**Pharmacophore**
(An International Research Journal)
Available online at http://www.pharmacophorejournal.com/

Original Research Paper

# MODELING AND MOLECULAR DYNAMICS STUDIES ON ESP1 AND ESP6 EPITOPES OF EARLY SECRETED ANTIGENIC TARGET PROTEIN ESAT-6 FROM *MYCOBACTERIUM TUBERCULOSIS*

**Rohan J Meshram[1]***, Pratik P Vikhe[1], Asmita J Gavhane[1], Suresh N Jangle[2] and Rajesh N Gacche[3]

[1]Center for Biotechnology, Pravara Institute of Medical Sciences, Loni (413736), Rahata, Ahmednagar, Maharashtra, India
[2]Department of Biochemistry, Pravara Institute of Medical Sciences, Loni (413736), Rahata, Ahmednagar, Maharashtra, India
[3]School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

## ABSTRACT

Tuberculosis (TB) has emerged as one of the leading health problem since last three decades. High rate emergence of extremely multiple drug resistant *Mycobacterium* strains results in inefficiency of available prophylactic and therapeutic means. Hence there is an urgent need to search for alternative, safe, effective and affordable preventive as well as therapeutic strategies against TB. ESAT-6 is an early secreted protein by *Mycobacterium tuberculosis* that can be used as target for efficient peptide vaccine design against TB. In present study, initially new epitopes were identified using online epitope prediction tools and structural models of both ESP-1 and ESP-6 epitopes derived from ESAT-6 were developed. Later on these epitopes were used for molecular dynamics analysis. Results obtained from molecular dynamics analysis on ESP1 and ESP6 epitopes direct need to focus further study on their interaction with MHC molecules.

**Keywords:** Immunodominant peptides, NAMD, HLA-DRB1*04 allele, Peptide vaccine.

## INTRODUCTION

World Tuberculosis (TB) is a highly infectious disease caused by *Mycobacterium tuberculosis* (MT) and continues to remain one of the most common and widely spreading infections, causing lot of economic burden on individual. Approximately 2.9 million people die from tuberculosis each year worldwide; about one fifth of them are from India alone. According to recent WHO report, India is the highest TB burden country in the world, accounting for an estimated 1.96 million cases annually. The only vaccine, Bacille Calmette Guerin (BCG) widely used since 1921 has now shown reduced efficienc[1] resulting in urgent need to investigate newer, effective and affordable immunoprophylactic strategies against tuberculosis. It is reported that at early stages of infection some surface proteins of MT are actively expressed[2,3] while some other proteins are secreted.[4] Then, it is logical that these proteins could be used as targets for vaccine design. ESAT-6 is an early secreted protein responsible for the increase synthesis for Gamma interferon and thought to be involved in the antituberculosis immunity development in host.[5]

Immunogenic peptides ESP1 (amino acid residue 1-20) and Esp6 (amino acid residue 51-70) were identified as protective epitopes of ESAT6 protein.[6] Structural knowledge of epitope is must for effective *in silico* design of peptide vaccine. Experimental structures of ESAT-6 proteins are available with PDB code 3FAV deduced by X-ray crystallography and 1WA8 deduced by NMR. The structure of ESP1 epitope is not available in PDB file 3FAV and the structural models of 1WA8 represent large variation in N terminal region, resulting in failure to have consensus structure of epitope. Identification of suitable epitopes from ESAT6 proteins is very crucial to design an effective vaccine. Computational tools like Propred1[7], BIMAS[8], RANKPEP[9], and SYFPEITHI[10] are available for identification of epitopes. An attempt has been made to find other epitopes apart from ESP1 and ESP6 from ESAT6 protein. During process of generation of antigenic peptide and its presentation on MHC Class II molecule, the native structure of epitope may not be maintained, as it has to pass through various cellular compartments with wide physiological conditions. Under such conditions, molecular dynamics concept can be utilized to take account of structural folding of epitope. NAMD can handle large system size and importantly has integration with VMD, the molecular visualization software.[11] VMD has the advantage of easy system preparation, post processing and analysis. NAMD has greater portability with Windows operating system and reduces computational time[12] hence; the same was included in the present study.

