Role of pH change on the dynamics of Carbon nanotube and Protein interactions

Sandeep Kumar[1], Dwij Mehta[2], Sunita Negi[1*]

[1]School of Engineering & Sciences, G. D Goenka University, Gurugram, Haryana – 122103, INDIA

[2]Department of Physics, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat-364002, INDIA

[*]Corresponding author: negisunita.81@gmail.com

Abstract

Proteins are made up of amino acids. Protein has basically primary (1-D), secondary (2-D), and tertiary (3-D) structures exhibiting different types of functions. 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 temperature and pKa values of the amino acids in the proteins have great importance about the pH-dependence of the protein stability. Many experiments have been carried out in the last few decades to re-engineer the temperature and pH-activity and pH-stability profile of the proteins. Some of the earlier results predicts a sets of point mutations that will change the pKa values of a set of target residues in each direction, thus allowing for targeted re-design of the pH-dependent characteristics of the proteins. In this work, molecular dynamics (MD) studies are carried out by re-designing pKa values of some of the protein's amino acids in the carbon nanotube and the protein system. In our earlier studies, a variation in temperature was observed to play an important role in the dynamics of protein around the carbon nanotube. The pH value of the protein is modified with the help of H++ server. Detailed molecular dynamics simulations of 100 ns each are carried out to further study the behaviour of this protein under the influence of the pH change environment. A shape change is observed in the protein surrounding around the carbon nanotube at a pH value other than physiological pH. Towards the end of the simulation, very interesting results are observed in the interaction of CNT with protein at a low pH of 6.5. Protein is observed to be wrapping around the CNT thus forming a corona like structure. Comparatively very less interaction is observed between CNT and protein at a higher pH of 10.

Keywords- Protein, pKa, Molecular dynamics (MD), Visual molecular dynamics (VMD), Nano Scale molecular dynamics (NAMD), Carbon nanotube (CNT)

1. Introduction

1.1 Carbon Nanotube

Carbon nanotube (CNT) was firstly discovered by Sumio Iijima in 1991 [1]. Two years later SWCNT (single walled carbon nanotube) of diameter 1 nm was synthesized [2]. Carbon

Nanotube was a by-product in chemical vapour deposition (CVD) or Arc discharge techniques of fullerene with unrecognized properties. CNTs are predicted to be metallic or semiconducting and its electronic properties depends on their diameter and the helicity of the arrangement of graphitic rings in their walls [3]. It has unique atomic configuration, mechanical, optical & electrical properties [4]. Because of the vast application of the carbon nanotubes, they are a potential candidate in the field of electronics, mechanics, textiles etc.

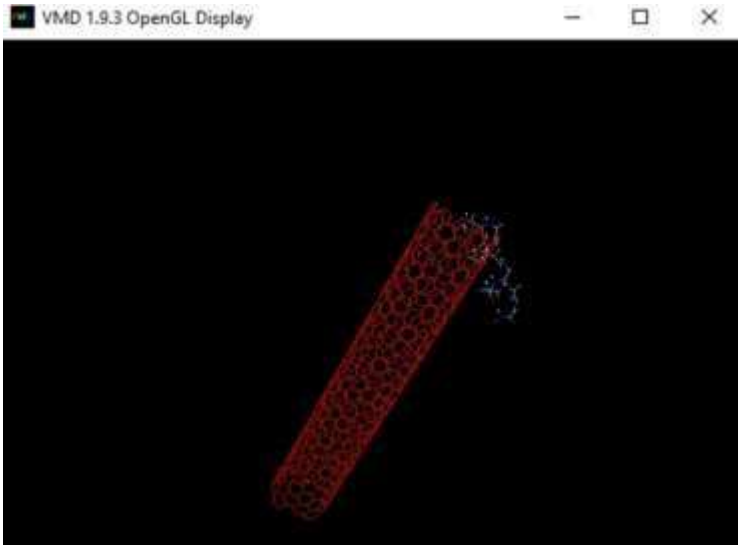

Fig.1: Structure of the protein with carbon nanotube

The interaction of nanoparticles CNT (Carbon Nanotube) with proteins is the basis of nanoparticle bio-reactivity. 3CLN (Calcium Modulated Protein) protein plays an important role in the calcium signaling inside the eukaryotic cell structure [5, 6]. pH-dependent conformation changes in the Calmodulin protein are studied with detailed molecular dynamics simulations by using VMD and NAMD software. The quantitative comparison of the simulation data with the analysis of different aspects of the folding process can help with the correctness of the calculations. It can also provide a detailed structural interpretation for the experimental observations as well as a physical interpretation of the theoretical concept without actual experimentation.

