Molecular Dynamics Simulation Study of Denaturation of Protein Under the Influence of Electric Field

S. P. Deshmukh[1], K.S. Kubade[1], N.P. Ambekar[1], M.S. Panchbhai[2]

[1]Department of Physics, Institute of science, Nagpur, Maharashtra, India

[2]Department of Forensic Biology, Institute of Forensic Science, Nagpur, Maharashtra, India

Corresponding Author Email: deshmukhsp18@gmail.com

Abstract: The present study investigated the effect of static electric field on the structural changes in the Ubiquitin protein using molecular dynamics simulations. Structural transitions of Ubiquitin are observed under the influence of an electric field such that at a higher electric field, helix and sheets turn into coil and the protein gets stretched and unfolded. The RMSD variation with respect to time clearly shows that the larger the electric field strength, the more rapid structural transition is observed. Increased value of dipole moment for higher electric field supports the charge re-distribution and thus the unfolding of Ubiquitin under the influence of the electric field.

Keywords: Ubiquitin, Protein Unfolding, Molecular Dynamics Simulation

I. INTRODUCTION

Proteins are vital biomolecules in living organisms, that carry varied biological functions. Various internal as well as external factors affect the structure of proteins and thus lead to their structural changes like folding and unfolding (Lapidus et al. 2017). Protein unfolding may result due to chemical processes (Freire et al. 2013), change in temperature (Privalov et al 1974, Moriyama et al. 2010), pressure, pH (Kishore et al. 2012), or due to applied electric field (Jiang et al. 2019). Unfolded proteins often lead to abnormalities and diseases in the host living organisms. (Chiti et al. 2017) Hence, understanding the folding and unfolding mechanism of proteins is important for deciphering their biological roles and developing respective therapeutic interventions.

Electric fields have been recognized as a tool to manipulate biomolecular structures, and studying their impact on protein unfolding is of paramount significance. Thus, the study of protein unfolding under electric fields, is crucial for understanding their stability, folding pathways, and interaction dynamics. The recent studies indicate that the intrinsic and extrinsic properties of protein may show different response towards higher electric field (Pereira et al. 2010, Ly et al. 2011) . The use of external field on given protein may leads to change of native structure of protein. The study of this response can be useful in various medical treatments.

Therefore, we intend to understand the effect of electric field on ubiquitin (PDB ID: 1UBQ) as a model protein. We choose ubiquitin protein as it is found in almost all cellular tissues in human and other eukaryotic organisms. Discovered in 1975 by Goldstein and the group, it is a small regulatory protein with a molecular mass of about 8.6 KD. Ubiquitin plays a vital role in disposal of unnecessary proteins, DNA transcription and repair. Being smaller in size, it's suitable for MD simulations. Ubiquitin structure contains 76 residues carrying 660 atoms (Pickart et al. 2004). Ubiquitin structure have 5 β-sheets, an α - helix and a short 3_{10} helix and coil. Figure 1 shows the structure of ubiquitin.