## MATERIALS AND METHODS

### Prediction of Epitopes

Sequence of ESAT-6 protein was retrieved from Swissprot Database[13] with ID P0A564. The sequence was then subjected for epitope prediction using PROPRED1 server taking 3 % of threshold value and SYFPEITHI parameter was set to 15-mers (15 amino acid residue) for MHC Type II only using HLA-DRB1*04 allele on both the servers, as it is found to be most common allele in Indian Population.[6] Deepview 3.7 was utilized to generate fully extended structural model of the identified epitope.[14]

### Molecular Dynamics Simulation of Epitope peptide

Initially, the PDB-PSF pair of files was generated for epitope using psfgen package implemented in VMD. top_all2_prot_lipid.inp topology file was utilized to generate psf file with complete structural information of the protein. New pdb file containing the complete coordinates of all atoms, including hydrogen was prepared. The peptide was further solvated by placing it in waterbox, having dimension such that it possess a layer of water of 5 Angstorm in each direction from the atom with the largest coordinate in that direction. To prepare protein in solvent system with periodic boundary conditions for minimization and equilibration, solvate package was utilized. A configuration file was constructed for both the epitopes, with values for Periodic Boundary Conditions and PME full electrostatics parameters and specific commands for the execution of simulation of 500 Ps. Simulation of ESP-1 epitope was carried on machine with Intel Core2Duo processor T5450/1.66 GHz having 2 GB RAM and that of ESP-6 on Intel core i3 processor/2.13 GHz with 3 GB RAM using WindowsXP operating System. From the resulting log files, energy values traced during simulation were parsed using Java based program NAMD Log Parser. Subsequently graphs were plotted. The epitopes were visualized in UCSF Chimera[15] (Fig 1).

## RESULTS AND DISCUSSION

### Epitope Prediction

There are 3959 estimated genes coding for proteins in MT genome.[16,17] A study on Molecular analysis of genetic difference, virulent *M. bovis* and the attenuated vaccine strain *M. bovis* BCG revealed three regions of difference, designated as RD1-RD3.[18] The RD1 region contains the genes for nine proteins (Rv3871-Rv3879c), which are thought to be involved in pathogenesis. The gene Rv3874 codes for a 95 residues ESAT-6 protein which is also termed as 6-kDa early secreted antigenic target. The experimentally observed ESP1 and ESP6 epitopes of ESAT-6 protein were also identified as top hits in

prediction of Propred (Fig 2) as well as SYFPEITHI (Table 1) servers. Here, we also suggest entirely new two potential epitopes $^{18}$IQGNVTSIH$^{26}$ and $^{43}$WGGSGSEAY$^{51}$. $^{18}$IQGNVTSIH$^{26}$ was detected as antigenic peptide in almost all HLA-DRB1*04 alleles. On the basis of these results obtained from online prediction and experimental evidences ESP1 sequence was constructed as $^{3}$EQQWNFAGIEAAASAIQ$^{18}$ and ESP6 sequence was considered as $^{48}$SEAQGVQQKWDATA$^{63}$.

### Molecular Dynamics Simulation of Peptide Epitope

Molecular Dynamics can be utilized to simulate the folding of peptide epitope when it is presented on MHC II molecule. Explicit solvent Molecular Dynamics (MD) is an atomistic means of simulating the behavior at room temperature of one or more solute molecules. Molecules can include an epitope peptide of defined geometry surrounded by an environment of solvent and ions. These simulations can be run over a timescale of one to several thousand nanoseconds.[19] MD explains in detail the individual and collective motion of atoms within a molecular system; thus MD can provide a dynamic picture of biomolecular systems. Subsequent to simulation, the NVT ensemble was further put through analysis for its thermodynamic states. Later on, peptide conformational stability was checked by calculating RMSD of protein backbone. Sudden increase was observed in bond angle energy during early steps of simulation of both ESP1 and ESP6 peptide but later on it was observed to converge around 130Kcal/mol in case of ESP1 and 50 Kcal/mol in ESP6 (Fig 3A and Fig 4A). While observing dihedral angle energy during simulation, it became evident that after rapid boost in energy, both the epitope peptides converge around 50 Kcal/mol (Fig 3B and Fig 4B), while similar observation can be made in case of improper dihedral energy which showed convergence in the order of 10 Kcal/mol (Fig 3D and 4D). In case of electrostatic energy, both the peptides showed congregation in energy but at different values i.e. ESP1 around -23000 kcal/mole and ESP6 in the region of -15500 kcal/mol (Fig 3C and Fig 4C). Kinetic energy of system, subsequent to initial rise in energy got converged more or less at 4000 Kcal/mol in ESP1 peptide and 2700 Kcal/mol in ESP6 peptide (Fig 3E and Fig 4E). All calculated thermodynamic terms showed union in energy values at some stage signifying that peptide has reached thermodynamic constancy. The RMSD of backbone of ESP1 and ESP6 peptides plotted against time also indicated achievement of conformational stability (Fig 5).

