CHAPTER 7

‘DNA-PROTEIN’ BASED COMPLEXES OF AD
7.1 General

Complexes of protein with DNA are of fundamental importance to living things. Protein-DNA based complexes are by their nature complicated assemblies of macromolecules and are therefore challenging subjects for study by the biophysicist. Certain proteins produced as a result of gene mutations target the DNA present the cell nucleus. These DNA binding proteins are known as histones. They have special binding domains, which bind DNA at particular sites. The interaction of DNA binding protein to DNA can be specific or nonspecific depending up on the nature of amino acid and nucleotide sequence and the environmental factors.

The transportation of most of the particles in and out of the cell will be regulated by ion channels, which are of nano dimensionality. Every channel carries a strong potential, which plays a critical role in its conduction mechanism. Many channels can selectively transmit or block ion species. Here, a 3D transport Monte Carlo ion channel simulation has been carried out using BioMOCA tool of nanoHub [214] to study the stability of protein-DNA complexes of AD.

These proteins generally distort the DNA and the resulting interaction may even lead into cell death. After the deactivation and distortion of DNA, the protein molecules may try to exit the cell through the cell membrane via the nuclear membrane. In most cases, the DNA that is bound to the protein may not be able to move out of the nucleus through the nuclear membrane as the pore size of the membrane being smaller than the size of DNA molecules. But in the process, the bond between the DNA and protein slowly gets weakened leading into complete dissociation of the complex and allowing the protein molecules to be separated completely leaving behind the dead or distorted DNA. Sometimes, the molecular interaction between the protein and the DNA may be very strong so that complete separation of the complex may not be possible. However, with the interaction, DNA loses its identity and the complex as a whole will be made inactive or dead. This phenomenon may be named as the ‘suicidal attack’ of the protein, where both the cell and the protein get distorted. Here the problem of the protein passing over to other cells does not occur through ion channel nanopores. In this chapter, the
DNA-protein based complexes of AD have been characterized to study the stability of these complexes.

The interaction between the DNA and the protein molecules mainly depends on the polarity of the amino acids making the protein molecules and the variation in concentration of ions between the intra or extra cellular matrices. The behavior of the complex structure has been simulated and analyzed based on Monte Carlo simulation technique.

7.2 Monte Carlo simulation

Monte Carlo Simulation is one of the largest and most important classes of numerical method for computer simulations or computer experiments. Monte Carlo simulation can be defined as a method to generate random sample data based on some known distribution for numerical experiments. In shortsighted view, Monte Carlo Simulation always involves random number (though actually not all methods that involve random number can be categorized as Monte Carlo Simulation, they may be better described as part of Monte Carlo Method). Generation of pseudo random number $R$ that distributed uniformly over interval $0 < R < 1$ is the heart of any Monte Carlo simulation. The random number generated must be independent (no correlation to other random number)

The major steps involved in a typical Monte Carlo simulation technique are:

1. Choose an initial set of atom positions.
2. Compute the energy for the system.
3. Randomly choose a trial move for the system.
4. Compute energy of the system in the new configuration.
5. Decide whether to accept the move or not based on the acceptance rule which ensures Botzmann distribution.
6. Iterate steps 3 to 5 until the system is equilibrated.
7.3 Monte Carlo simulation of the biological system.

The Protein-DNA based complexes of Alzheimer’s disease have been taken from the NCBI repository. All these simulations have been done with the ‘Biomoca suit of nanoHUB [215]’, where an artificial lipid rapper is designed mimicking the membrane and variation in potential and concentration heads has been provided to study the behavior of the complex.

Using Biomoca, particle flow through ion channel can be simulated (Fig. 7.1). In this tool, water and lipid molecules will be treated as background dielectric materials while ions and proteins are treated as discrete particles. Forces acting on each particle is computed from the Poisson equation, ion movement is allowed to occur in every time step to ensure that the movements are physically permitted without allowing overlapping of any two ions or overlapping of ions and protein molecules [216].

Prior to running BioMOCA, it is necessary to run the map generator and the lipid wrapper to model natural environment. The Monte Carlo simulation
follows the time dependence of the model in a stochastic manner, which depends on a sequence of random numbers.

