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Interactions of Transcription Factors in HLA Class I Transcriptosome

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***Abstract*: Promoter region of Human Leukocytic Antigen class I (HLA-I) has two main regions namely, enhancer A (EnhA) and enhancer B (EnhB). Various transcription factors (TFs) bind either to EnhA or EnhB region regulate the HLA-I expression. These TFs are associated with different diseases in human. Experimental evidences suggest that EnhA is responsible for the maintenance of basal expression, while EnhB may be associated with the regulation of inducible expression of HLA-I. Though 3D structural information of EnhA binding TFs are available; however, structural information of EnhB region binding TFs are not yet available. Therefore, comparative functionality between these two regions at the molecular level is yet to determine. Hence, we have predicted 3D protein structure of several EnhB region binding TFs first, then performed molecular dynamics simulation followed by molecular docking and hypothesize that EnhA region binding TFs are more potent than the EnhB region binding TFs in regulating the HLA-I expression.**

***Keywords*:** HLA, gene regulation, transcription factor, molecular modeling, molecular docking.

I. Introduction

The HLA class I molecules are ubiquitously expressed on the surface of most of the nucleated cells of human. It plays a crucial role in immunological recognition. With the availability of the upstream promoter sequences of different HLA classes including their different allelic forms, it was possible to make an alignment of the promoter sequences that revealed presence of two broad regions –EnhA (also known as CRE, Class I Regulatory Element) and EnhB (also known as MARM, MHC antigen regulatory module). EnhA located between -150 to -200 bp upstream of transcription initiation site [1] responsible for the constitutive expression of HLA-I. EnhB, located between -60 to -120 bp upstream of transcription initiation site, is responsible for inducible expression of HLA and has a sequence similarity in different classes of HLA promoter region [2, 3].

Different transcription factors (TRFs) are identified that regulate the HLA-I gene transcription by binding to these promoter regions. Recombinant DNA technology based study established that different members of Rel family, either as heterodimer or homodimer bind to the EnhA region of HLA-I promoter region. RelA (p65) is a strong transactivator of HLA-I genes in the form of heterodimer with NF-κB1 (p50) [4, 5]. The dominant homodimeric form of NF-κB1 (p50-p50) was shown to inhibit basal and to a laser extent cytokine mediated HLA-I expression [6, 7, 8]. NF-κB1 and NF-κB2 are closely related and both synthesized as large precursor protein (MW 105 and 100 KD, respectively), have the Rel homology domain at their N-terminal, and an ankyrin repeats structure in C-termini. by which they are sequester in the cytoplasm by IκB, until they are stimulated with some agents like TNF or IL-1 [9]. The large precursor form of NF-κB can bind with Rel protein but it appears to be localized predominantly in cytoplasm [10, 11]. Although RelA may function as a homodimer, its activity is potentiated by association with NF-κB1 (p50) and/or NF-κB2 (p52) possibly because the heterodimeric forms have a higher affinity for DNA [12]. The altered or aberrant binding activity of NF-κB /RelA by different oncogenes is reported in several human diseases including cancer [13-17].

Several TFs namely RFX5, RFXB (RFXANK), RFXAP and CIITA binds to the X1 box of MARM regulate expressions of different classes of HLA genes. Several workers have shown the altered expression/binding of these proteins in several human diseases. [13, 18-20].

X2 box of MARM is bound by X2BP, a complex which contains factors related to or identical to members of the ATF (activating transcription factor) or CREB (cAMP response element binding protein) family of proteins. It is reported that CREB along with X1 binding protein can transactivate the HLA gene [21].

A region in the promoter of HLA-I, called TATA box is present just proximal to the transcription initiation site. TBP binds to this region. This transcription factor is association with other general transcription factor such as TFIIA, TFIIB and other TBP associated factors occupies a central place in the general transcriptionary complex and regulate basal transcription of genes. Mutation in the TBP disrupts CIITA mediated transcription [22].

Crystal structures of the following proteins are available: 1. combinations of p50-p65 (RelA) [23], 2. combinations of p50-p50 (NF-κB1) [24], 3. partial crystal structure of CREB1 sequence 1-55 [25] and 4. TBP [26].

Several biochemical studies establish interactions among the X1 and/or X2 box binding TFs [27-30]. However, high-resolution 3D (crystal and/or NMR) structures of several transcription factors that bind to HLA-I promoter are still not available. Recently a bioinformatics based study predicts the 3D structure of X1 box associated TFs (Figure 1) and labile

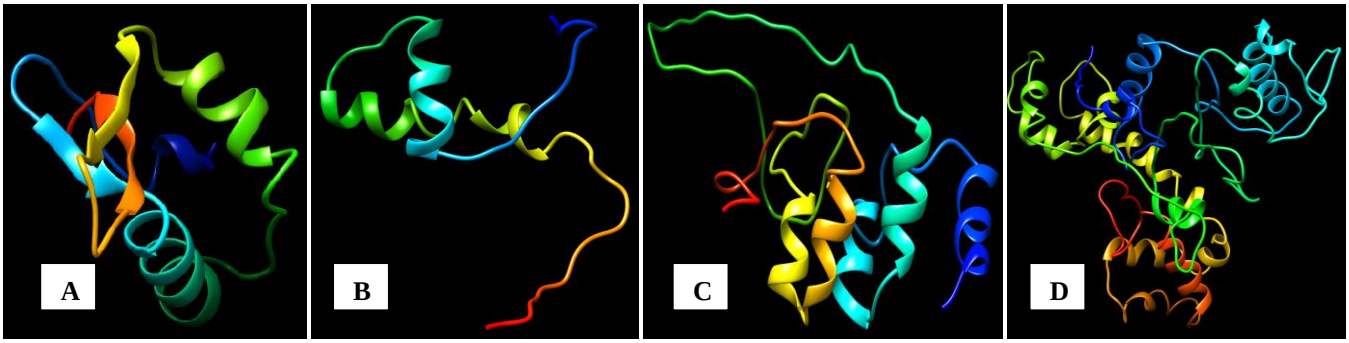

Figure 1. Predicted model of X1-box associated TFs: RFX5 (A), RFXAP (B), RFXANK (C), CIITA CARD domain (D).