## CONCLUSION

In quest for finding new epitopes from ESAT-6 sequence, two novel epitopes were identified that are not reported earlier. Using Molecular dynamics, thermodynamically and conformationally stable ESP1 and ESP6 peptides were obtained. Results from this report can be effectively utilized to study interaction of ESP1 and ESP6 epitope with MHC-II molecule as promising antigenic peptide.

## ACKNOWLEDGEMENTS

Authors are thankful to Pravara Institute of Medical Sciences for providing their computation facility and Microsoft Office Package.

**Table 1:** Epitope prediction results obtained SYFPEITHI server showing starting amino acid residue positions, sequence and score for each identified epitope.

| Amino acid residue starting Position | Peptide Sequence | Score |
|---|---|---|
| 8 | FAGIEAAASAIQGNV | 26 |
| 62 | ATELNNALQNLARTI | 26 |
| 66 | NNALQNLARTISEAG | 26 |
| 73 | ARTISEAGQAMASTE | 26 |

| | | |
|---|---|---|
| 3 | EQQWNFAGIEAAASA | 22 |
| 5 | QWNFAGIEAAASAIQ | 22 |
| 48 | SEAYQGVQQKWDATA | 22 |
| 55 | QQKWDATATELNNAL | 22 |
| 19 | QGNVTSIHSLLDEGK | 20 |
| 22 | VTSIHSLLDEGKQSL | 20 |
| 25 | IHSLLDEGKQSLTKL | 20 |
| 26 | HSLLDEGKQSLTKLA | 20 |

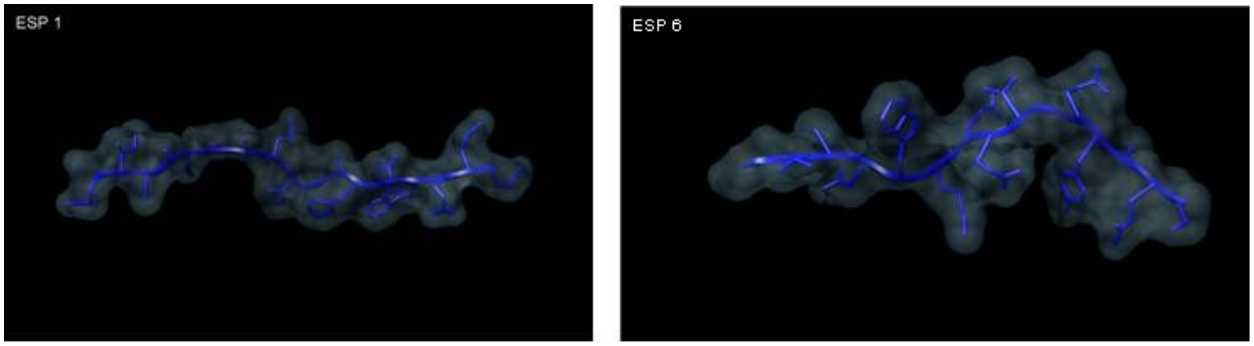


**Figure 1:** Three dimensional visualization of the ESP1 and ESP6 peptides through UCSF Chimera software.

```
                ---------10--------20--------30
DRB1_0401:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0405:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0408:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0426:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0402:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0404:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0410:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0421:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
DRB1_0423:  MTEQQWNFAGIEAAASAIQGNVTSIHSLLDE
```

