Corona Structure Formation by Molecular Docking Studies between a Protein and Carbon Nanotube

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Abstract
Proteins are made up of amino acids. Protein has basically primary (1-D), secondary (2-D), and tertiary (3-D) structures. In the case of, linear (1-D) structure amino acids are considered as beads of a necklace and they can join together using peptide bons to form the protein structure. Linear chain undergoes twists and turns to form secondary structures namely, alpha-helix and beta-sheet. When the x, y, and z axis come into play 3-D structure of a protein is formed. Nanoparticles (NPs) have unique properties that may be useful in a diverse range of applications, and consequently, they have attracted significant interest. Particularly in the bio-medical field, the use of nano-vaccines and nano-drugs is being intensively researched. The interaction of nanoparticles with proteins is the basis of nanoparticle bio-reactivity. This interaction gives rise to the formation of a dynamic nanoparticle-protein corona. The protein corona may influence the cellular uptake, inflammation, accumulation, degradation, and clearance of the nanoparticles.

The main aim of this work is to study the interaction of proteins with nanoparticles using molecular docking process. Visual Molecular Dynamics (VMD) and Molecular Docking Software, Autodock are used extensively to study this interaction. VMD is a molecular modeling and visualization computer program which is freely available. Docking software is used to predict the interaction of small molecules such as substrate or drug candidates to a receptor protein of a known 3Dstructure. The interaction between proteins and nanomaterial is important as this interaction can led pathways for the application of nanomaterials in drug delivery procedures. As per the literature, a corona structure is observed to be formed when a nanomaterial interacts with the protein.In this work, the interaction of myoglobin protein is studied with a carbon nanotube using AutoDock software. In Auto Dock software, repeated runs were performed to find out the interaction between CNT and myoglobin protein. A corona-like structure is formed which looks similar to the structure reported by other workers.

Keywords: Protein, Nanoparticles, Corona, Molecular Dynamics, AutoDock, Simulation, Visual Molecular Dynamics (VMD)

1. Introduction

Protein structure[1] is a three-dimensional polymer structure of amino acids. These polymers can specifically be polypeptides formed from the sequences of amino acids. A single amino acid, monomer may also be called as a residue indicating a repeating unit of a polymer. The primary structure of a protein consists of amino acids arranged in a polypeptide chain. The primary structure of protein starts from the amino acid terminal(N)end and ends in the carboxyl-terminal (C) end. The Secondary structure of a protein is a local, regularly occurring structure in proteins as shown in Fig.1. This structure is mainly formed through hydrogen bonds formed between backbone atoms. Pauling & Corey [2] studied the secondary structure of proteins and proposed two conformations namely α-helix & β-sheets. The tertiary structure defines the specific overall 3-dimensional shape of the protein. Tertiary structure is based on various types of interactions between the side-chains of the peptide

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chain.

The quaternary protein structure involves the clustering of several individual peptide or protein chains into a final specific shape. Each amino acid has 4 different groups attached to α- carbon (which is C atom next to COOH).These 4 groups are: amino group, COOH group, Hydrogen atom and side chain(R). The side chain group R is unique to all amino acids.