Earlier these kinds of studies were also performed experimentally using fluorescence measurements as in [7]. The Molecular Dynamics (MD) approach was used to study changes in pH-dependent conformation change of the Calmodulin protein [8]. In this work we study the interaction of CNT with proteins after changing pH of the protein 3CLN (Calcium Modulating Protein) by using VMD (Visual Molecular Dynamics) and NAMD (Nano Molecular Dynamics) Software. Visual Molecular Dynamics (VMD) is a molecular modeling and visualization computer program. As per the literature, the interaction between a carbon nanotube and 3CLN (Calmodulin) protein [9-11] was studied with the help of the software VMD and NAMD. This interaction will be helpful in drug delivery. The need for this interaction will give a pathway for further research work it will help to study the role of

environmental conditions on a specified protein [12]. Carbon nanotube (CNT) has a cylindrical structure with a nanoscale diameter and appears like a rolled Graphene sheet. VMD is developed as a tool to view and analyze the results of molecular dynamics simulations. It also includes means for working with volumetric data, sequence data, [13] and arbitrary graphics objects etc. NAMD enables interactive simulations with the Visual Molecular Dynamics (VMD) molecular visualization software. So, in this work we study the interaction of CNT with protein as it has a small number of atoms and plays an important role in drug delivery.

1.2 Proteins

The protein is made up of amino acids. Proteins are of different shapes some are of dumbbell-shaped proteins composed of two globular domains [14] connected by a flexible linker. Amino acid availability is a good regulator for muscle protein synthesis. Protein structure [15] is a three-dimensional arrangement of atoms. Proteins are polymers – specifically polypeptides – formed from sequences of amino acids, the monomers of the polymer. Biophysics [16] will play an important role in medical science as it will help in the treatment with much better ways. It will play important role in drug delivery as CNT is a very small tube of nano-scale diameter that makes it easily pass through the organs.

The interaction between the carbon nanotube and the protein at pH changes is an important aspect to study for its application in drug delivery Proteins, are made up of different types of amino acids. Some proteins can be found in the cytoplasm of all eukaryotic cells. Amino acid availability is a good regulator [17] for muscle protein synthesis. For a particular type of drug delivery through CNT interaction with the protein needs to be studied under different environmental conditions like pH. Earlier work is done on the interaction of CNT and proteins [18] but the effect of environmental conditions is not studied in detail for the 3CLN protein.

2. Methodology

2.1. Protein Data Bank

Before starting the work, we need to choose a particular protein and nanomaterial for this interaction. In the case of protein after choosing it, we download it using the protein database website namely (https://www.rcsb.org/) in the (.pdb) format and after choosing a particular nanomaterial we synthesize it using VMD software and save it in the supported format. After various literature reviews, the choice of proteins is various namely albumin, p-gp, calmodulin, myoglobin, kinase, etc. From these various proteins, our choice for docking was calmodulin because of its relatively small structure and small number of carbon structures. Proteins were downloaded from the protein database website in the (.pdb) format. Similarly, in the case of nanomaterial choice we choose Carbon Nanotubes (CNTs) to get the best results. Preparation of CNT is done using Visual Molecular Dynamics (VMD) software and it is saved in all files format. Molecular dynamics (MD) is a computer simulation method for analyzing the physical movements of atoms and molecules in a

system. Molecular dynamics (MD) is a computational technique widely used in the studies of biomolecules (like proteins), and nanoscale systems. Following are the two software (NAMD And VMD) as materials software to be used to study the interaction of CNT with proteins. Visual Molecular Dynamics (VMD) Nano Scale Molecular Dynamics (NAMD).

2.2 Computational details

Visual molecular dynamics (VMD)

VMD is software with the capability of showing molecular shapes in different ways. VMD is designed for showing and studying molecular assemblies, particularly in biopolymers such as proteins and nucleic acids, etc. VMD can 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 volumetrics and sequence of data, also for arbitrary graphics objects. VMD can support computers running MacOS X, Unix, or Windows and is distributed free of cost, including source codes.VMD is software with molecular modeling and visualization computer programing. VMD was developed as a tool to observe and analyze the results of molecular dynamics simulations. VMD includes tools for observing volumetrics and sequence of data, also for arbitrary graphics objects. Figure 1 shows the structures of a CNT and CNT interacting with protein created with the help of the VMD program.