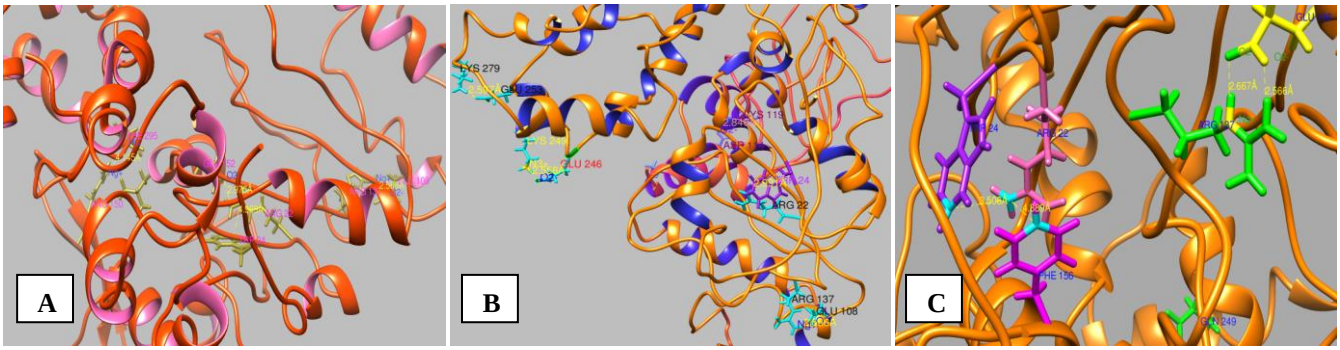

Figure 2. Interaction between CIITA (CARD domain) & RFX5 (in A), Interaction between CIITA (CARD domain) & RFXANK (in B), Interaction between CIITA (CARD domain) & RFXAP (in C)

interactions among them (Figure 2) [31]. Therefore, relative potentiality among these two HLA-I promoter regions remains to be elucidated. Hence, the molecular interactions study between these TRFs may hint towards this direction.

II. Materials and Methods

3D structure of several proteins specially, EnhA region binding TFs are already available. However, 3D structure of EnhB region binding TFs are not available. For predicting those protein structures we have used MODELER 9v8. After getting all the structures of proteins we have performed molecular docking using HEX v6.3.

A. Finding of EnhA region binding TFs

Different transcription factors like RelA, NF-κB1 and TBP bind to the different regions of HLA-I promoter region. The crystal structures of those TFs are available obtained from the Research Collaboratory for Structural Bioinformatics Protein databank (RCSBPDB). The PDB ID of those structures is depicted in Table 1.

Table 1. Enhacer A Binding Protein Sequences (Human).

Protein	PDB ID
Combination of p50-p65 (RelA)	1VKX
Combination of p50-p50 (NF-κB1)	1NKF
TBP	1TGH

B. X1-Box of EnhB region binding TFs

Recently a bioinformatics based study predicts the 3D structure (Figure 1) of X1-box binding TFs [31] and predicted interactions among them (Table 5).

C. Prediction of X2-Box of EnhB binding TF: Molecular Modeling

For predicting protein structures of X2-Box binding TF CREB1, we have followed the previously mentioned methods [31]. The sequential steps are –

1. Download the sequence of the protein of interest (target) from NCBI, Gi No: 4758054, NCBI ID: NP_004370.1, Length: 327aa (http://www.ncbi.nlm.nih.gov/);
2. Sequence search based homology or physiochemical similarity by using Blast, Phyre, JPRED, Modbase and Fugue [32-36]. Template which showed the highest e-value or Z-score (Fugue) was chosen;
3. Sequence similarity between target and template was checked by ClustalW [37, 38];
4. If there is >30% sequence similarity in between target and template in ClustalW, 3D model building of target sequence is done using MODELER 9v8 [39, 40]. Out of five, single model are selected according to the lowest DOPE (Discrete Optimized Protein Energy) and highest GA341 (Score for the reliability of a Model having the probability of the correct fold is larger than 95% [41] assessment score.
5. Missing side chain in the generated protein structure is checked and if needed structure refinement is done by WHAT IF [42].

6. Then protein structure was validated through Ramachandran plot (>80% in favorable/allowed region) or by G score (>0.5) using PROCHEK [43, 44].

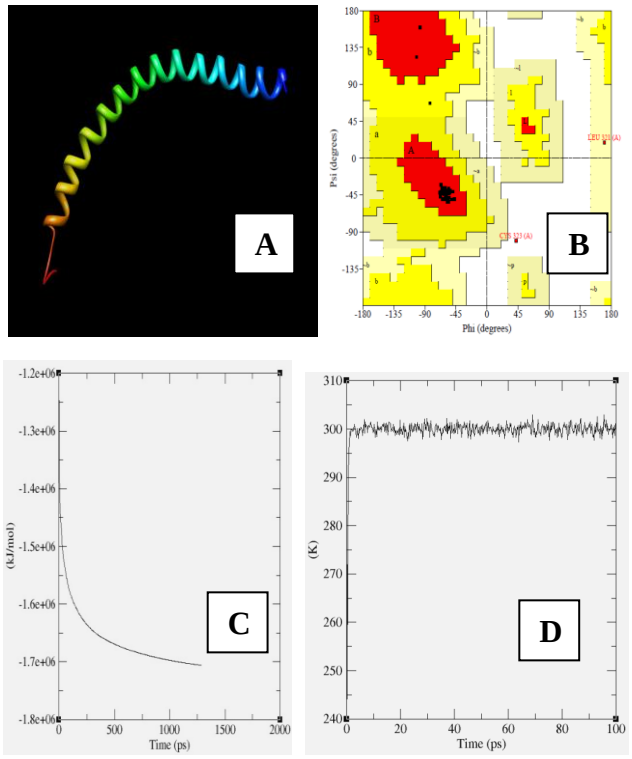

Figure 3. CREB1: Predicted model (in A), Ramachandran plot for the predicted model (in B), Potential energy minimization graph (in C), and Temperature graph (in D).

D. Molecular Dynamics: Energy Minimization

Molecular dynamics (MD) simulations for adding of ionic solution were performed using GROMACS (v4.5.4) following GROMACS manual [45]. Briefly, the steps are:

1. Converting the .PDB file into gromacs file and generate topology followed in OPLS-AA/L all-atom force field (2001 amino-acid dihedrals).
2. For solvate the protein, a cubic box is set; then water and ions are added into the box using gromacs.mdp file;
3. Energy minimization is performed by using GROMACS energy minimization input file that is depicted in potential energy minimization graph. GROMACS uses steepest descent minimization algorithm and simulation is performed with maximum number (50,000) of iterative steps for minimization, energy step size (0.01) and stop minimization when the maximum force <1000.0 kJ/mol/nm.
4. After minimization the solvent and ions around the protein is equilibrated by applying temperature (based on kinetic energies), and pressure (system until it reaches the proper density). Equilibration is often conducted in two phases. The first phase is conducted under an *NVT* ensemble (constant Number of particles, Volume, and Temperature). This ensemble is also referred to as "isothermal-isochoric" or "canonical". This process performs in 0.002 picoseconds (ps). The second phase of equilibration process is *NPT* ensemble, wherein the Number of particles, Pressure, and Temperature are all constant. The ensemble is also called the "isothermal-isobaric" ensemble, and most closely resembles experimental conditions.
5. Lastly 1 nanosec. MD simulation is performed with 5,00,000 steps with an integration time 0.002 picoseconds (ps). The lowest energy was selected for docking studies.