**Figure 2:** Top hits obtained from epitope prediction from Propred server

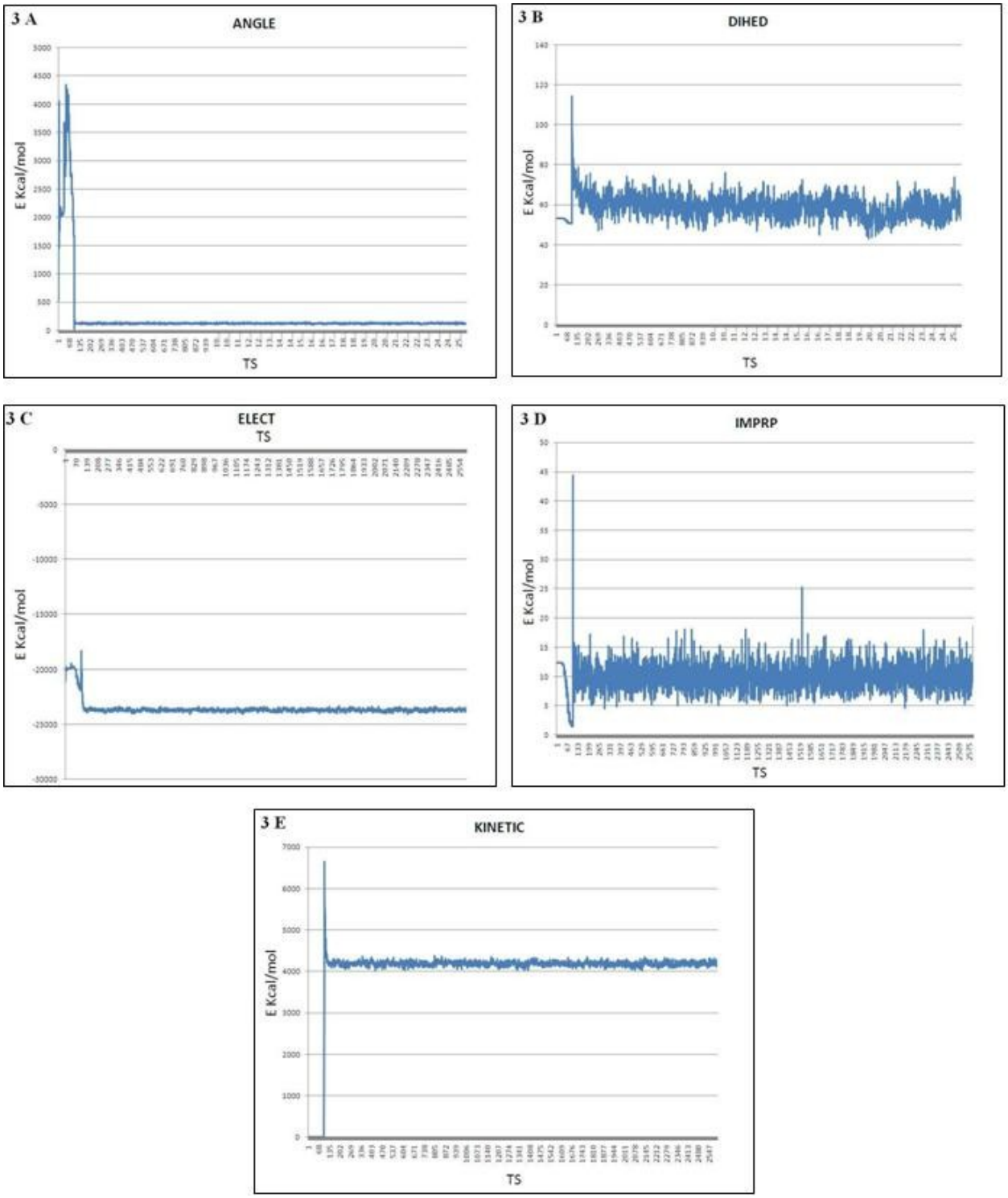


**Figure 3:** Plots of Time Step of simulation (ps) against Energy (kcal/mol) for ESP1 peptide. 3A: Angle, 3B: Dihedral, 3C: Electrostatic, 3D: Improper, 3E: Kinetic Energy T.S:- Time step