Nanomaterials, on the other hand are very promising materials because of their vast applications in the various fields. A carbon nanotube (CNT) has a cylindrical structure with a nanoscale diameter and appears like a rolled graphene sheet[3, 4]. Interaction between a carbon nanotube and 3CLN (Calmodulin) protein was studied [5,6] with the help of molecular dynamics and visualization software. This interaction will be helpful in drug delivery. The need for this interaction will give a pathway for further research also as it will help to study the role of environmental conditions on a specified protein. It will play important role in the drugs delivery as CNT is a very small tube of nano scale diameter make it to easily pass through the organs.
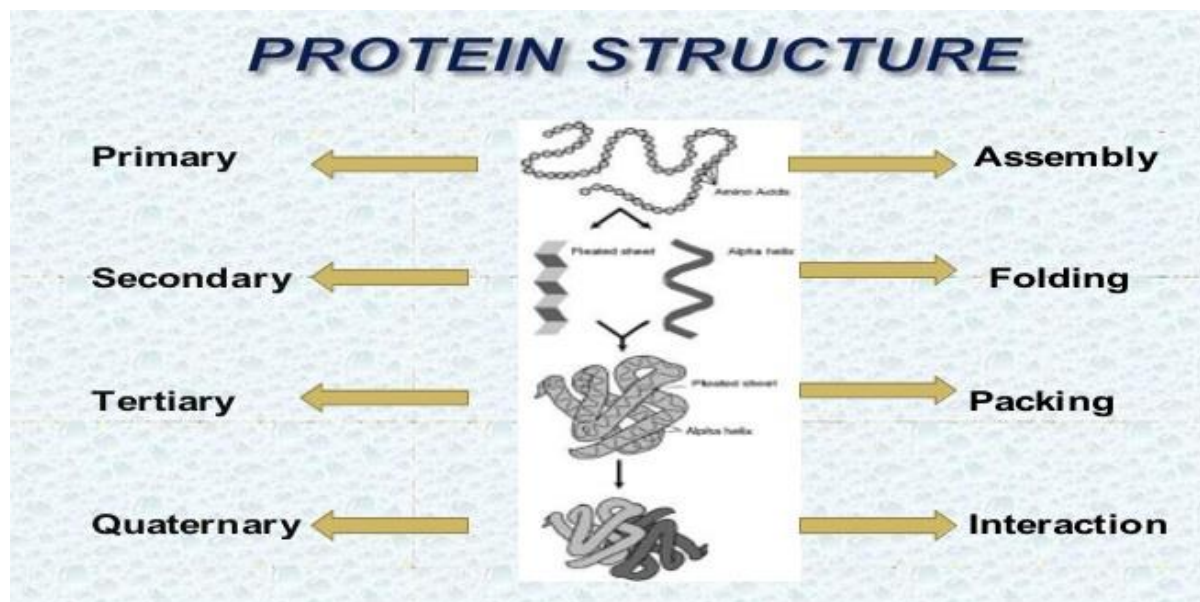

Fig.1: Protein Structure showing Primary, Secondary, Tertiary and Quaternary forms

2. Methodology

2.1 Protein Data Bank

The process of docking starts with choosing a particular type of protein and nanomaterial for the docking process. The protein after choosing it, can be downloadusing the protein database website namely (https://www.rcsb.org/) in the (.pdb) format.The particular type of nanomaterial can be generated using Visual Molecular Dynamics(VMD) software [7] by savingit in the supportedformat. The choice of proteinscan be any protein whose pdb structure is available in the protein data bank namely albumin, calmodulin, myoglobin, kinase,etc. From the various proteinsstructures available in the protein data bank, we choose myoglobinprotein (1MBN) because of its relatively small structure and a lesser number of the atoms. These proteins are then downloaded from the protein database website in the (.pdb) format. Similarly, in the case of nanomaterial[8], we choose Carbon Nanotubes (CNTs) to get the best docking results. Preparation of the CNT structure is also done using VMD software as shown in Fig 2.
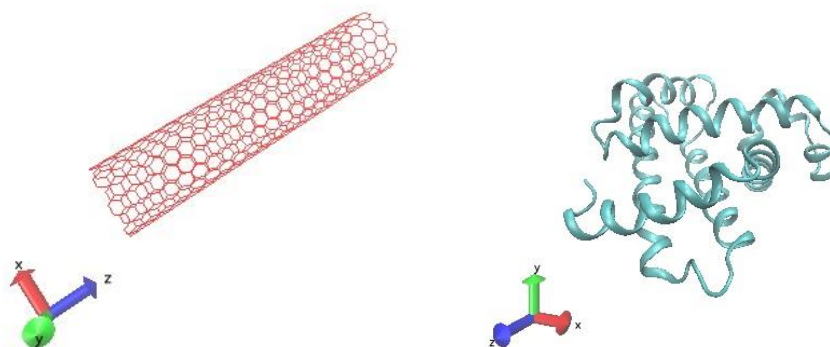
Fig.2:Initial Structure of (a) Carbon nanotube (CNT) and (b) a Myoglobin protein (1MBN.pdb) created using VMD

2.2 Visual molecular dynamics (VMD)

VMD is visualization software with the capability of showing molecular shapes in different ways and patterns. VMD is designed for showing and studying molecular assemblies, particularly in biopolymers such as proteins and nucleic acids,RNA, DNA etc. VMD can be used to simultaneously display any number of structures using a wide variety of rendering styles and colouring methods. VMD is developed as a tool to observe and analyze the results of molecular dynamics simulations as well. It includes tools for observing volumetric and sequence of data, also for arbitrary graphics objects.As an example, Figure 2 shows the structure of a CNT and a Myoglobin protein created with the help of the VMD program. VMD has the advantage of supporting computers running MacOS X, Unix, or Windows and is distributed free of cost, including source codes.

