1.ABSTRACT

Natural killer (NK) cells are large granular lymphocytes that are involved in the demoliation of the cells infected by virus and the tumour cells. Natural killer (NK) cells can be defined as set of innate immune cells , carrying out continuous surveillance to detect the presence of virally infected or tumor transformed cells. In context to inhibitory signals, the human NK cells express different kinds of inhibitory receptors that are class I human leukocyte antigen (HLA) specific. These NK inhibitory receptors that are MHC specific exhibit abasic and conserved function in regulating cytolysismediated by NK cell.NK cells procure both the receptors, activating and inhibitory that comprise the total NK activity , and the summation of these opposing signals determines whether the target cell will be spared by the NK cell or not. Inhibitory signals very often override the activating signals and hence downregulate the cytotoxicity of NK cells on being recognizedby specific classI MHC molecules present on target cells. NK inhibitory receptors and non classical MHC interactions has been shown to have better inhibition of NK cell cytoxicity than the classical MHC molecule .The multifarious recognition pattern of classical and non classical MHC proteins by NK inhibitory receptors and every accessible surface involved in this binding event should be explored . The knowledge of affinity of NK cell receptor with MHC I ligands will help in understanding the binding patterns required for the inhibition of NK cell activity. The interactions between these classical and non classical MHC with their inhibitors are potentially addressable by computational approaches and can further help to develop NK based cancer therapeutic strategies.

2. INTRODUCTION

Natural killer (NK) cells have the ability that theycandiscriminate between the cells that are healthy and the abnormal ones through a repertoire of their cell surface receptors that regulate their growth, activation, and other effector functions . Natural killer (NK) cells wereoriginally, recognised on a functional basis as this specification was earlier given to lymphoid cells because they were able to causelysis of tumor cell lines if no stimulation was present (Herberman*et al*.,1982). The NK cells portray large granular morphology includesminor population of the lymphocytes found in the peripheral lymphoid tissues and blood , Functionally , NK cells are known to have 'natural' cytolytic activity against virally infected cells and certain tumor (Herberman*et al* .,1982). Different human leukocyte antigen (HLA)-class I-specific inhibitory receptors are expressed on human NK cells. Killer Ig-like receptors (KIR) are a family of these receptors (Wagtmann N *et al* .,1995)., recognizing the shared allelic determinants of the HLA molecules of class I MHC, whereas loose recognition patterns are being displayed by other receptors and so they are distinguished by broad specificity for different HLA class I molecules and identify the non classical HLA-E molecules (CD94/NKG2A).

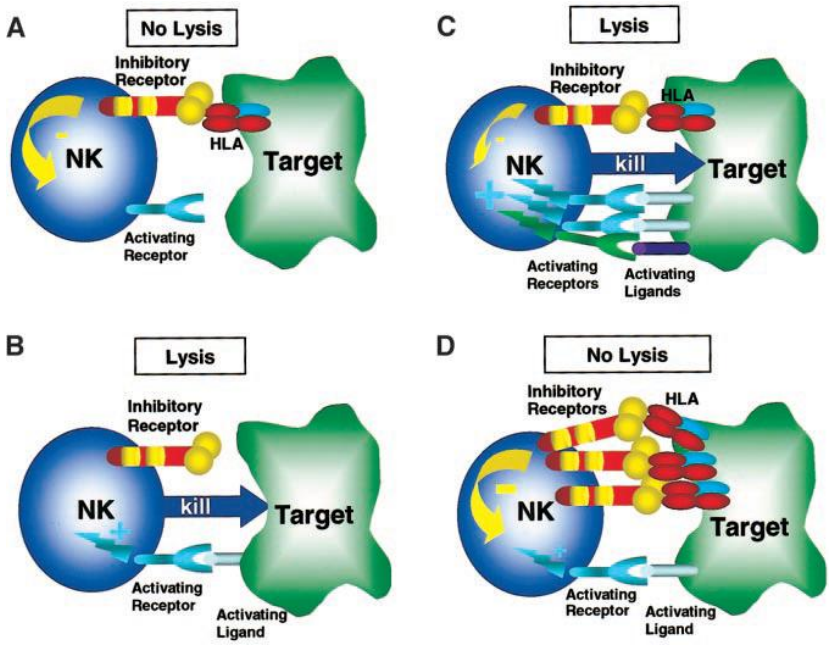


Fig 1:Activating and inhibitory receptors regulating NK cell response .