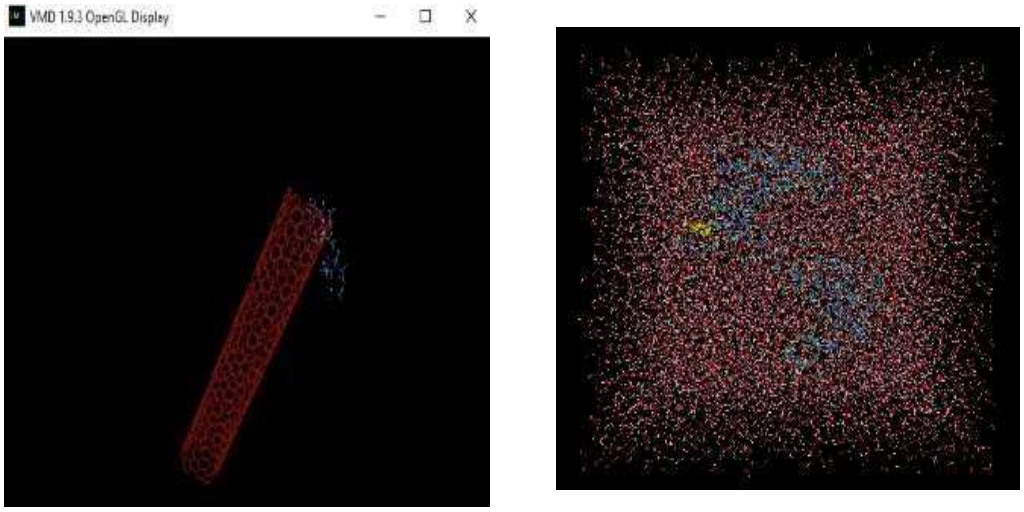

Fig 2. (a); Structure of 3CLN protein with CNT (b) Structure of 3CLN Protein with CNT in a water box

Nanoscale Molecular Dynamics (NAMD)

NAMD is used to run efficiently on parallel machines for simulating large molecules. NAMD helps in an interactive simulation tool with the Visual Molecular Dynamics (VMD) molecular software. NAMD is a parallelly designed software for high-performance simulation of large biomolecule systems. NAMD has ORCA and MOPAC (utilized in

chemistry also) and can also acts as a process interface to many other quantum packages. Together with VMD and QwikMD, NAMD's interface helps to access hybrid QM/MM simulations in a unit, is descriptive, customizable, easy to handle, and easy to use. NAMD is also available as free tool for the non-commercial tool used by a single person or academic institution, also for a corporation's in-house uses.

2.3 Software for pH change:

The pH of the protein system is changed through the web link http://newbiophysics.cs.vt.edu/H++/. We can change pH and ionic concentration by software through a web link in which H++ works as an automatic system i.e. H++ is an automated system that can computes pK values of ionizable groups in macromolecules and can add missing hydrogen atoms according to the specified pH of the environment. That gives a (PDB) structure file as an input, H++ outputs the completed structure in several common formats (PDB, PQR, AMBER inpcrd / prmtop) and provides a set of tools for analysis of electrostatic-related molecular properties**.**

3. Results & Discussion

Two sets of simulations are performed to study the interaction of 3CLN protein and carbon nanotube at different pH conditions. The snapshots for pH 6.5 at different time intervals is as shown in Fig.3. An interaction is observed to be happening between the CNT and the protein towards the end of the simulation as the protein starts attaching to the carbon nanotube. The root mean square deviation (RMSD) for the same is as shown in Fig.4. A fluctuation of about 6 Angstrom is observed in the overall structure of the protein around the carbon nanotube. This structure is like the corona structure formation reported earlier by other workers.