Following the minimization process, the protein-protein binding site of X1-Box of EnhB binding TFs were noted [31]; and predicted binding between EnhA, X1- and X2-Box occupying TFs with TBP by predicting servers – PPI-Pred [46].

E. Molecular Docking: Molecular Interaction Study

Molecular docking is performed using Hex program – to predict the intermolecular complex formed between two constituent molecules and interactions between them [47, 48]. It uses spherical polar Fourier (SPF) correlations to accelerate the calculations. Using this program we docked those proteins in different receptor ligand combination. Previously, we have performed docking and interaction study among the X1 box of EnhB (MARM) binding TFs. These are: 1. RFX5 (R) & RFXANK (L); 2. RFX5 (R) & RFXAP (L); 3. RFXANK (R) & RFXAP (L); 4. CIITA (CARD domain) (R) & RFX5 (L); 5. CIITA (CARD domain) (R) & RFXAP (L); 6. CIITA (CARD domain) (R) & RFXANK (L) (Table 4, Figure 2). [31] Here we have followed similar methods to study the interactions among other proteins along with the previously predicted proteins in different combinations. These are:

1. NF-κB1 - NF-κB1 (R) & TBP (L); 2. RelA - NF-κB1 (R) & TBP (L); 3. CIITA (CARD domain) (R) & TBP (L); 4. RFX5 + RFXAP + RFXANK + CIITA (CARD domain) (R) & TBP (L); 5. CREB1 (R) & TBP (L); 6. RFX5 + RFXAP + RFXANK + CIITA (CARD domain) (R) & CREB1 (L); 7. CIITA (CARD domain) (R) & CREB1 (L), where R and L represent receptor and ligand respectively.

III. Results

A. Selection of suitable template for CREB1

Using step 1-2 as mentioned in section II.C, the chosen templates are depicted in Table 2.

Table 2. E-score and Z-score of Different Template Search Programs for CREB1

Server	E-values	HIT
PDB-blast	1e-24	1dh3
FUGUE	22.85(Z-score)	1dh3
Phyre (V0.2)	0.44	1dh3
Swiss-model	8e-20	1dh3

B. Protein structure prediction

Using step 3-6 as mentioned in section II.C, the predicted model for CREB1 are depicted in Figure 3 (A). Predicted structure of CREB1 protein molecules are checked through Ramachandran plot. The results of PROCHECK for representation of Ramachandran plot (Figure 3(B)) shows 94.3% of residues in the most favoured regions, 1.9% in additionally allowed regions.

C. Molecular Dynamics

Using MD, energy minimization for the predicted protein molecule is done for CREB1 (Figure 3 (C) and (D)).

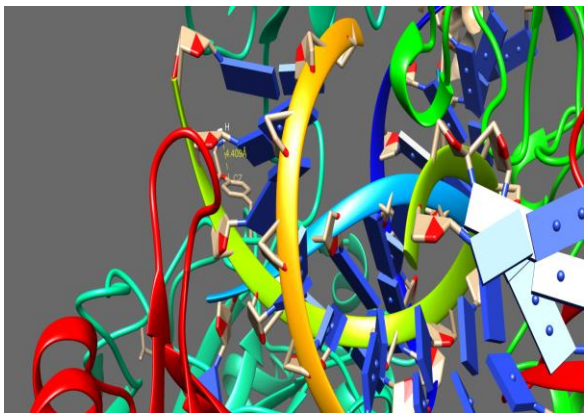

Figure 4. Docking complex between NF-κB1-NF-κB1 and TBP protein. Yellow dotted indicate salt bridge.

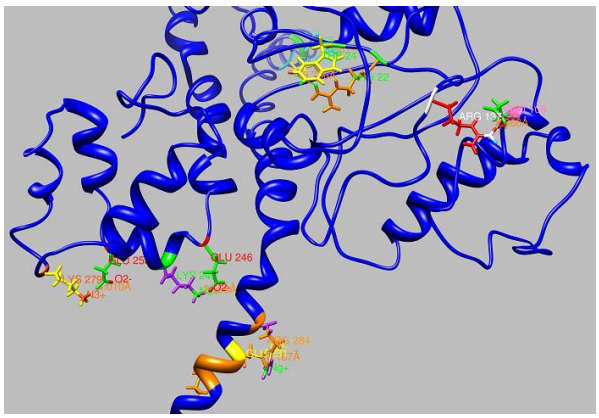

Figure 5. Interaction between CIITA (CARD domain) and CREB1 protein. Yellow color indicates the pi cation.

Table 3. Predicted Protein-Protein binding Site Pocket.