On target tumor cell surface , the inhibitory receptors (KIR , NKG2A) recognize their ligands HLA class I molecules and then engage them which is followed by the trigerring of the

inhibitory signals whereas the activating receptors on the other hand trigger the activation of NK cell and cause lysisof NK cell on binding to its ligand present on the surface of target cell. In the figure mentioned above the 'A' part depicts that, target cell will not be lysed when inhibitory receptor ligand engagement occurs in absence of interaction of an activating receptor and ligand and a net negative signal is generated, the part 'B' represents that lysis of the target cell will occur when activating receptor ligand engagement occurs in absence of interaction of an inhibitory receptor and ligand and a net activation signal is generated.. In part 'C' the when activating receptor/ligand interactions occur they prevail over the inhibitory receptor/ligand signals which happens to be weak and the outcome is activation of NK cell and ultimately killing the target cell. For eg. In case of occurrence of viral infection or transformation, ultimately the expression of self MHC ligands will be decreased , causing generation of a positive signal and causing lysis of cell. In the last part 'D' interactions occurring between inhibitory receptor and their ligandinitiate a negative signal ,therefore causinglysis of the target cell.

In the early studies of NK cells ,it was suggested that the specific MHC antigens do not restrict the activity of NK cell on the target cells (Herberman*et al* .,1982). However, the recent studies have suggested that higher is the expression of certain MHC class I molecules by the target cells lower is their susceptibility to lysis mediated by NK cells . (KIRs)come under a family of Ig-like extracellular domain receptors that have recently evolved and they are usually present in the nonhuman primates, and happen to be the main receptors for the classical MHC I (HLA-A, HLA-B, HLA-C) . Killing by the NK cell is inhibited when KIR identify MHC I that is expressed on the regular cells. (Iannello*et al* .,2008). These (HLA) class I molecules have been found to be helpful in determining the specificity of the immune response. Because of this diversitya number of advantages are being conferedto them in the way they response to pathogens, but simultaneously they also presenthinderence in distinguishing effects in particular to HLA allele-specificity. Thus ,classification of different HLA alleles overlapping in the peptide-binding specificities is done by HLA class I supertypes. The recognition of the new antigenic peptides, that are derived from infectious agents or cancer cells, that bind to human leukocyte antigen (HLA) class I and II molecules, is of great importance for the development of novel and effecacious vaccines that are capable of activating the cellular arm of immune responses. However, the barrier to this development of peptide-based vaccines with high population coverage is that the restricting HLA genes are highlydistinctiveso their are diverse peptide-binding HLA specificities and minor population coverage for any of the given peptide–HLA specificity. A way to reduce this complexity is by grouping thousands of these different HLA molecules into so-called HLA supertypes. By understanding the correlation of these HLA supertypes with the propensity to certain viral diseases and cancers in different geographical regions of the world , the molecular basis of these diseases could be understood . The genes present in HLA class I and II encode those molecules that areinvolved in the immune responses a against infectious diseases. Such associations between these molecules and the infectious diseases are difficult to track down in history ,comparatively to those associations that are more obvious with the autoimmune diseases. Different combinations of HLA and KIR have affect the outcomes of

the viral diseases, implicatingHLA class I diversity in both the !nnate immune response and the @cquired immune response.

In the ethnic population these HLA are found to highly distinctive including Black, Hispanic, American Indian, Australian aboriginal, Mixed race, Pacific Islander and alsotheirspecificities for peptide binding is different in different allelles. However, majority of alleles come under these HLA supertypes,such that different members of aonesupertype bind to a single peptides,and still exhibiting different repertoires.

Further ,the classical HLA supertypes were found to be associated with different diseases ., studies demonstrated that different HLA supertypes were found causing immune responses to the mumps and measles components of the MMR vaccine. Also , it was observed in patients suffering with pulmonary TB and miliary TB, frequency of A3-like supertypes was high and frequency of A1-like supertypes was high, which suggests that there is activity of natural killer cellis inhibited against the target cells that are infected(A.Balamurugan et al.,2004).