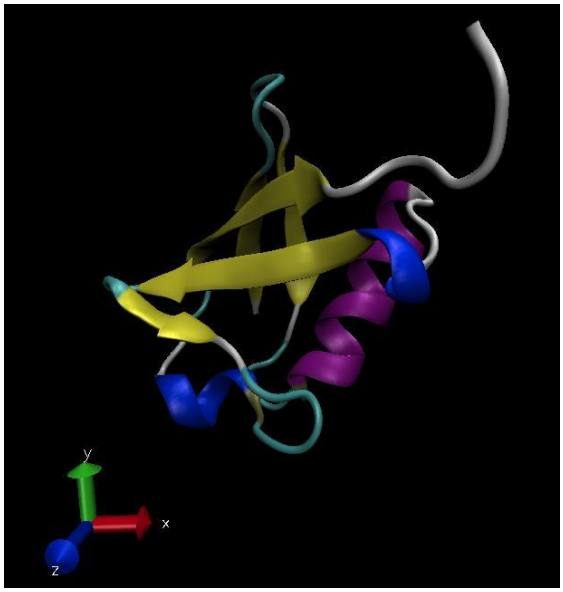


Figure 1: The structure of Ubiquitin protien

II. METHODS

The molecular dynamic simulation is carried out using GROMACS v2021 (Groningen Machine for Chemical Simulation) software developed by University of Groningen Royal Institute of Technology, Uppsala (Justin A.L. 2018). The PDB file for ubiquitin protein is retrieved from RCSB (Vijay-Kumar et al. 1987, Berman et al. 2000). The PDB file is first cleaned for the water molecule and then used for further study. We used CHARM36 force field for MD simulation (Huang et al. 2013). A cubic box is considered around Ubiquitin such that each periodic image of ubiquitin is at least 2 nm apart from each other. The box is filled with water molecules. Here we used TIP3P model for water molecules (Mark et al. 2001). The solvated, electroneutral system is now assembled. Before we begin MD simulation, we confirm that the system has no steric clashes or inappropriate geometry. The structure is relaxed by means of energy minimization. Energy minimisation is carried for 500 ns. The maximum force on each atom is restricted to 1000 kJ/mol/nm. NVT equilibration is carried out using V-rescale coupling scheme (Bussi et al. 2007) and temperature set is set to 300 K. Pressure equilibration is carried out using Parrinello-Rahman coupling scheme (Parrinello et al. 1981). System pressure is set to be 1 Bar. After setting the system at desired temperature and pressure, we perform production MD simulation for 0.5 ns. To analyze the dynamics of ubiquitin structure, we used the Root Mean Square Deviation (RMSD) concept. RMSD indicates the changes in the structure with respect to the original reference structure. It is given by the formula

$$RMSD = \left\{ \frac{1}{N} \sum_{i=1}^{N} \left[r_{final}(i) - r_{initial}(i) \right] \right\}^{0.5}$$

RMSD is a good tool to understand the structural changes in the protein during the MD simulation. Larger the deviation, greater will be the value of RMSD which represents relatively unstable structure. Practically the RMSD values less than 0.2 nm represents relatively stable structure. Flattened/constant RMSD over the simulation time indicates the stability of the system.

In order to study the effect of electric field on the protein structure, we applied the static electric field to the stabilized ubiquitin protein along Z-direction. The electric field strength we have chosen for present study is

E0 = {0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 3, 3.2} V/nm.

Due to the applied electric field, there will be rearrangement of charges within the protein. Here we calculate the dipole moment as follows.

$$\bar{d} = \sum_{i=1}^{N} q_i\,(i)\bar{r}_i$$

III. RESULT AND DISCUSSION

The main results of the present study are as follows:

PROTEIN UNFOLDING

First, to analyse the structural changes we present the variation in RMSD during the MD simulation. The variation of RMSD with respect to time for different applied electric field strength is as shown in the Figure 1. The results were obtained by exposing the ubiquitin protein to the external electric field along z direction. We find that, for lower electric field RMSD tends to saturate, however, as we increase the electric field, RMSD value keeps on increasing. This indicates that the increasing strength of electric field causes the structural deformation, which leads to unfolding of the protein structure. From figure, it is clear that the average value of RMSD increases from 0.07 nm to 0.4 nm for field strength 3.2 V/nm suggesting the deviation of protein structure due to applied electric field. These average values of RMSD are computed for the time duration 0.1ns to 0.2 ns. Even for 3.2 V/nm field strength, the RMSD value ~ 1nm suggests the stability of protein in presence of applied electric field. Noticeable jumps are observed in the RMSD variation of ubiquitin at applied electric field strengths 1.2 v/nm up to 3.2 V/nm. It implies the drastic structural changes in the protein under study. Moreover, the characteristic time at which the RMSD shows jump, decreases with increasing electric field strength.