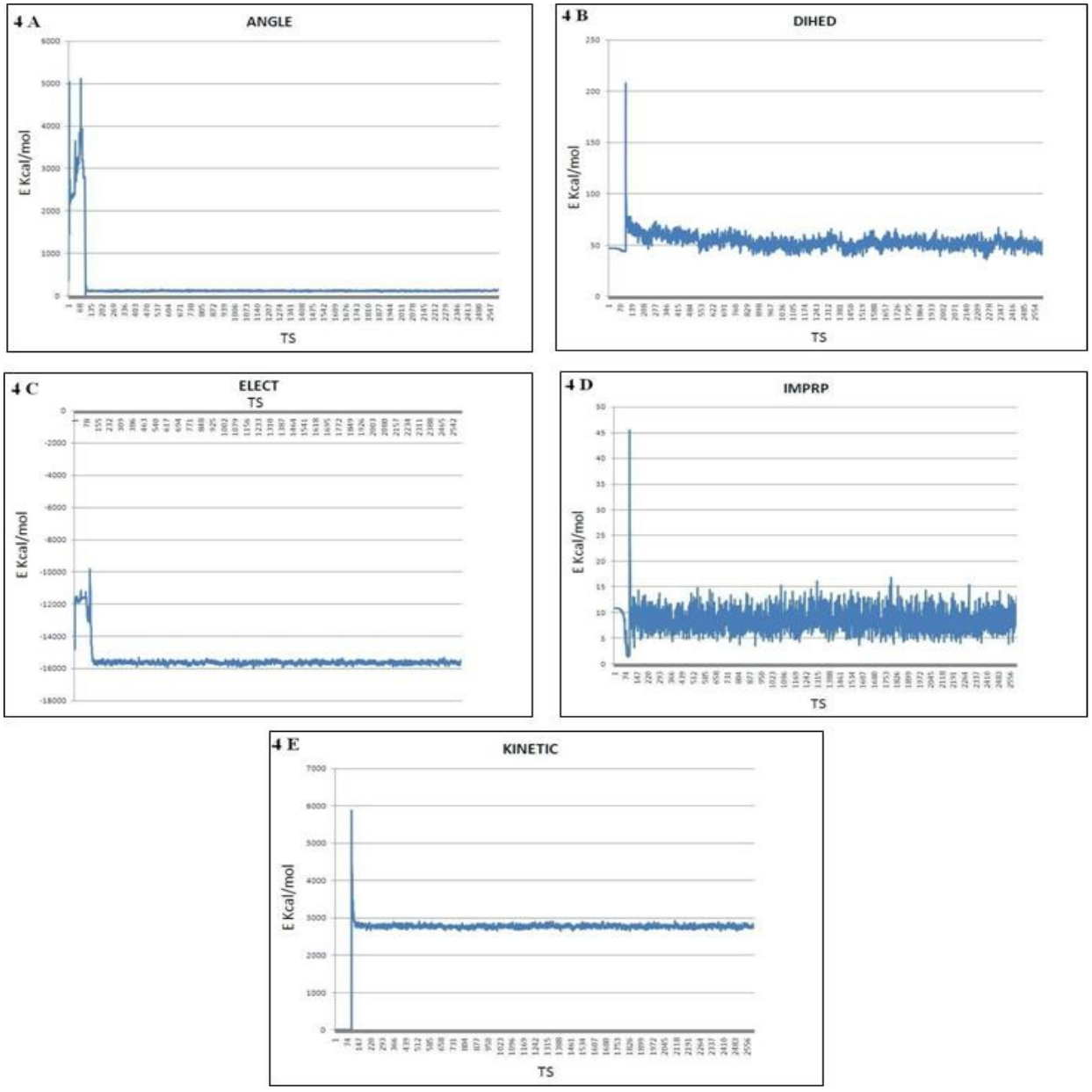


**Figure 4:** Plots of Time Step of simulation (ps) against Energy (kcal/mol) for ESP1 peptide. 4A: Angle, 4B: Dihedral, 4C: Electrostatic, 4D: Improper, 4E: Kinetic Energy T.S:- Time step