A comparative analysis of these classical and non- classical MHC supertypes is done to understand the binding affinities of these HLA molecules with their inhibitory receptors to further understand their inhibition and activation mechanisms. Also, knowledge of these functional studies can be acquired to ascertain the immunological relevance of associations found with several diseases.

3. REVIEW OF LITERATURE

3.1 Identification of MHC –I by NKinhibitory receptors:

Originally , NK cells were defined as large granular lymphocytes that have the ability to lyse tumour cells. Later they were identified as cells having both effector function produced by cytokines as well as cytotoxic effect (Trinchieri et al.,1989). Cells that are infected with virus or tumourare more prone to cytolyticactivity causing NK cells to release chemokines and cytokines thus initiating inflammatory responses and ultimately regulatory effects are applied on adaptive immune responses (Biron CA et al .,1999).

NK cells are able to kill virus or tumor infected cells leaving the normal cells (Moretta A et al.,2001). The recognition of various receptors that cause the activation and inactivation of NK cells played a major role.(Bottino C et al.,2005). Immune responses that are mediated by NK cells are a result of interaction of NK receptor and its respective ligand..

The inhibitory receptors present on NK cells can whether self MHC molecules are present on target cell or not. This happens because NK cells bear receptors that are MHC class I–specificthat release inhibitory signals whenever they encounter a cell that lacks MHC class I(Yokoyama et al.,2003) .This was defined as the "missing self hypothesis".(Karre et al.,1986). The inhibitory receptor that are MHC class I specific include the NKG2A-CD94 heterodimer and killer cell immunoglobulin like receptors (KIR)(Yokoyama et al.,2003). These inhibitory receptors bear one or two intracytoplasmic inhibitory signaling domains called ITIM's (immunoreceptor tyrosine-based inhibition motifs)(Vivier et al.,2004) . These domains can differentiate between healthy and stressed cells because they interact with MHC –I that is present on healthy cells only.These inhibitory receptors of MHC class I enable the NK cells to protect self molecules while causing lysis of the stressed cells.

Identification of the target cells by NK cells has been a guiding principle for missing self hypothesis from years(Karre K et al.,1986). This hypothesis was discovered during the studies that were carried out for understanding the role of MHC in response to NKintumour cells and it was understood that if appropriate levels of MHC I molecules is not present in host cells it will be lysed by NK cells (Bix M et al.,1991).

3.2 MHC (classical and non classical) :

A group of genes called major histocompatibility complex (MHC), encodesfor the proteins present on cell surfacecan detect foreign substances thus helping the immune system(Iannello*et al* .,2008). Although the MHC proteins are found in all the higher vertebrates , human leukocyte antigen (HLA) system is what they are called in human beings.

Classical MHC	Non classical MHC
HLA A	HLA E
HLAB	HLA F
HLA C	HLA G
	HLA H

Table1: different classical and non-classical MHCfound in human

MHC class I and II that are found in human are also called human leukocyte antigen (HLA). In literature HLA refer specifically to HLA protein molecules while the word MHC reserved for that part of the gene that is encoding this molecule, but this doesn't prove to be very conventional.

Most highly studied HLA genes known are the nine classical MHC genes: HLA-DPA1,HLA-A, HLA-B, HLA-C, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA and HLA-DRB1. Three main regions are characterized for them .The A, B and C genes come under MHC class I, while the remaining six D genes come underclass II.

This Natural Killer inhibitory receptor that is MHC specific regulate cytolysis mediated by NK cells in a very conserved manner. This is of great importance as the structure of these receptors regulate inhibition caused by NK cells. This information is being used in various therapies including therapy of leukemias, but still a lot remains to be done. Currently,attention is needed towards role of activating KIRs and how they interact with ligand(s), since their involvement in course of viral diseases is stillunclear.

When inhibitory receptors recognize that specific MHC –I molecules are present on the target cells they downregulate the cytotoxity of NK cells. Their are a variety of activating and inhibitory receptor that are present on NK cells , includingKIR, NKG2D, Ly49 orheterodimers of CD94–NKG2 and also theNCR's, as well as co-stimulatory receptors.