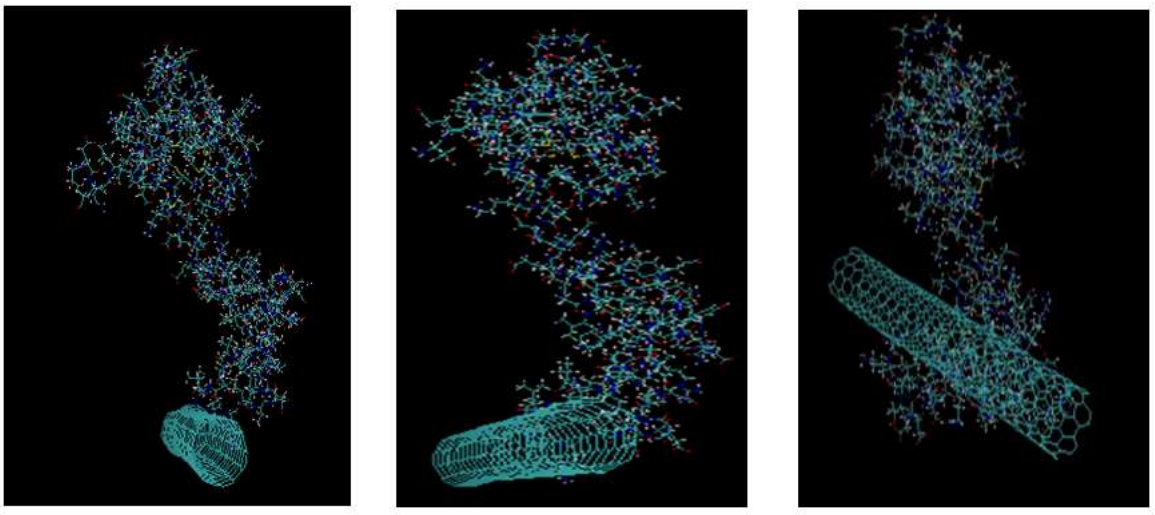

Fig 3. (a) Snapshot at 10 ns (b) Snapshot at 60 ns (c) Snapshot at 90 ns

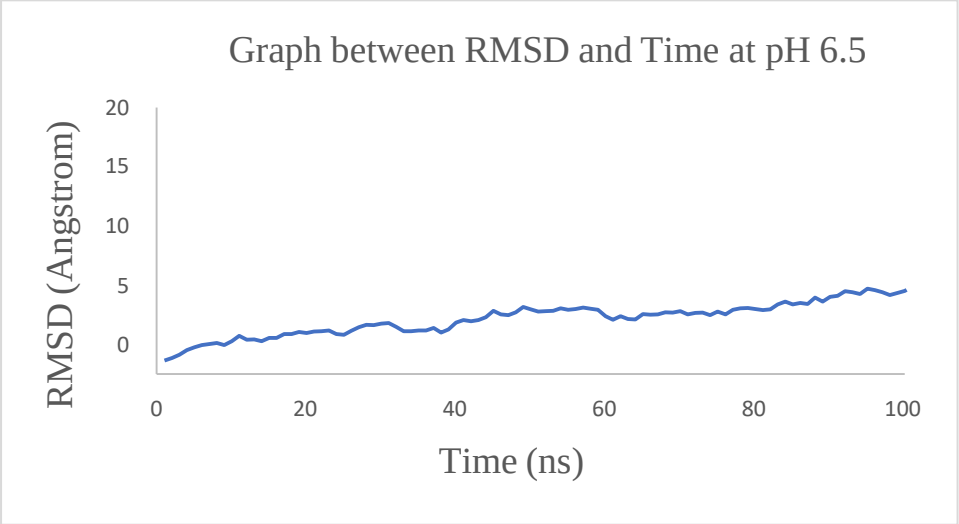

Fig 4. Root Mean Square Deviation (RMSD) as a function of time at pH 6.5

The interaction between the CNT and 3CLN protein is also observed at pH 10 to see the effect of higher pH values on the interaction of these two structures. The snapshots of this interaction are as shown in Fig. 5. At this pH as well, the protein appears to start sticking to the CNT surface. The root mean square deviation is as shown in Fig. 6. A fluctuation of about 5 Angstrom is observed in the overall structure of the protein around the carbon nanotube which is less than the fluctuation observed in the case of pH 6.5. This means that the acidic pH condition changes the pKa values of the amino acids significantly which then modifies the overall structure of the protein thereby leading to a shape change of the protein structure. Repetitive simulations are performed to confirm this corona structure formation towards the end of the simulation.