2.3 Nanoscale Molecular Dynamics (NAMD)

Nanoscale Molecular Dynamics (NAMD) package [9] is a freely available source code used to run efficiently on parallel machines for simulating large molecular interactions. NAMD helps in an interactive simulation with the help of Visual Molecular Dynamics (VMD) as a molecular software used for visualization. NAMD is a parallelly designed software for high-performance simulation of large biomolecular systems. NAMD has ORCA and MOPAC (utilized in chemistry also), and can also act as a processes interface to many other quantum packages. Together with VMD and QwikMD,NAMD's interface helps to access hybrid Quantum Mechanical (QM)/Molecular Mechanics (MM) simulations in a single unit, is descriptive, customizable, easy to handle, and easy to use.NAMD is also available asa free tool for non-commercial use by a single person or academic institutions, also for a corporation's in-house uses.

2.4Molecular DockingMethod

The associations between different biologically relevant molecules such as proteins, peptides, nucleicacids, carbohydrates, and lipids play a central role in the signal transduction procedure. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism) in the system. Therefore, molecular docking method is used for predicting both the strength and type of signal produced.

Molecular docking is a very significant method in the field of molecular modeling that predicts the preferred orientation of one molecule with respect toasecondmolecule. Docking can be used when aligand and a target that are interacting and bound to each other to form a stable complex [10]. Knowledge of the preferred orientation in turn may be used to predict the strength of the association or the binding affinity between the two molecules using, for example, scoring functions.

Molecular docking is therefore one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site as shown in Fig. 3.Characterization of the binding behavior plays an important role in the rational design of the drugs as well as to elucidate fundamental biochemical processes. Docking can also be used to predict the interaction between the proteins and nano materials [11,12] which would be helpful in the targeted drug-delivery purposes.
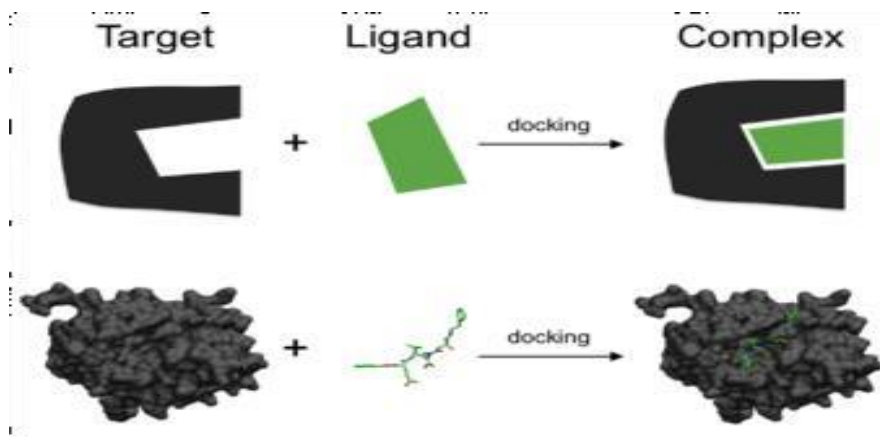

Fig. 3:Schematic illustration of docking a small molecule ligand (green) to a protein target (black) producing a stable complex.

Docking Approaches andtools

Two approaches are particularly popular within the molecular docking community[13]. One approach uses a matching technique that describes the protein and the ligand as complementary surfaces. The second approach simulates the actual docking process in which the ligand-protein pairwise interaction energies are calculated. Both the approaches have significant advantages as well as somelimitations. In this work we have used the docking process to study the interaction between the protein and carbon nanotube.