HLA –A , HLA-B , HLA-C are types of classical MHC molecules while the non classical MHC molecule include HLA-E , HLA-F ,HLA-G,HLA-H. The interaction of these classical MHC and non-classical MHC molecule with the inhibitory receptors is studied.

Studies are being focused on how KIR interacts with its respective MHC ligand and consequently it causes the regulation of killingfunction of these cells and enables detection of virally infected cells .

Non – classical MHC interaction with NKG2 family which are C-type lectin receptors , usually found on the surface of NK cells andeven on$CD8^+$ T-lymphocyte subset . These receptors eitherstimulate or inhibit the cytotoxic activity of NK cells, and so they are divided into activating and inhibitory receptors depending to their function. CD94/NKG2 is known to

detect and bind to the non classicalMHC glycoproteins class I which includes HLA-E ,HLA-F,HLA-G(Jay Iams*et al*.,2004).

3.3 NK inhibitory receptors specificity for ligands :

The CD94/NKG2A inhibitory receptoris found specific for HLA-E molecule(class Ib) (Brand VM et al.,1998). It is the availability of peptides , of the leader sequences of different HLA-class I alleles that determines the surface expression of HLA-E.Also , the surface expression of HLA-E depends upon the overall expression of HLA-class I molecules on the cells (Long EO et al.,1998).

A distinctive and a rapidly progressing family of genes is known to encode KIR's that recognize the allelic determinants of the HLA-class I molecules (Trowsdate J et al.,2001) . In human NK cells the expression expression of CD94- NKG2A and KIR genes are extremely diverse. According to the the facts , KIR displays are clonally distributed on the NK cells, such that each KIR is expressed only by a particular fraction of NK cellsenabling them to sense even the loss of the single alleles on the potential target cells (Shilling HG et al.,2002). Therefore, proinflammatorychemokines or cytokines are being released as a result of NK cell activation and causing the lysisof target cells lacking HLA-class I and thus provides an efficient mechanism for defence against theinfections (Zingoni A et al.,2005). Cytolytic T lymphocytes(CTLs) also express KIR , NKG2Awhere they have a regulatory role on TCR-mediated functions (Mingari MC et al.,1995).

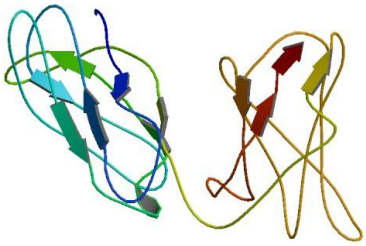


Fig 2: Killer cell immunoglobulin likreceptor(2DL2)

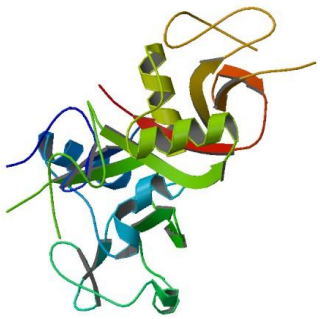


Fig 3 : Human CD94/NKG2A

3.4 Inhibition of NK cell via class I HLA specific inhibitory receptor :

Group of HLA classI specific inhibitory receptors is found in humans which is called as the killer immunoglobulin Ig like receptors (KIRs) ,that are able to distinguish between different groups of classical HLA molecules including HLA – A,B,C .

Another NK cell inhibitory receptor in humans whose involvement is reported in recognition of non- classical HLA molecules is represented by CD94/NKG2A . This NKG2A molecule is known to show interaction with HLA-E(A.Moretta et al.,1993).

The chromosome 19 encodes a family of genes known asKIRs and they are known to causevariation in haplotype in gene contentandnumber also they exhibit allelic polymorphism for the individual genes (M.Uhrberg et al.,1997). While chromosome no. 12 in the human NK complex (NKC) encodesNKG2andCD94 family members which are also

found to be conserved in human population. Therefore ,it is the collection ofthese conserved CD94/NKG2 genes and variable KIR and HLA class I genes that constitute the human NK cell repertoir.

