. It can induce a structural change in the finger region, distorting the spatial arrangement of the domain. As the finger domain is linked with the thumb domain, a change can occur in the positioning of the thumb domain as well. Disturbed positioning and structure of the thumb and finger domains result in the deformation of the template and nucleic acid binding channel. The attached ligand can also act as a blocker for newly formed rNTPs. Furthermore, the regulatory mechanism of NS5B through the GTP molecule can be breached and damaged by the ligand by disfiguring the GTP binding site, resulting in allosteric inhibition. Considering the current study showing Ag nanoparticles against the NS5B protein of the hepatitis-C virus, there are many docking studies conducted on HCV inhibition concerning pharmacology, pharmacokinetics, and other interactions. Mathew et al. showed that the drug N-Butyldeoxynojirimycin (NB-DNJ) established the greatest number of hydrogen bond interactions and high interaction energy with p7 proteins, portraying that the predicted p7 protein molecule of HCV from GT3 and GT4 targets the structure-based antiviral compounds [46]. Similarly, in another study, Ejeh et al.'s discoveries indicated that the specified bioactive molecules

used in the study differ considerably in docking scores against Sofosbuvir, the NS5B enzyme inhibitor. In comparison with Sofosbuvir, which had a drug score of 0.31, the ADMET analysis of the bioactive compound (1c) depicted better results with a drug score of 0.88 [47]. Pierre et al. described that mutations close to the template entrance (K98E, K100E), and in the center of the RNA binding channel (R394E), lowered the population of RNA-bound enzymes and the fluctuations linked with the binary complex. The study disclosed a representation of the association-dissociation events of HCV-NS5B with RNA, and the interaction between HCV NS5B, its RNA template, and finger loop inhibitors [44]. Wadood et al. showed that, using complex-based pharmacophore mapping and virtual screening, simultaneous inhibition of protease and helicase activities of HCV NS3/4A protease were revealed [48]. El-Sokkary et al. performed a study that identified many antiviral drug resistance mutations of HCV genotype 4a and presented a mechanism through which the T282S mutation may contribute to Sofosbuvir and ribavirin resistance [49].

The limitations in the present study related to molecular docking included a restricted sampling of ligands and conformations of the receptor. Additionally, ensuring appropriate scoring functions and algorithms with binding affinities is often a limitation observed for docking studies.

5. Conclusion:

All the results obtained from the computational docking of the three ligands individually to the NS5B protein showed promising effects on inhibiting protein activity. The NS5B protein is an RNA polymerase and plays a key role in HCV replication. HCV becomes inactive without this protein molecule. There are three domains of the protein, i.e., the palm, fingers, and thumb, which collectively form the active site. All three ligands were docked near the active site, inhibiting the RNA replication process. The most effective ligand was tyrosine-capped silver nanoparticles. Its relative highest binding energy, i.e., -5.29 kcal/mol, showed its intense binding with the protein molecule, causing more damage to the integral residues forming the active site.

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