Figure 2 shows the visual outcome of the final structure after 0.5 ns. From Figure 2 and 3, it is evident that unfolding progresses as we increase the electric field. For ubiquitin, the maximum unfolding is found for applied electric field equal to 3.0 V/nm. The structural deformation in ubiquitin under the influence of electric field can be observed by using VMD (Humphrey et al. 1996). The secondary structure analysis provides a quantitative approach towards understanding the protein structure and function. Although, α-helix and β-sheets are the well known forms of secondary structure, it also includes C_{10} sheets, turns and bends. Secondary structure of a protein includes hydrogen bonding which assists the formation of tertiary structure. The evolution of secondary structure of ubiquitin under the application of electric field of different strength is as shown in Figure 3.

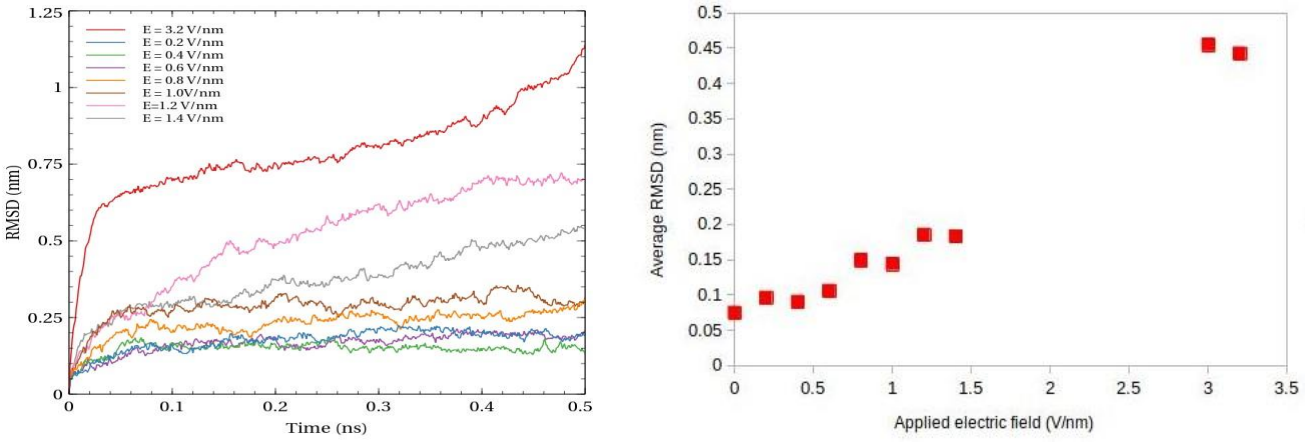


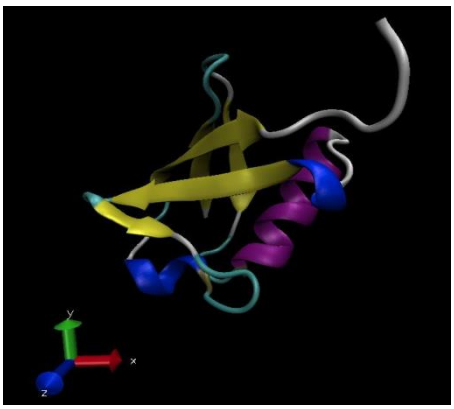
Figure 2: Left panel shows the variation of RMSD over the time period of MD simulation. Right panel shows the average RMSD for applied electric field. The average is taken over 0.1 ns to 0.2 ns.

III B. DIPOLE MOMENT VARIATION

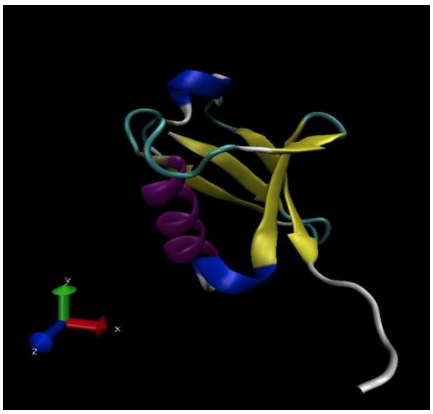
Next, we present the variation of dipole moment of the protein as a function of time at a given applied electric field in figure 5 a) and b). The variation of dipole movement is observed to be consistent with that of the RMSD variation. The average dipole moment is observed to increase from 450 Debye to 1350 Debye. It is also observed that beyond electric field of 3V/nm the dipole moment remains the same. The dipole moment of stable structure i.e. folded is expected to be minimum and is maximum for the unfolded structure. Therefore, we conclude that dipole moment variation is consistent with the unfolding as observed in figure 1 and 2.