Since HLA,KIR, and CD94/NKG2 are encoded by genes that arepresent on different chromosomes, both the ligands and the receptors can be segregated independently in the human pedigrees (C.Vilches et al.,2002). Consequently , there are certain KIR's for whichHLA ligands are not present. While all those NK cells that are mature will be expressing at least onereceptor that is specific for self HLA class I molecules thus the coexpression of these two or more self-reactive receptors is therefore found less frequent.

Therefore ,loss of even a single HLA class I alleles on self cells in whole NK cell pool of the given individual can be predicted (whichwas frequent cause in tumor transformation or infectionwith certain kind of viruses).

3.5 Role of HLA-I specific inhibitory receptors in NK cell inactivation:

Studies were carried out in human and mice in response to HLA-I specific inhibitory receptors that explained thatcytolytic function of NK cell is mediated by the surface receptors that deliver signals for activation rather than inhibition. Activation of NK cell that causes the lysis of NK cells occurs when there is no engagement between inhibitory receptor and MHC-I (Moretta A et al.,1996). This is found to be the cause of tumor transformation or even infection with certain kind of viruses and results in case if either the target cells have lost MHC –I or have expressed it in inappropriate amounts (Alcami A et al.,2000).

3.6 HLA ligands of KIR:

The fact that there is an inverse relationship between class I surface MHC molecule and target cell lysis susceptibility has provided with the evidence that class I molecules happens to be possibleligands for NK inhibitory receptors. Crystal structures of the extracellular domainsof KIR family membranes including , KIR2DL1, KIR2DL2 , KIR3DL2 andKIR2DL3, has been published (Fan et al., 1997; Snyderet al., 1999; Maenaka et al., 1999b).

The HLAsupertypes- Cw2,4,5 and were found to be specific for KIR2DL1. Subsequently HLA-Cw 1,3and 7 for KIR2DL2 for and HLA-A3 and A11 for KIR3DL2 .(Wagtmann et al., 1995a,b; Longet al., 1996; Colonna and Samaridis, 1995).However these interactionsbetween KIRs and theirrespective class I HLA-A , -B and -C ligands has been observed by directly using the soluble forms of the receptors(Wagtmann et al., 1995b; Boyington et al., 2000). No direct binding evidence has been observed.

3.7 HLA ligand for NKG2A/CD94:

A type of humanNK cell inhibitory receptor that is involved in HLA recognition is being represented by CD94/NKG2A and is found specific for the non classical MHC ligand HLA E and HLA G(A.Moretta et al.,1993).

3.8 Association of HLA-C in different human diseases:

The different biochemical properties of HLA-C are found different from other classical HLA molecules. Therefore the expression of HLA-C at the cell surface and their diversity is found much lower compared to its counterparts and consequently HLA-C restricted responses are infrequently detected, also HLA-C has been found to be associated with various autoimmune diseases , chronic infections and cancers.

Because of the characteristically low expression of HLA-C antigens their physiological relevance is questioned , particularly in context to antigen presentation to CD8+.The HLA-C molecules play a clear role in the NK cell activation through binding with the KIRs, but involvement of HLA-C inthe T-cell-mediated responses is poorly defined.The HLA-C alleles are implicated in numberof human diseases, but still it is not always clear that whether this relationship is the result of function of HLA-C as a consequence of its interactionwith the KIR on NK cells or T-cell restriction element.

Some of the associations are clearly attributed to HLA-C/KIR interactions, as they have both an HLA-C as well as a KIR component. The first description of this HLA-C/KIR association with a clinicaloutcome was observed in hepatitis C virus infection, which lead to chronic viral infection in about 80% of infected people. In a large study conducted on hepatitis C

virus-exposed individuals(Khakoo et al.,2004)found that there is a strongassociation between presence of KIR2DL3, along withhomozygosity for C1 group of the HLA-C molecules that bind to the KIR2DL2 and 2DL3, and cause clearance of infection.The interaction between C1 molecules and KIR2DL3 isnotably weaker than that compared to KIR2DL2, and thus these twovariants of KIR act as alleles of one another, that is why the protectiveeffect that occurs is thought to be mediated by NK cells that arerelatively less inhibited than they would have been inhibited byKIR2DL2 interacting with C1 (Parham P et al.,2004). Also a number of autoimmune diseases have shown HLA-C associations. One of these is association of psoriasiswith HLA-Cw6, which was first shown in the candidategene studies and it was subsequently confirmed in the genome-wideassociation studies(Cao J et al.,2008) that people expressing this particular HLA-Callele will not only have an earlier onset, but also are prone to more extensiveand severe skin disease.