The pores of the complexes collected from the NCBI were oriented in random directions. Proper reorientation of the complexes has been made by using Visual Molecular dynamic (VMD) tool. Molecular channels with dimensionality of nanopores separate the intracellular and extracellular compartments. The compartments may have different concentrations of K+, which provides a potential head as the driving force for molecular movements.

7.4 Computation method.

Only three DNA-protein based complexes are available in the repository, 3DXC, 3DXE and 3DXD with complexes in the APP intracellular domain and the adapter protein in the P2B2 domain. Adapter protein forms a transcriptionally active complex with the gamma-secretase-derived amyloid precursor protein. These complexes play central role in the response to DNA damage by translocating to the nucleus and inducing apoptosis. They act by specifically recognizing and binding histone H2AX phosphorylated on 'Tyr-142' (H2AXY142ph) at double-strand breaks (DSBs), recruiting other pro-apoptosis factors such as MAPK8/JNK1. These complexes lead in to histone H4 acetylation at double-strand breaks (DSBs) leading into binding with modified histones.

Initially the computations were carried out at a concentration of 0.2 M K+ in the intracellular and extracellular compartments without making any concentration gradient. It has been found that ion crossing through the membrane was absent for all these samples. Different concentration heads have been setup to study the effect of change of concentration on movement of complexes through the pores. It has been found that ion crossing takes place for the sample 3DXC, at a concentration head of 0.6 M K+ (0.8 M K+ ion and 0.2 M K+ in the intracellular and extra cellular compartments.) Still there was no movement of particles for 3DXE and 3DXD. An electric potential head is developed to study the effect of movement of complexes through the channels. At a minimum potential head of 240 mV, there was movement of particle for 3DXC while even on increasing the potential up to 1000mV, there was no movement for 3DXE and 3DXD. This
clearly indicates that the proteins 3DXE and 3DXD are stable complexes. Even 3DXc is stable up to 240 mV. Hence these complexes may not be considered as propagating the disease from cell to cell. Their actions are expected to be localized. The high stability of the complexes suggest the biological neutralization of the bioactive AD protein molecules which are responsible for the disease.

7.5 Modeling and simulation

The thermodynamic stability of these complexes has been studied with the help of molecular modeling in the ‘molecular mechanics level’ by using CHARMM forcefield. Molecular dynamic simulation has been conducted to study the temperature evolved or time evolved behavior of the modeled structures and all of them are found to attaining stability fast. All these samples are found to be stable (Table 7.1).

<table>
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<th>Sl.no.</th>
<th>Name</th>
<th>Potential energy (k.cal/mol)</th>
<th>van der Waal’s energy(k.cal/mol)</th>
<th>Electrostatic energy (k.cal/mol)</th>
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<td>3DXE</td>
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<td>-2355.92794</td>
<td>-28317.64492</td>
</tr>
</tbody>
</table>

7.6 Subcellular location

In normal conditions, all these three proteins mainly localizes to the cytoplasm, while a small fraction of these proteins is tethered to the cell membrane via its interaction with APP. Following exposure to DNA damaging agents, it is released from cell membrane and translocated to the nucleus. Nuclear translocation is under the regulation of APP.
7.7 Analysis of protein-DNA complexes.

The amino acids present in 3DXC, 3DXD and 3DXE have been identified. No abnormality is identified on measuring the stability of various residues present in the protein molecules (Fig. 7.2).

Fig. 7.2 Protein residues and their energies (3DXC)

In the study of the variation of pH of the protein with the charge, almost linear relationship is identified (Fig. 7.3). Hence no sudden configurational or structural change is expected on the protein molecule.
7.8 Summary

The protein-DNA complexes of AD disease have been characterized. The complexes 3DXE and 3DXD are found to be unaffected by variation in potential and ionic concentration. 3DXC was found to be dissociated at a potential head of 240 mV or at a concentration head of 0.6 M K+. All these molecules are found to thermodynamically and structurally stable. Moreover, no drastic change in structure, geometry or configuration is expected on changing the pH.