Proteins	Residues
CIITA (CARD) & RFX5	127, 126, 113, 45, 17, 16, 43, 44, 53, 209, 145, 42, 41, 40, 116, 49, 13, 39, 38, 37, 36, 118, 143, 144, 32, 33, 10, 9, 34, 31, 30, 8, 35, 5, 212, 213, 180, 7, 6, 184, 4, 3, 2, 183, 1
CIITA (CARD) & RFXANK	86, 87, 85, 84, 88, 89, 96, 97, 98, 90, 99, 102, 106, 83, 82, 81, 95, 100, 101, 105, 137, 109, 93, 94, 132, 128, 134, 136, 139, 108, 110, 79, 78, 75, 67, 103, 48, 135, 50, 123, 129, 125, 133, 167, 111, 112, 71, 80, 72, 68, 64, 63, 70, 119, 120, 130, 121, 164, 165, 166, 131, 156, 138, 114, 113, 77, 73, 69, 51, 155, 21, 140, 45, 19, 20, 18, 74, 107, 47, 116, 49, 118
CIITA (CARD) & RFXAP	123, 119, 135, 50, 100, 67, 128, 129, 130, 132, 99, 48, 70, 71, 95, 121, 98, 102, 103, 80, 145, 214, 143, 133, 101, 97, 73, 49, 118, 36, 155, 131, 134, 105, 106, 85, 86, 87, 77, 47, 116, 154, 157, 156, 165, 136, 137, 109, 88, 81, 44, 45, 40, 160, 164, 167, 166, 139, 108, 83, 84, 82, 79, 74, 111, 110, 112, 113, 153, 159, 161, 162, 163, 107, 114, 43, 16, 168, 21, 138, 140, 18, 17, 39, 24, 158, 169, 19, 20, 171, 170, 14
RFX5 & RFXANK	106, 101, 102, 105, 143, 109, 116, 120, 142, 112, 119, 123, 124, 130, 141, 115, 122, 125, 121, 138, 137, 118, 126, 129, 134, 110, 127, 182, 133, 179, 196, 178, 212, 200, 204, 208, 202, 205, 136, 135, 139
RFX5 & RFXAP	167, 99, 98, 97, 100, 143, 116, 120, 95, 94, 142, 119, 123, 124, 117, 214, 215, 122, 125, 121, 128, 217, 220, 118, 126, 127, 129
RFXANK & RFXAP	105, 102, 109, 106, 139, 136, 138, 101, 119, 116, 143, 110, 142, 127, 130, 134, 251, 120, 122, 121, 135, 137, 204, 124, 125, 123, 133, 126, 179, 129, 196, 178
NFκB1 - NFκB1 & TBP	55, 57, 114, 116, 137, 102, 466, 240, 141, 143, 144, 145, 147, 148, 179, 220, 226, 248, 249, 271, 273, 199, 202, 203, 205, 206, 207, 208, 239, 241, 242, 243, 244, 274, 358, 302, 312, 359, 360, 361, 362, 364, 365, 366, 367, 369, 391, 392, 396, 397, 399, 401, 403, 406, 407, 408, 409, 410, 412, 420, 422, 423, 424, 425, 426, 427, 428, 429, 457, 482, 484, 487, 488, 490, 492, 494, 501, 511, 513, 514, 518, 519, 522, 525, 526
RelA - NFkB1 & TBP	428, 100, 113, 115, 116, 196, 226, 271, 272, 78, 79, 153, 156, 224, 494, 495, 500, 231, 233, 257, 258, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 41, 42, 43, 44, 45, 46, 47, 49, 51, 52, 53, 56, 57, 58, 91, 92, 115, 116, 117, 118, 119, 186, 222, 223, 275, 276, 278, 371, 372, 373, 397, 398, 412, 414, 416, 418, 419, 452, 454, 456, 516, 221, 225, 236
CIITA (CARD) & TBP	169, 214, 248, 201, 204, 205, 211, 188, 214, 224, 14, 19, 20, 170, 10, 12, 13, 16, 121, 146, 148, 256, 275, 310, 311, 158, 159, 206, 207, 218, 74, 80, 103, 107, 24, 25, 144, 146, 151, 152, 97, 111, 112, 109, 167, 187, 190, 110, 193, 194, 210, 136, 156, 165, 167, 169, 178, 179, 182, 240, 249, 292, 293, 294, 262, 265, 266, 301, 302, 97, 111, 114, 126, 243, 245,247, 263, 305, 308, 309, 14, 170, 333, 279, 11, 12, 15, 22, 23, 25, 27, 28, 29, 30, 31, 32, 33, 35, 36, 39, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 65, 66, 69, 102, 249, 245
CREB1 & TBP	274, 275, 276, 277, 278, 281, 284, 285, 287, 288, 290, 291, 169, 214, 248, 110, 193, 194, 210, 206, 207, 208, 218, 293, 319, 323, 109, 167, 214, 215, 277, 107, 310, 312, 272, 266, 267, 279, 302, 333, 113, 293, 294, 297, 178, 179, 182, 240, 271, 274, 106, 114, 115, 297, 300, 301, 121
CIITA (CARD) & CREB1	292, 293, 57, 296, 126, 291, 295, 299, 300, 238, 125, 128, 56, 151, 124, 130, 129, 132, 123, 63, 62, 54, 302, 306, 307, 304, 235, 294, 150, 154, 155, 121, 133, 100, 135, 119, 50, 55, 298, 305, 309, 301, 297, 156, 131, 48, 70, 71, 67, 69, 66, 52, 51, 53, 149, 153, 159, 165, 136, 122, 236, 232, 213, 308, 147, 26, 146, 24, 167, 73, 49, 118, 143, 145, 120, 209, 148, 28, 29, 210, 212, 228, 27, 158, 36, 144, 243, 31, 2, 207, 30, 32, 174, 173, 171, 25, 170, 169, 168, 21, 138, 44, 45, 47, 116, 40, 37, 33, 34, 8, 5, 3, 176, 205, 175, 11, 172, 19, 20, 140, 114, 41, 35, 7, 6, 4, 177, 180, 13, 14, 18, 43, 42, 38, 184, 9, 10, 17, 16, 39

D. Molecular Interaction Study

The docking results between different combinations of protein molecules are tabulated in Table 4. Interactions and docking complex between different combinations of proteins are depicted in Figure 4-10. The figures are prepared using UCSF Chimera software. The combinations of the interacting residues are tabulated in Table 5. We have made an attempt to find out the protein protein interaction binding site of docking complex between RFX5+ RFXAP+RFXANK+CIITA (CARD domain) and TBP; and RFX5+ RFXAP+RFXANK+CIITA (CARD domain) and CREB1, however, are not predicted due to larger size of complex (atom size more than 10,000). The predicted protein-protein binding site residues are listed in Table 3.

IV. Discussion.

Several TFs bind to the specific region of the HLA class I promoter region. Several members of Rel family, in different combinations, namely Rel-NF-κB1, NF-κB1- NF-κB1, NF-κB1- NF-κB2 bind to the EnhA region. It is reported that these TFs are responsible for the maintenance of basal expression level of HLA class I [2, 5]. Several oncogenic proteins make hindrance in binding of Rel to the EnhA region. This may be the cause of reduction of HLA class I surface

expression by the cancer cells, which help them to escape from immune attack [13, 14].

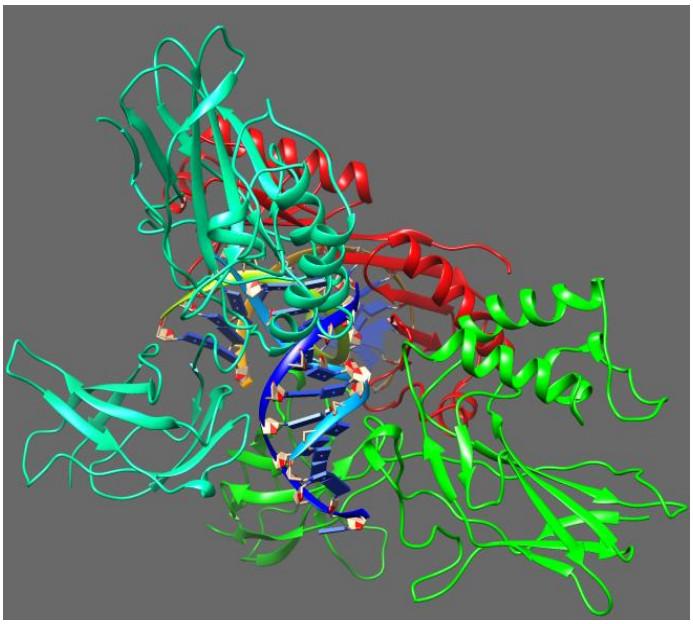

Figure 6. Docking complex of RelA-NF-κB1 & TBP protein.