IV. CONCLUSION

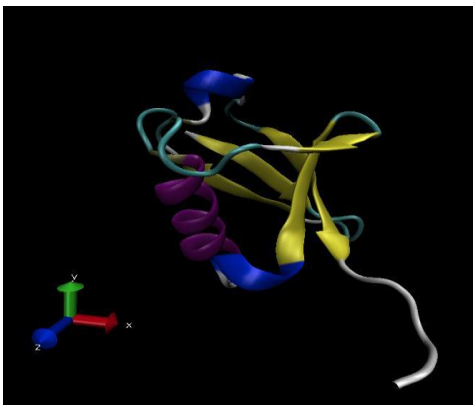
We investigate the effect of electric field on unfolding of protein by using ubiquitin as a model system via molecular dynamics simulation of 1ns. In general, we find that the electric field induces the structural deformations in the protein structure and therefore can be used for structural modulation of proteins thereby their biological activity. We also find that the electric field above 3 V/nm and above could unfold the protein. The electric field induced unfolding is evident in the final structure and is supported by the increase in RMSD value, secondary structural analysis and increase in dipole moment values with increasing electric field. In conclusion, this study provides comprehensive insights into the unfolding dynamics of ubiquitin under the influence of an electric field.



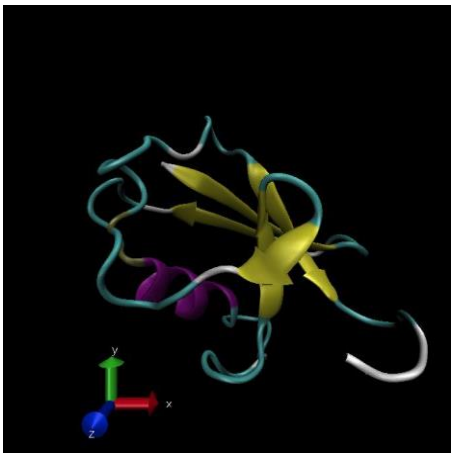
No electric field



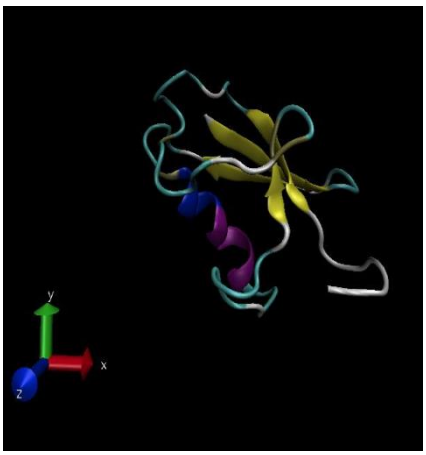
E = 0.2V/nm



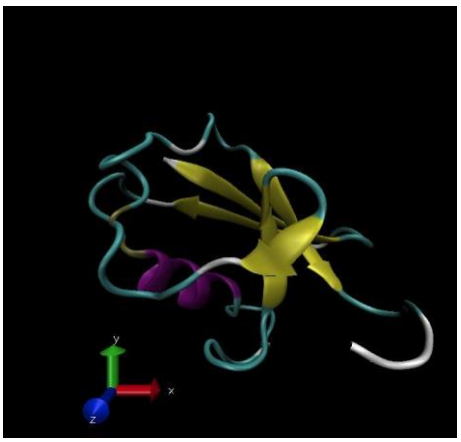
E=0.4 V/nm



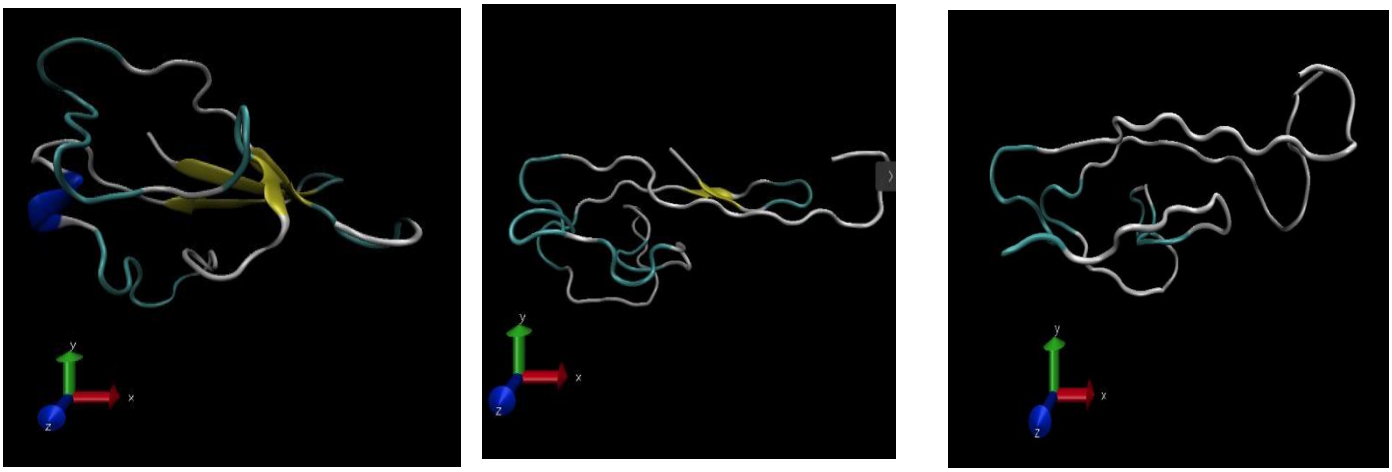
E=0.6 V/nm



E=0.8 V/nm



E = 1.0V/nm



E=1.2V/nm E=3.0V/nm E=3.2 V/nm

Figure 3: Ubiquitin structure under the influence of electric field.