3.9 Clinical settings that are associated with HLA-C alleles:

Disease	Type Of Disease	Type of Association	HLA-C Allele	Reference
Genital herpes simplex type 2	Infection	Severity of infection	Cw*02	Lekstrom-Himes et al., 1999
		Susceptibility	Cw*04	Lekstrom-Himes et al., 1999
Human immunodeficiency virus	Infection	Protection (with B*8101)	Cw*0401	Leslie et al. 2010
Hepatitis C virus	Infection	Protection in presence of KIR2DL3	HLA-C1 homozygosity	Khakoo et al.
Graves' disease	Autoimmunity	Protection	Cw*03	Simmonds et al., 2007
		Susceptibility	Cw*07	Simmonds et al., 2007
		Protection	Cw*16	Simmonds et al., 2007
Type I autoimmune hepatitis	Autoimmunity	Susceptibility	Cw*0701	Strettell et al., 1997
Nasopharyngeal carcinoma	Cancer	Protection	Cw*0401	ButschKovacic et al., 2005

TABLE 2: Association of HLA-C with Human Diseases

3.10 NK cells response to pathogens :

Although it is clear that NK cells recognize tumors and theymay be instrumental in a number of therapeutic approaches towards cancer, the major evolutionary pressure that is found responsible for the development of these NK cells and for tailoring their receptors is most likely by pathogen infections. NK cells have shown important role in this context from sometime (A.Alcami et al.,2000). Their role against viruses that cause downregulationof the MHC class I
molecules to escape the CTL-mediated killing of the infected cellscan be easily understood. These include herpes viruses andadenoviruses which are known to evolve various proteins

that are capable of retaining the MHC moleculeswithin the cells by using different type of mechanisms.
Even the viruseswhich do not induce the downregulation of MHC class I molecules can cause binding of viral peptides ultimately provoking steric alteration of the MHC molecules restraining their ability to engage MHC-specific inhibitory receptors(M.S.Malnati et al.,1995).
Also, upon activation, NK cells produce large amount of IFN-c that along with displaying antiviral activity , also regulates various cells of the immune system (e.g. theyinduce polarization of T cell and macrophage activation towardsTH1 effectors).

3.11 HLA-A3 and HLA-A11 interact with NK inhibitory receptor KIR3DL2:

Among all the KIR receptors KIR3DL2 is known to interact with HLA-A3 and A11.though Direct evidence is provided that HLA-A3 and-A11 bind to KIR3DL2 receptor, while the other HLA allotypes, thatinclude HLA-A2, -B7, -B8, -B27, -B58, -E and -G, show no interaction(P Hansasuta et al.,2004).
The interaction is very much dependent on peptides present in the groove . HLA-A3 refolded with selfpeptide didn'tinteracted with KIR3DL2 but the HLA-A3 molecules that are associatedwith the self peptides are expected to prevent the NK cell lysis ofautologous healthy cells by interacting withKIR3DL2. However, this cannot be excluded that only a limited set of self peptides permit KIR3DL2 recognition. The limited peptide specificity of KIR3DL2 that is
observed explains the difficulty of demonstrating the inhibitionmediated by MHC class I- through KIR3DL2 and whether it recognize HLA-A allotypes or not(Valiante et al.,1997).

3.12 Non-Classical MHC (HLA-E and HLA-G) interact with NKG2A/CD94:

The non classical molecule HLA-G impairs inhibition of NK lysis along with the specific cytolytic T cell functions. HLA-G has been identified as a key mediator in immune tolerance by giving protection to HLA-G target cells from NK cytolysis by interacting with KIR(killer inhibitory receptors).
HLA-E which is the only ligand known for CD94/NKG2 receptor family plays a broad role , it confers protection to cells from lysis by NK , also it is known to be involved in peripheral tolerance.