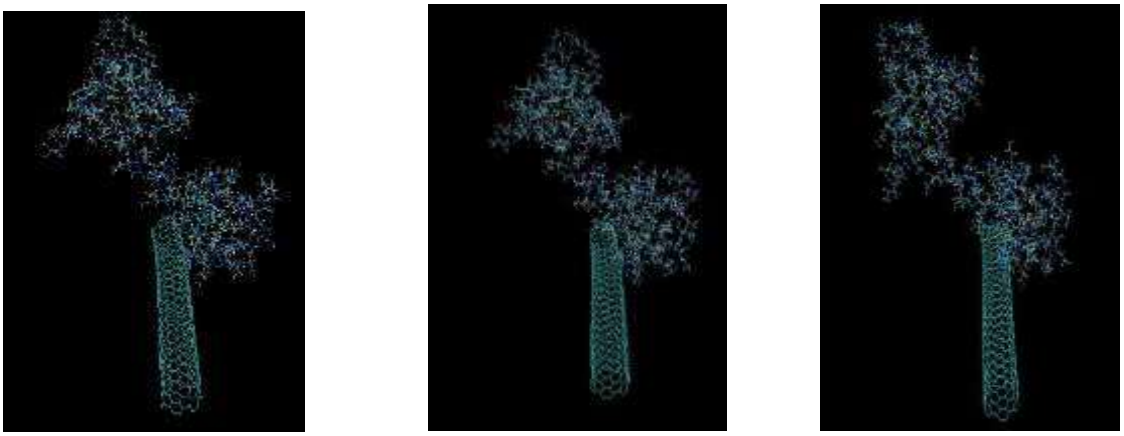

Fig 5. (a) Snapshot at 15 ns (b) Snapshot at 55 ns (c) Snapshot at 95 ns

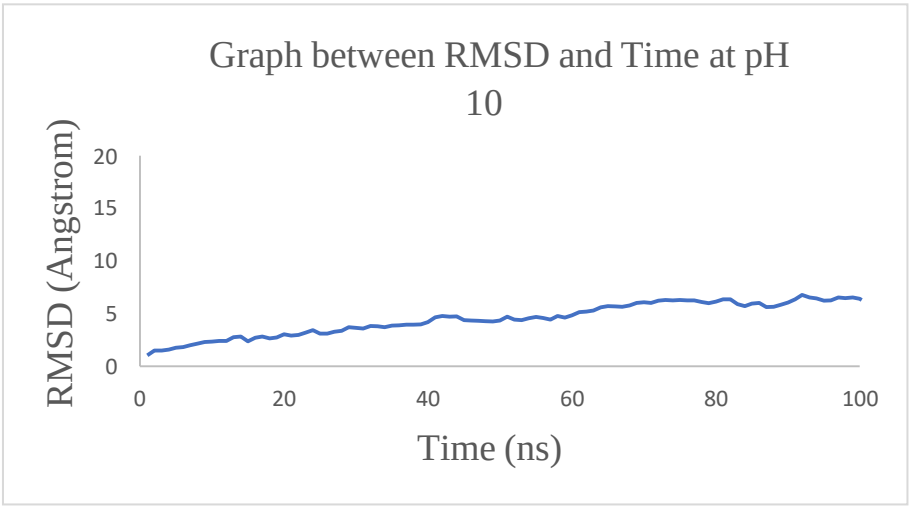

Fig 6. Root Mean Square Deviation (RMSD) as a function of time at pH 10

Summary & Conclusion

Simulation studies are carried out by re-designing pKa values of some of the protein's amino acids in the carbon nanotube and protein system using H++ server. Detailed molecular dynamics simulations of 100 ns each are carried out to study the behaviour of this protein under the influence of the pH change environment. Towards the end of the simulation, very interesting results are observed in the interaction of CNT with protein at different pH values. At low pH of 6.5, a very interesting interaction between CNT with protein is observed as it forms a corona like structure around the CNT. At a high pH of 10, a comparatively less interaction of the CNT is observed with the protein. These kind of interactions between protein and nanomaterial are important from the point of view of the application of nanomaterials in targeted drug delivery processes.

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Compliance with Ethical Standards

This article does not contain any studies involving humans or animals performed by any of the authors.

Conflict of Interests

The authors declare no conflict of interest.

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