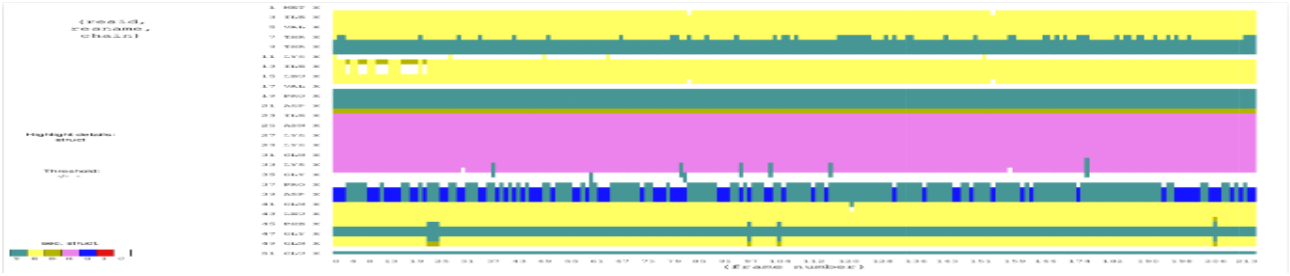
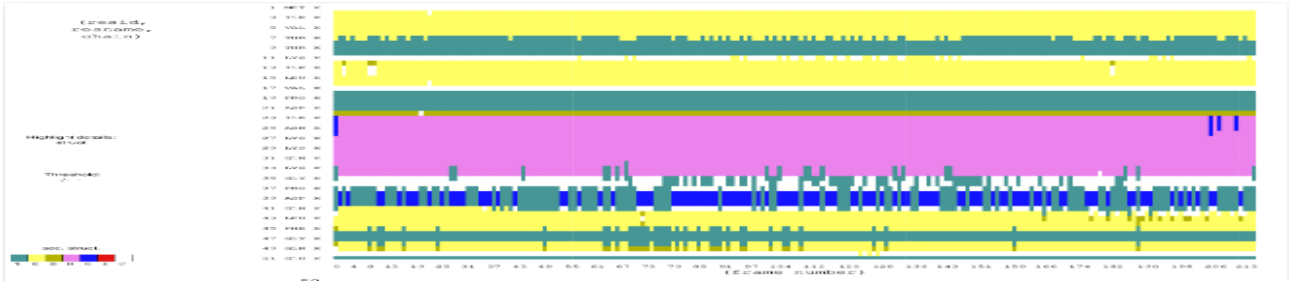
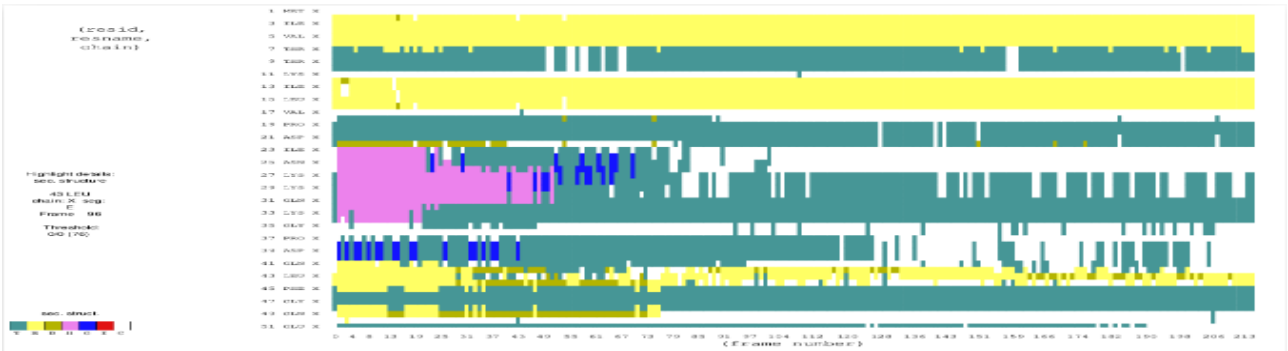