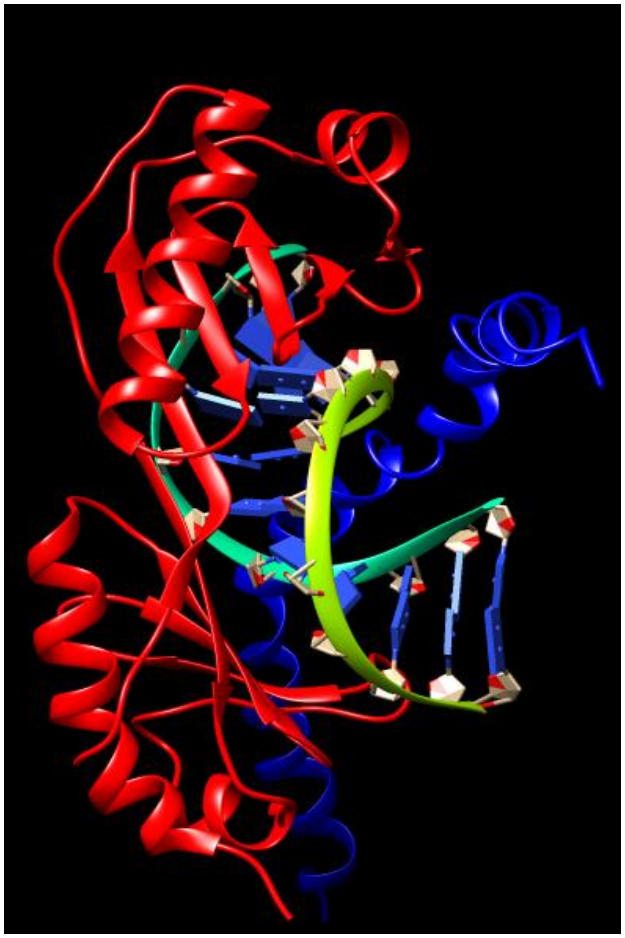

Figure 7. Docking Complex of CREB1 protein and TBP.

Our docking study shows that the binding efficiency of heterodimer Rel - NF-κB + TBP is same that of homodimer NF-κB – NF-κB + TBP. This indicates that the homodimeric form of NF-κB is capable of inhibiting heterodimer Rel – NF-κB functionality in transactivating HLA class I genes. This corroborates the earlier findings [2, 8, 49].

Table 4. Molecular Interaction Study Using Docking.

Interaction Study	Protein-Protein Docking		E-Value
	Receptor Protein	***Ligand Protein***	
1	NFκB1 - NFκB1	TBP	-702.5
2	RelA - NFκB1	TBP	-702.5
3	CIITA (CARD domain)	TBP	-682.1
4	CIITA (CARD domain)	RFX5	-634.2
5	CIITA (CARD domain)	RFXANK	-608.3
6	RFX5 + RFXAP + RFXANK + CIITA (CARD domain)	TBP	-605.3
7	CIITA (CARD domain)	RFXAP	-602.03
8	CIITA (CARD domain)	CREB1	-598.6
9	CREB1	TBP	-587.6
10	RFX5	RFXANK	-586.1
11	RFX5 + RFXAP + RFXANK + CIITA (CARD domain)	CREB1	-550.92
12	RFX5	RFXAP	-541.7
13	RFXANK	RFXAP	-531.4

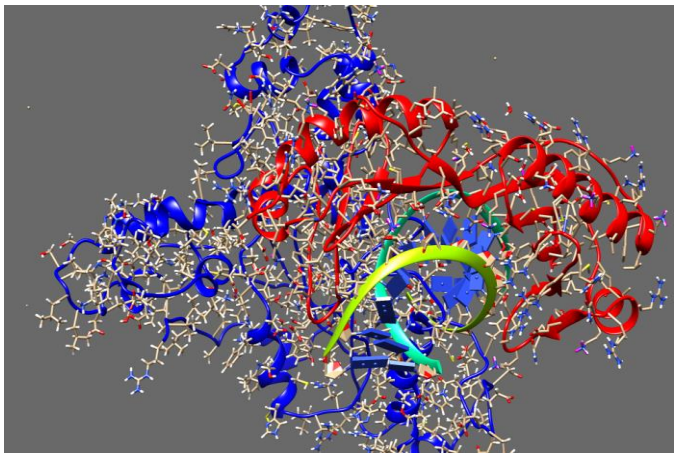

Figure 8. Docking complex of CIITA CARD domain and TBP protein. Blue color indiactes CIITA and Red color indicates TBP.

Table 5. Docking results of different combination proteins and their interact residue.