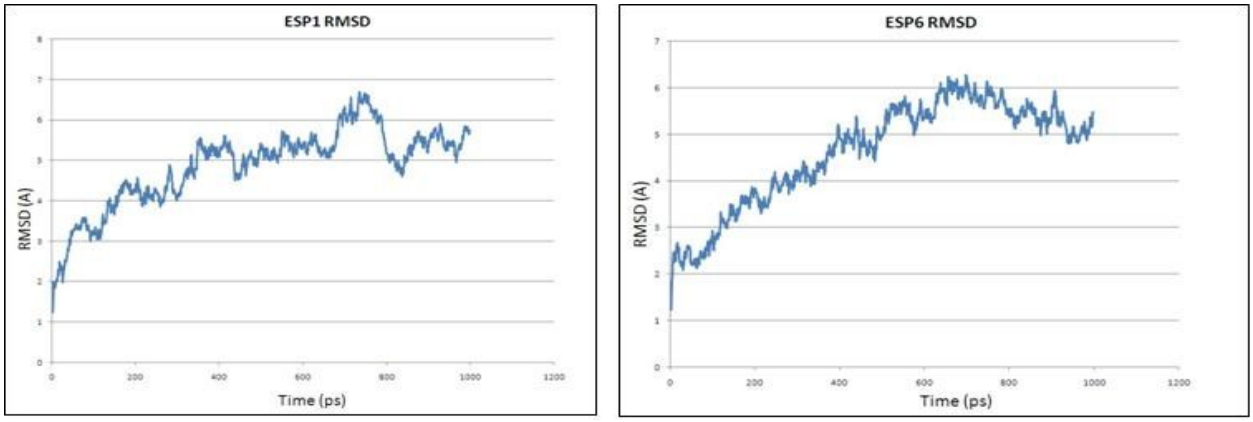


**Figure 5:** Plot of RMSD (A) of peptide backbone against Time (ps)

## REFERENCES


1. Colditz, G; Brewer, T; Berkey, C; Wilson, M; Burdick, E; Fineberg, H and Mosteller, F (1994), "Efficacy of BCG vaccine in the prevention of tuberculosis ", ***JAMA***, Vol. 271, 698-702.
2. Andersen, P; Askgaard, D; Gottschau, A; Bennedsen, J; Nagai, S; and Heron, I (1992), "Identification of immunodominant antigens during infection with Mycobacterium tuberculosis", ***Scand J Immunol***., Vol.36, 823-831.
3. Andersen, P and Heron, I (1993), "Specificity of a protective immune response against *Mycobacterium tuberculosis*", ***Infect Immun***., Vol.61, 844-851.
4. P, Andersen (1994), "Effective vaccination of mice against Mycobacterium tuberculosis infection with a soluble mixture of secreted mycobacterial proteins", ***Infect Immun***, Vol.62 2536-2544.
5. Andersen, P; Andersen, A; Serensen, A and Nagai, S (1995), "Recall of long-lived immunity to Mycobacterium tuberculosis infection in mice", ***J Immunol***, Vol.154, 3359-3372.
6. Kumar, M; Meenakshi, N; Sundaramurthi, J; Kaur, G; Mehra, N and Raja, A (2010), "Immune response to Mycobacterium tuberculosis specific antigen ESAT-6 among south Indians", ***Tuberculosis***, Vol.90, 60-69.
7. Singh, H and Raghava, G (2001), "ProPred: Prediction of HLA-DR binding sites", ***Bioinformatics***, Vol.17, 1236-1237.
8. Parker, K; Bednarek, M and Coligan, J (1994), "Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains", ***J Immunol***, Vol.152, 152-163.
9. Reche, P; Glutting, J; Zhang, H and Reinherz, E (2004), "Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles", ***Immunogenetics***, Vol.456, 405-419.
10. Rammensee, H; Bachmann, J; Emmerich, N; Bachor, O and Stevanovic, S (1999), "SYFPEITHI: Database for MHC ligands and peptide motifs", ***Immunogenetics***, Vol.50, 213-219.
11. Humphrey, W; Dalke, A and Schulten, K (1996), "VMD - Visual Molecular Dynamics", ***J. Mol Graphics***, Vol.14, 33-38.
12. Adcock, SA and McCammon, JA (2006), "Molecular Dynamics: Survey of Methods for Simulating the Activity of Proteins", ***Chem. Rev.***, Vol.106, 1589-1615.
13. Boeckmann, B; Bairoch, A; Apweiler, R; Blatter, M; Estreicher, A; Gasteiger, E; Martin, M; Michoud, K; O'Donovan, C; Phan, I; Pilbout, S and Schneider, M (2003) "The Swiss-Prot Protein Knowledgebase and its supplement TrEMBL", ***Nucleic Acids Res***, Vol.31, 365-370.
14. Guex, N and Peitsch, M (1997), "SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling", ***Electrophoresis***, Vol.18, 2714-2723.
15. Pettersen, E; Goddard, T; Huang, C; Couch, G; Greenblatt, D; Meng, EC and Ferrin, TE (2004), "UCSF Chimera--a visualization system for exploratory research and analysis",***J Comput Chem***, Vol.25 (13), 1605-1612.
16. Cole, ST (1999), "Learning from the genome sequence of Mycobacterium tuberculosis H37Rv", ***FEBS Lett***, Vol.452, 7-10.
17. Cole, ST; Eiglmeier, K; Parkhill, J; James, KD; Thomson, NR; Wheeler, PR; Honore, N; Garnier, T; Churcher, C; Harris, D; Mungall, K; BashamBrown, DD; Chillingworth, T and Connor, R (2001), "Massive gene decay in the leprosy bacillus", ***Nature***, Vol.409, 1007-1011.
18. Maheiras, GG; Sabo, PJ; Hickey, MJ; Devinder, C and Stover, CK (1996), "Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis", ***J Bacteriol***, Vol.178, 1274-1282.

19. Flower, DR; Phadwal, K; Macdonald, IK; Coveney, PV; Davies, MN and Wan, S (2010), "T-cell epitope prediction and immune complex simulation using molecular dynamics: state of the art and persisting challenges", ***Immunome Res***, Vol.6, S2-S 4.