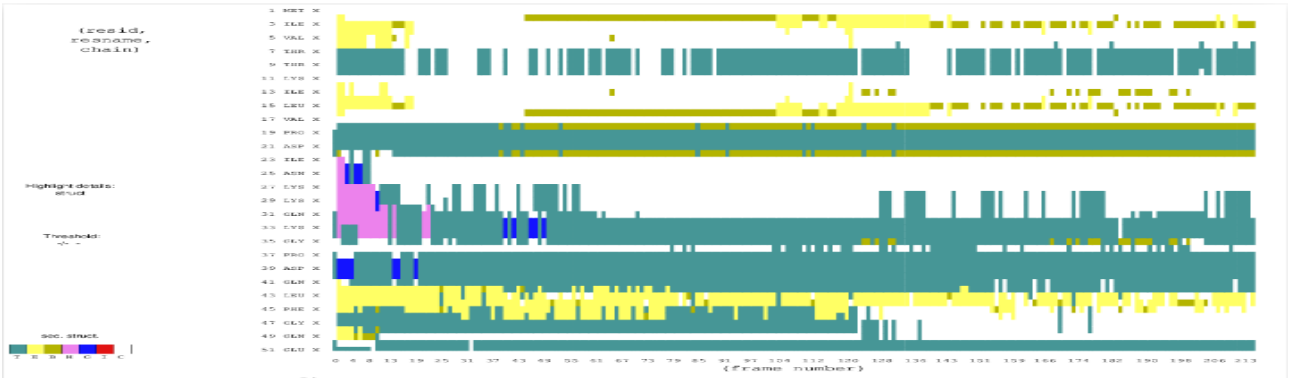
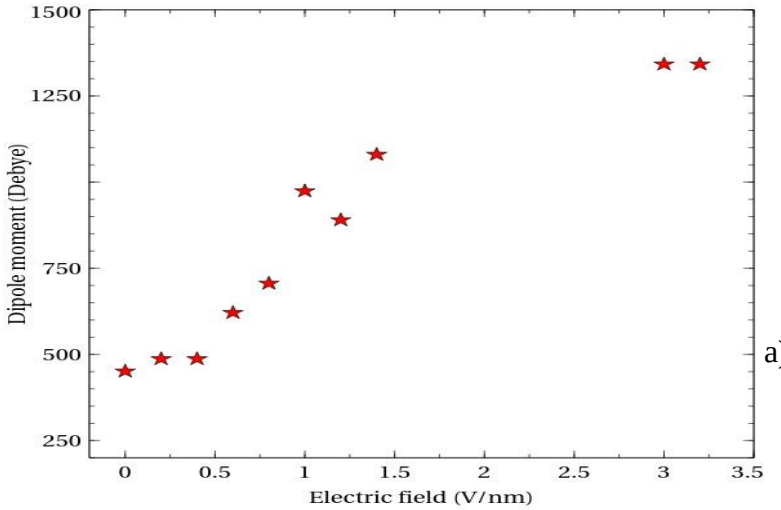