Protein A (Receptor)	**Protein B (Ligand)**	**E total**	**Intermolecular interaction (Protein A <-> Protein B)**		
			Residue Name & Atom name	***Distance (Å)***	***Bond***
NFκB1 - NFκB1	TBP (Figure 4)	-702.5	ASP 172 OD2 <-> HIS 170 ND1	3.60	Salt-Bridge1
			ASP 172 OD2 <-> HIS 170 NE2	3.31	Salt-Bridge2
			ASP 220 OD2 <-> ARG 227 NE	4.07	Salt-Bridge3
			ASP 259 OD2 <-> LYS 261 NZ	3.27	Salt-Bridge4
			ASP 259 OD1 <-> LYS 305 NZ	3.56	Salt-Bridge5
			ASP 276 OD2 <-> ARG 290 NH1	4.27	Salt-Bridge6
			ASP 302 OD2 <-> ARG 252 NH2	4.94	Salt-Bridge7
			GLU 60 OE2 <-> ARG 54 NH2	4.64	Salt-Bridge8
			GLU 60 OE2 <-> ARG 56 NH1	4.65	Salt-Bridge9
			GLU 60 OE1 <-> ARG 54 NE	4.35	Salt-Bridge10
			GLU 152 OE2 <-> ARG 195 NE	3.62	Salt-Bridge11
			GLU 187 OE2 <-> ARG 199 NE	4.41	Salt-Bridge12
			GLU 190 OE1 <-> ARG 184 NH2	3.32	Salt-Bridge13
			GLU 192 OE2 <-> ARG 195 NH2	3.33	Salt-Bridge14
			GLU 202 OE2 <-> LYS 232 NZ	4.03	Salt-Bridge15
			GLU 223 OE1 <-> ARG 314 NE	3.29	Salt-Bridge16
			GLU 224 OE2 <-> ARG 227 NE	3.93	Salt-Bridge17
			GLU 284 OE2 <-> LYS 317 NZ	3.70	Salt-Bridge18
			GLU 293 OE1 <-> ARG 281 NE	4.13	Salt-Bridge19
			GLU 338 OE1 <-> ARG333 NH1	4.14	Salt-Bridge20
			ARG 332 CB <-> PHE 262 CD1	4.64	Pi-cation1
			TYR 238 CZ <-> ARG 51 HH21	4.40	Pi-cation2
RelA - NFκB1	TBP (Figure 6)	-702.5	ASP 172 OD2 <-> HIS 170 ND1	3.60	Salt-Bridge1
			ASP 172 OD1 <-> HIS 170 ND1	3.31	Salt-Bridge2
			ASP 220 OD1 <-> ARG 227 NE	4.07	Salt-Bridge3
			ASP 259 OD2 <-> LYS 261 NZ	3.27	Salt-Bridge4
			ASP 259 OD2 <-> LYS 305 NZ	3.56	Salt-Bridge5
			ASP 276 OD2 <-> ARG 290 NE	4.27	Salt-Bridge6
			ASP 302 OD2 <-> ARG 252 NH2	4.94	Salt-Bridge7
			GLU 60 OD2 <-> ARG 54 NH2	4.64	Salt-Bridge8
			GLU 60 OE2 <-> ARG 56 NH1	4.65	Salt-Bridge9
			GLU 60 OE1 <-> ARG 54 NE	4.35	Salt-Bridge10
			GLU 152 OE2 <-> ARG 195 NE	3.62	Salt-Bridge11
			GLU 187 OE1 <-> ARG 199 NH2	4.41	Salt-Bridge12
			GLU 190 OE1 <-> ARG 184 NH2	3.32	Salt-Bridge13
			GLU 192 OE2 <-> ARG 195 NH2	3.33	Salt-Bridge14
			GLU 202 OE2 <-> LYS 232 NZ	4.03	Salt-Bridge15
			GLU 223 OE1 <-> ARG 314 NE	3.29	Salt-Bridge16
			GLU 224 OE2 <-> ARG 227 NE	3.93	Salt-Bridge17
			GLU 284 OE1 <-> LYS 317 NZ	3.70	Salt-Bridge18
			GLU 293 OE1 <-> ARG 281 NE	4.13	Salt-Bridge19
			GLU 338 OE1 <-> ARG 333 NH1	4.14	Salt-Bridge20
			ARG 332 NH1<-> PHE 262 CE2	4.23	Pi-cation1
			ARG 51 NH1 <-> TYR 238 HD2	3.59	Pi-cation2
			ARG 281 NH2 <-> TYR 283 CE2	4.14	Pi-cation3
CIITA (CARD domain)	TBP (Figure 8)	-682.1	ASP 259 OD1 <-> LYS 261 NZ	3.27	Salt-Bridge1
			ASP 259 OD1 <-> LYS 305 NZ	3.56	Salt-Bridge2
			ASP 271 OD2 <-> HIS 258 ND1	4.25	Salt-Bridge3
			GLU 108 OE2 <-> ARG 137 NH2	3.27	Salt-Bridge4
			GLU 108 OE1 <-> ARG 139 NH2	3.46	Salt-Bridge5

Table 5. Contd.

Protein A (Receptor)	Protein B (Ligand)	E total	Intermolecular interaction (Protein A <-> Protein B)		
			Residue Name & Atom name	Distance (Å)	Bond
CIITA (CARD domain)	TBP (Figure 8)		GLU 152 OE1 <-> ARG 22 NH1	3.30	Salt-Bridge6
			GLU 152 OE2 <-> LYS 146 NZ	3.82	Salt-Bridge7
			GLU 187 OE2 <-> ARG 199 NH1	4.41	Salt-Bridge8
			GLU 202 OE2 <-> LYS 232 NZ	4.03	Salt-Bridge9
			GLU 223 OE1 <-> ARG 314 NE	3.29	Salt-Bridge10
			GLU 224 OE2 <-> ARG 227 NE	3.93	Salt-Bridge11
			GLU 253 OE2 <-> LYS 279 NZ	3.20	Salt-Bridge12
			ARG 22 HH1 <-> PHE 156 HD2	5.29	Pi-cation1
			ARG 227 HH1 <-> PHE 249 CE2	2.91	Pi-cation2
			ARG 332 HH1 <-> PHE 262 CE2	4.57	Pi-cation3
			ARG 22 HH2 <-> TRP 24 CZ3	2.99	Pi-cation4
			LYS 146 HZ <-> TRP 24 CH2	2.56	Pi-cation5
RFX5 + RFXAP + RFXANK + CIITA (CARD domain)	TBP (Figure 9)	-605.3	ASP 54 OD1 <-> LYS 137 NZ	2.88	Salt-Bridge1
			ASP 104 OD1 <-> ARG 111 NH1	1.93	Salt-Bridge2
			ASP 114 OD1 <-> LYS 119 NZ	2.69	Salt-Bridge3
			ASP 115 OD1 <-> ARG 118 NE	4.78	Salt-Bridge4
			ASP 171 OD2 <-> ARG 157 NH1	4.55	Salt-Bridge5
			ASP 187 OD1 <-> ARG 179 NH1	2.32	Salt-Bridge6
			ASP 259 OE2 <-> LYS 261 NZ	3.27	Salt-Bridge7
			ASP 259 OD2 <-> LYS 305 NZ	3.56	Salt-Bridge8
			ASP 271 OD1 <-> HIS 258 NE2	4.25	Salt-Bridge9
			GLU 101 OE2 <-> ARG 165 NH2	4.09	Salt-Bridge10
			GLU 101 OE2 <-> LYS 167 NZ	2.60	Salt-Bridge11
			GLU 108 OE1 <-> ARG 111 NH1	4.05	Salt-Bridge12
			GLU 108 OE1 <-> ARG 137 NH2	3.27	Salt-Bridge13
			GLU 108 OE1 <-> ARG 139 NH2	3.46	Salt-Bridge14
			GLU 138 OE1 <-> ARG 141 NH1	4.60	Salt-Bridge15
			GLU 145 OE1 <-> ARG 212 NH2	4.46	Salt-Bridge16
			GLU 152 OE1 <-> ARG 22 NH1	3.30	Salt-Bridge17
			GLU 152 OE1 <-> LYS 146 NZ	3.82	Salt-Bridge18
			GLU 187 OE1 <-> ARG 199 NH2	4.41	Salt-Bridge19
			GLU 202 OE1 <-> LYS 232 NZ	4.03	Salt-Bridge20
			GLU 223 OE1 <-> ARG 314 NE	3.29	Salt-Bridge21
			GLU 224 OE2 <-> ARG 227 NE	3.93	Salt-Bridge22
			GLU 246 OE2 <-> LYS 249 NZ	2.99	Salt-Bridge23
			GLU 253 OE2 <-> LYS 279 NZ	3.20	Salt-Bridge24
			GLU 267 OE1 <-> LYS 333 NZ	3.03	Salt-Bridge25
			GLU 280 OE1 <-> ARG 234 NH2	2.95	Salt-Bridge26
CREB1	TBP (Figure 7)	-587.6	ASP 259 OD2 <-> LYS 261 NZ	3.03	Salt-Bridge1
			ASP 259 OD2 <-> LYS 305 NZ	2.82	Salt-Bridge2
			GLU 187 OE2 <-> ARG 199 NH2	2.67	Salt-Bridge3
			GLU 202 OE1 <-> LYS 232 NZ	2.98	Salt-Bridge4
			GLU 223 OE1 <-> ARG 314 NH2	2.64	Salt-Bridge5
			GLU 224 OE1 <-> ARG 227 NH2	2.91	Salt-Bridge6
			GLU 267 OE2 <-> LYS 333 NZ	2.78	Salt-Bridge7
			GLU 273 OE1 <-> ARG 280 NH2	2.82	Salt-Bridge8
			GLU 280 OE1 <-> LYS 289 NZ	2.22	Salt-Bridge9
			GLU 281 OE1 <-> ARG 284 NH2	2.67	Salt-Bridge10
			GLU 305 OE2 <-> ARG 199 NH1	2.83	Salt-Bridge11
			GLU 313 OE2 <-> LYS 316 NZ	2.58	Salt-Bridge12
			ARG 227 CD <-> PHE 249 CZ	4.13	Pi-cation1
			ARG 332 CB <-> PHE 262 CE2	5.83	Pi-cation2
			LYS 295 HZ1 <-> PHE 284 CD1	4.79	Pi-cation3