AutoDock Software

AutoDock is a suite of automated docking tools[14, 15]. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Over the years, it has been modified and improved to add new functionalities, and multiple engines have been developed. Current distributions of AutoDock consist of two generations of software: Auto Dock 4 and Auto Dock Vina. AutoDock 4 consists of two main programs: auto dock performs the docking of the ligand to a set of grids describing the target protein; autogrid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists design better binders. Auto Dock Vina does not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates the grids internally, for the atom types that are needed, and it does this virtually instantly.

3.   Results And Discussion

In the software Autodock, it is very important that all the files related to the docking are in a single folder to get the docking results accurately without any error. After downloading the required protein

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i.e., receptor and making the CNT i.e., ligand, we need to make some further changes in them and save them in the (pdbqt) format. So that, they can we used in AutoDock to give us the desired and accurate docking results.B ecause in most of the cases, water molecules are not involved in the binding, they are therefore deleted to make the computations easier as shown in Fig. 4. It will also clear the binding pockets of the possible water molecules that would distort the pose search.
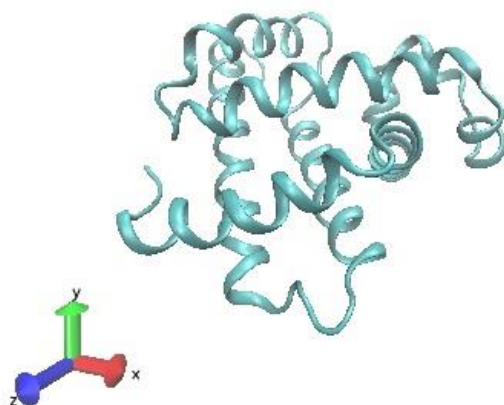

Fig. 4: Myoglobin (1MBN.pdb) protein molecule with deleted water molecules

In order to prepare a receptor file for docking, we need to remove the ligand associated with the protein; as in the pdb files from Protein Data Bank, coordinates for ligand are also mentioned as shown in Fig. 5.
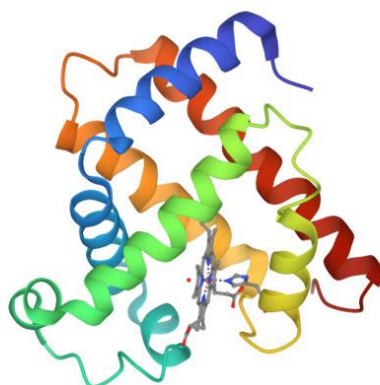

Fig. 5: Ligand associated with Myoglobin protein molecule downloaded from protein data bank

Inside the human/animal body the macromolecules are present in the charged form with no atom missing. So, it is necessary and sensible to add charges and missing atoms (hydrogen and in some cases non-hydrogen) to protein before proceeding for docking experiment, irrespective of the docking tool/software and algorithm we use.Further, we need to make sure that the charges are distributed equally over the whole protein molecule. A grid box needs to be defined for this where the grid box position defines the region of the protein where the docking procedure will be performed. Any region

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outside the box is not explored duringdocking.Docking is not an easy task particularly when we do not know the binding site location.So, if we do not know the specific binding site of a protein, we perform the blind docking procedure. The blind docking can be performed in two ways:

a.) creating multiple smaller grid boxes eventually covering the entire protein
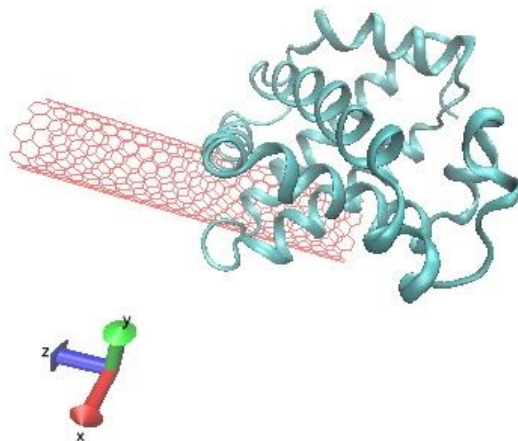b.) fitting the entire surface of your protein into one large grid box.