Figure 4: Secondary structure analysis of Ubiquitin under the influence of electric field. It shows the frame wise distribution of residues of Ubiquitin protein under applied electric field strength 0 V/nm, 0.8 V/nm, 1.4V/nm, 3.2 V/nm respectively from top to bottom.

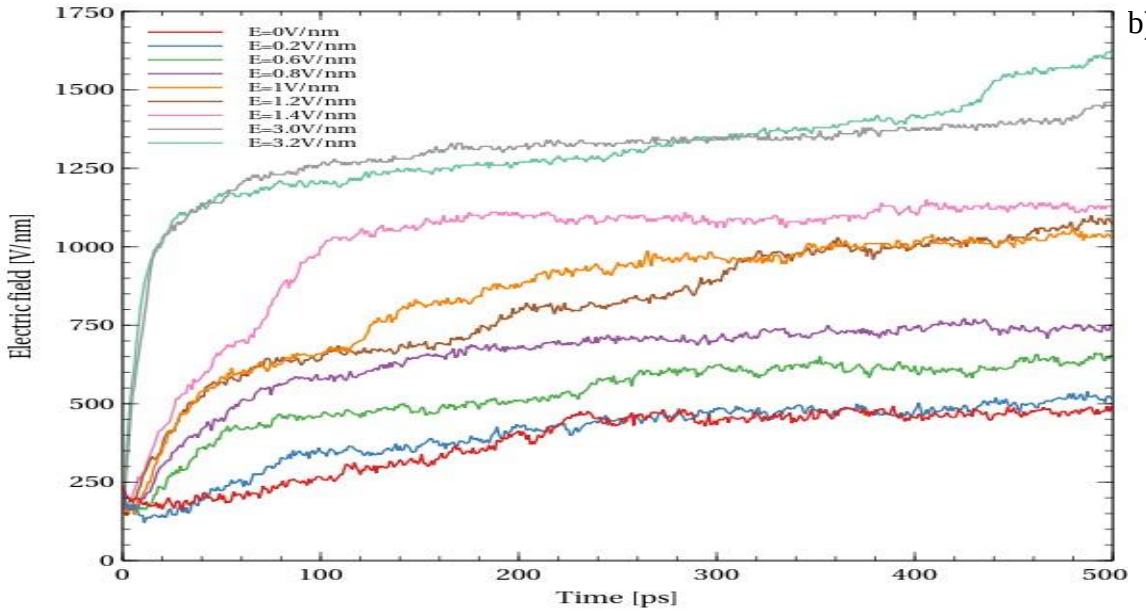








a)



b)

Figure 5: Dipole moment variation with time for a given electric field.

V. ACKNOWLEDGEMENT

The PDB structure of Ubiquitin protein is retrieved from www.rcsb.org. Also, use make use of GROMACS and VMD software for molecular dynamics simulations and results. SPD is thankful to the organisations that maintain and updates these websites and software.

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