Table 5. Contd.

Protein A (Receptor)	Protein B (Ligand)	E total	Intermolecular interaction (Protein A <-> Protein B)		
			Residue Name & Atom name	Distance (Å)	Bond
RFX5 + RFXAP + RFXANK + CIITA (CARD domain)	CREB1	-550.92	ASP 54 OD2 <-> LYS 137 NZ	2.72	Salt-Bridge1
			ASP 104 OD1 <-> ARG 111 NH2	3.08	Salt-Bridge2
			ASP 114 OD1 <-> LYS 119 NZ	2.98	Salt-Bridge3
			ASP 115 OD1 <-> ARG 118 NH2	2.77	Salt-Bridge4
			ASP 171 OD1 <-> ARG 157 NH1	2.63	Salt-Bridge5
			ASP 187 OD1 <-> ARG 179 NH1	2.61	Salt-Bridge6
			ASP 271 OD2 <-> HIS 258 NE2	2.73	Salt-Bridge7
			GLU 101 OE1 <-> ARG 165 NE	2.87	Salt-Bridge8
			GLU 101 OE2 <-> LYS 167 NZ	2.60	Salt-Bridge9
			GLU 108 OE1 <-> ARG 111 NH2	2.58	Salt-Bridge10
			GLU 108 OE1 <-> ARG 137 NH2	2.57	Salt-Bridge11
			GLU 108 OE2 <-> ARG 139 NH2	2.95	Salt-Bridge12
			GLU 138 OE2 <-> ARG 141 NH1	2.66	Salt-Bridge13
			GLU 145 OE1 <-> ARG 212 NE	2.82	Salt-Bridge14
			GLU 152 OE2 <-> ARG 22 NH2	2.97	Salt-Bridge15
			GLU 152 OE1 <-> LYS 146 NZ	2.73	Salt-Bridge16
			GLU 170 OE1 <-> LYS 290 NZ	2.68	Salt-Bridge17
			GLU 246 OE1 <-> LYS 249 NZ	3.72	Salt-Bridge18
			GLU 253 OE2 <-> LYS 279 NZ	2.60	Salt-Bridge19
			GLU 273 OE1 <-> ARG 280 NH2	2.82	Salt-Bridge20
			GLU 281 OE1 <-> ARG 284 NH2	2.67	Salt-Bridge21
			GLU 292 OE2 <-> ARG 180 NH1	2.92	Salt-Bridge22
			GLU 313 OE2 <-> LYS 316 NZ	2.58	Salt-Bridge23
CIITA (CARD domain)	CREB1 (Figure 5)	-598.6	GLU 246 OE2<-> LYS 249 NZ	2.558	Salt Bridge1
			GLU 108 OE1<-> ARG 137 NH2	2.566	Salt Bridge2
			GLU 281 OE2 <->ARG 284 NH2	3.167	Salt Bridge3
			GLU 313 OE2 <-> LYS 316 NZ	2.581	Salt Bridge4
			ARG 22 1HH2 <-> TRP 24 HZ3	3.756	Pi-cation1

*Salt Bridge: If the distance between any of the oxygen atoms of acidic residues and the nitrogen atoms of basic residues are within the cut-off distance (3.2 Angstroms) in at least one frame.
**Cation Pi-Interaction: Distance within 6.0 Å of the face of an aromatic ring may engage in polar interactions.
***Hydrogen bond: Distances between donor acceptor distance 3.0 Å and Angle cut-off -20

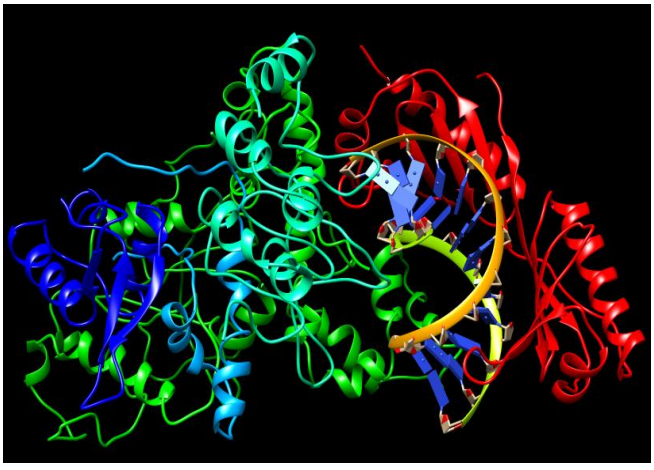

Figure 9. Docking Complex of RFX5, RFXANK, RFXAP, CIITA (CARD domain) and TBP.