Fig. 6: Carbon Nanotube (Ligand) associated with protein molecule

Out of the two procedures mentioned above, the second one is less complicated and quite popular. So, in this work the docking of the protein and carbon nanotube has been performed using the blind docking process. A total of 10 conformations were generated out of which the best one was analyzed. The best conformation was chosen based on the various properties which are provided to us on the left side of the docking screen. As we change the various conformations the value of these properties changes accordingly. The various properties are binding energy, ligand efficiency, intermolecular energy, torsional energy, total energy, unbound energy, and various other properties. Inthis work, as we analyzed the various conformations, the binding energy was highest in the case of conformation no. 5. The snapshots for the same are as shown in Fig.7.A Corona like structure is observed in which the protein is observed to wrap itself around the carbon nanotube. This structure is quite like the corona structure as reported by other workers as shown in Fig.8.
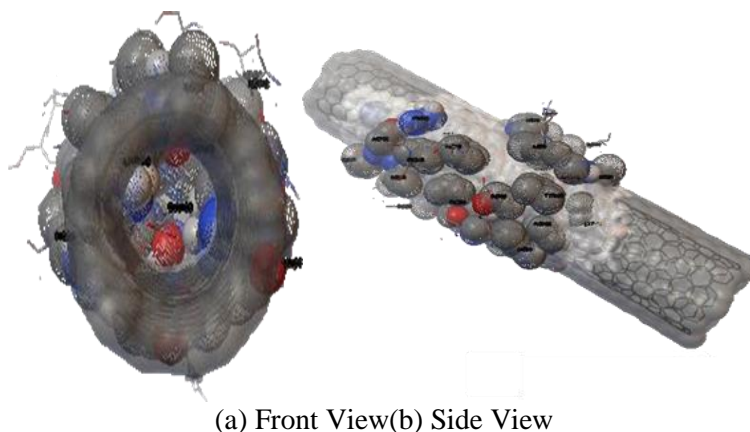
(a) Front View(b) Side View
Fig. 7: Final configuration of protein docked with CNT showing a Corona structure formation
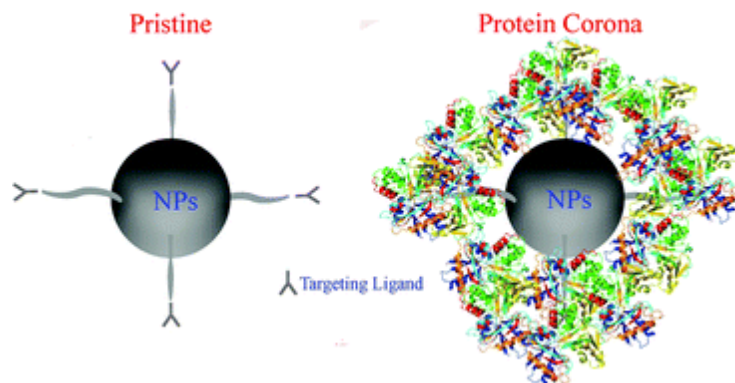
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Fig. 8:Actual protein corona structure when nanoparticle interacts with protein.

4. Summary and Conclusions

The interaction between a protein and nanomaterial is important from the point of view of application of nanomaterials in targeted drug delivery. As per the literature, a corona like structure is observed to be formed when a nanomaterial is interacted with a particular protein. The thickness of the corona structure depends on the shape and size of the nanomaterial. In this work, the interaction between a myoglobin protein with a carbon nanotube is studied using AutoDock software. In AutoDock, repeated runs are performed to check the statistical validity of the interactions between CNT and myoglobin protein. A corona like structure is observed to be formed in the maximum binding energy case which looks quite similar to the structure reported by other workers. The myoglobin protein is observed to completely wrap around the carbon nanotube as reported in our earlier stuides. In future these types of studies can be performed with the different types of proteinsto validate the formation of the protein corona structure.

Declarations
Ethical Approval
Not applicable as the present work does not include any human and/or animal studies.

Competing interests
Authors have no competing interest in the present work.

Authors' contributions
Sandeep Kumar has written the manuscript, Avinash Jha has made all the images and tables and Sunita Negi has done the final compilation of the manuscript.

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Availability of data and materials
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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