Clinical findings suggest that EnhA binding TFs are also associated in several other human diseases like atherosclerosis, coronary artery disease and schizophrenia [15, 16, 17]. Therefore it is to be interest regarding the role of the other enhancer region that is, EnhB region regarding the maintenance of HLA class I expression [20]. In other words, it is of the natural query whether EnhB has any protective role in different pathophysiological state.

To the EnhB region several TFs – RFX5, RFXANK, RFXAP, CIITA and CREB1 bind to transactivate the HLA class I gene. Altered bindings of these TFs are also reported to the associated in several cancers and some auto-immune disorders [13, 18, 19, 50, 51]. Therefore it is needless to point out here that EnhB region binding TFs are also associated with human diseases. There are very few systematic studies have been carried out so far involving both region bindings TFs to establish the differential involvement among these promoter regions and/or TFs. One such study based on the measurement of the expression levels of their TFs in human leukemic cases suggests that the major involvement of EnhA region binding TFs for the proper maintenance of HLA class I surface expression [13]. However, there are no structure based information are available so far regarding this issue. The reason may be due to absence of 3D structural information of the most of the EnhB region binding TFs.

A solution based study with some partial portions of RFX5, RFXANK and RFXAP established interactions among these proteins. In one such attempt, study was conducted with

25-90 amino acid residues of RFX5, 215-272 amino acid residues of RFXAP and 88-260 amino acid residues of RFXB (RFXANK) established that RFXAP can form complex with RFX5 and RFXB. This study identified a glutamine rich region in C-terminal region of RFXAP and a leucine rich region in RFX5 that explain the possible binding among them and postulates further that binding of RFX5 to RFXAP enhances binding to the RFXB and in absence of RFX5, RFXAP does not bind to RFXB and RFXB remains in unfolded state [52, 53]. Recently a bioinformatics based study involving protein structure prediction, molecular dynamics simulation followed by molecular docking reveals whole RFXANK can bind with whole RFXAP but most feebly compared to other interactions within the HLA transcriptosome (Table 4) [31]. This study reveals the possible interactions among the X1-box of EnhB binding TFs (Table 4). Docking studies in this work reveals that the binding capabilities among these TFs are in the following order: RFX5 and RFXANK (E-value -586.1) > RFX5 and RFXAP (E-value -541.7) > RFXANK and RFXAP (E-value -531.4).

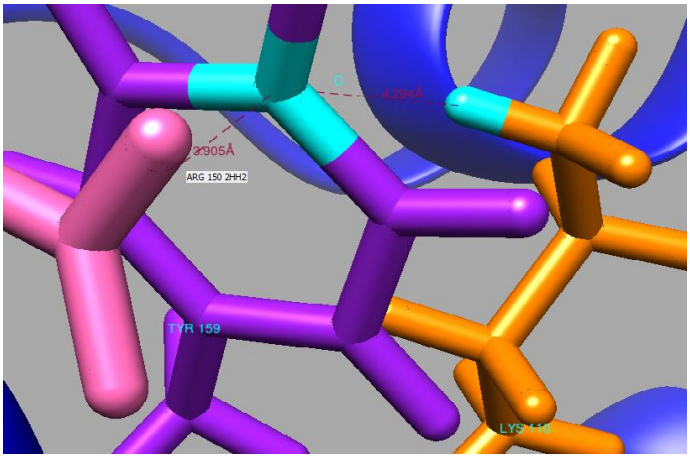

Figure 10. Close view of interaction of docking complexes.Red dotted showing the distances between two residues.

It is hypothesized that X2-box binding TFs CREB1 alone is able to transactivate the HLA class II gene when applied exogenously [54]. Our docking study indicates that the transactivation capabilities of CREB1 (CREB1 + TBP) is much less compared to the EnhA binding region (like Rel-NF-κB + TBP) or X1-box binding regions TFs (like CIITA-CARD domain + TBP) (Table 4). Another important finding of these studies is that though CIITA (CARD domain) is much potent to bind with the TBP, however its binding capability is less than EnhA region binding TFs. It is interesting to note that both EnhA and EnhB regions are present in the promoter region of HLA class I while EnhA is absent in the promoter region of HLA class II. This observation may signify that the origin of HLA class I is much earlier than the HLA class II [3]. The increased binding efficiency of EnhA region binding TFs compared to EnhB binding TFs as revealed by our study may support this hypothesis.

V. Conclusion

Available information indicate that different interactions profiles among the X1 box of EnhB (MARM) binding TFs; however, with partial (specific regions of the proteins) structural information. However, there is no comparative structural information available regarding the transactivation capacity among the EnhA and EnhB binding TFs. Our docking studies indicate that EnhA binding TFs are more potent compared to EnhB binding TFs in the transactivation of HLA class I genes.

By using conventional bioinformatics tools we have predicted the overall 3D structure of the EnhB (X1- and X2-box) region binding TFs. The 3D conformations of the predicted protein models of different TFs qualify the criteria of Ramachandran plot and displayed several meaningful features like secondary structure, charge distribution, conserved residues engaged in non-bonded interaction. As our docking study was done with the molecular dynamic simulation therefore it could be expected that the interaction study among the proteins may mimic the *in vivo* situation of the physiological system.

One important finding of our study indicates that there is no hydrogen bond between any combinations of protein-protein interactions. This confirms the functionality of these TFs are labile in nature in the transactivation of HLA class I genes and thereby, for the regulation of transient immunological regulation [20]. The predicted structure and interactions among these proteins may have an importance for the designing of new drug targeting to these TFs and would be helpful in immune-modulation in future.

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Durjoy Majumder is working as an Assistant Professor of Physiology, West Bengal State University. He received his Ph.D. from Jadavpur University in 2006. He gained his research expertise by working in different premier institutes of India like Calcutta School of Tropical Medicine, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow and Saha Institute of Nuclear Physics, Kolkata. Before joining to his present position, he held a faculty position of Bioinformatics at the School of Information Technology, Bengal Engineering & Science University, Shibpur. His cross-disciplinary research experience led him to be interested in the area of Systems Biology & Systems Medicine. He was the recipient of several prestigious national level fellowships from Dept. of Health & Family Welfare (Govt. of W.B.), DST-SRF (Govt. of India) and CSIR-UGC NET (Govt. of India) fellowship. He has supervised several M.Tech. theses, become the editorial board member of several international peer-reviewed journals, and invited speakers of different international conferences.