4. MATERIALS AND METHODS

4.1 Receptor Modelling:

NK inhibitory receptor KIR (KIR2DL1, KIR2DL2 , KIR3DL2) and NKG2A are studied. The structure of NKG2A and KIR2DL2 were available at PDB while KIR2DL1 and KIR3DL2 were not available so they were predicted using MODELLER 9.14.

KIR2DL2 : The crystal structure of KIR2DL2 was available at RCSB Protein Data Bank with PDB ID (2DL2). The structure was directly downloaded from PDB (Brooks AG *et al*.,2000)

NKG2A : The crystal structure of inhibitory receptor NKG2A was available at PDB with PDB ID (3BDW).The structure was directly downloaded from PDB (Snyder*et al*.,1999).

The structure of KIR2DL1 and KIR3DL2 was modelled using modeller9.14 by the following steps:

MODELLER isbeen used for comparative or homology modeling of protein 3-D structures (N.Eswar et al.,2006). User provides alignment of the sequence that is to be modeled with the known related structures and then MODELLER calculates a model which contains all the non-hydrogen atoms. MODELLER implement a comparative structure modeling for protein by the satisfaction of spatial restraints (A.Sali et al.,1993), and also it can perform many additional tasks that includes optimization of the various model of protein structure ,de novo modelling of loops in protein structure , alsomultiple alignment of protein sequences and structures, clustering, comparison of the protein structures, etc

- Modeller 9.14 was downloaded and installed in the C drive.
- The query sequence whose structure is to be modelled is searched at NCBI or Uniprot in FASTA format
- The query FASTA sequence was then copied in qseq1.
- A BLAST search was performed with query sequence to find the template sequences using BLASTp.
- The top 4 structures were picked with highest query cover value and were renamed as tseq1 , tseq2 , tseq3 and tseq4
- The scripts were prepared for modelling of query sequence.

Alignments | Download | GenPept | Graphics | Distance tree of results | Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain D, Crystal Structure Of The Human Natural Killer Cell Inhibitory Receptor Kir2dl1 Bound To Its Mhc Ligand Hla-Cw4	466	466	64%	5e-166	100%	1IM9_D
Chain A, Crystal Structure Of The Human Killer Cell Inhibitory Receptor (Kir2dl3) Specific For Hla-Cw3 Related Alleles	434	434	64%	2e-152	93%	1B6U_A
Chain A, Inhibitory Receptor (P58-Cl42) For Human Natural Killer Cells	417	487	60%	9e-147	100%	1NKR_A
Chain G, Crystal Structure Of Killer Cell Immunoglobulin-like Receptor Kir2ds2 In Complex With Hla-a	385	461	60%	4e-134	93%	4N8V_G
Chain A, Crystal Structure Of The Human Natural Killer Cell Activator Receptor Kir2ds2 (Cd158j)	384	461	60%	6e-134	93%	1M4K_A
Chain D, Structure Of A Complex Between The Human Natural Killer Cell Receptor Kir2dl2 And A Class I Mhc Ligand Hla-Cw3	377	520	60%	5e-131	92%	1EFX_D
Chain A, Killer Immunoglobulin Receptor 2dl2,Trigonal Form	370	512	60%	2e-128	92%	2DLI_A
Chain A, Killer Immunoglobulin Receptor 2dl2	369	510	60%	6e-128	91%	2DL2_A
Chain A, Crystal Structure Analysis Of Kir2ds4	355	483	60%	2e-122	89%	3H8N_A
Chain G, Kir3dl1 In Complex With Hla-B5701	342	464	63%	1e-115	73%	3VH8_G
Chain G, Kir3dl1 In Complex With Hla-b*57:01.j80t	329	512	93%	9e-111	72%	3WUW_G

Fig 4: Results obtained after BLAST of query sequence

- The scripts were prepared for modelling of query sequence.
- A folder named test is made and all the test sequences (tseq1 , tseq2 , tseq3 and tseq4) and the query sequence (qseq1) along with the scripts are copied into it.
- This test folder is then copied into the bin folder of modeller file in C drive.

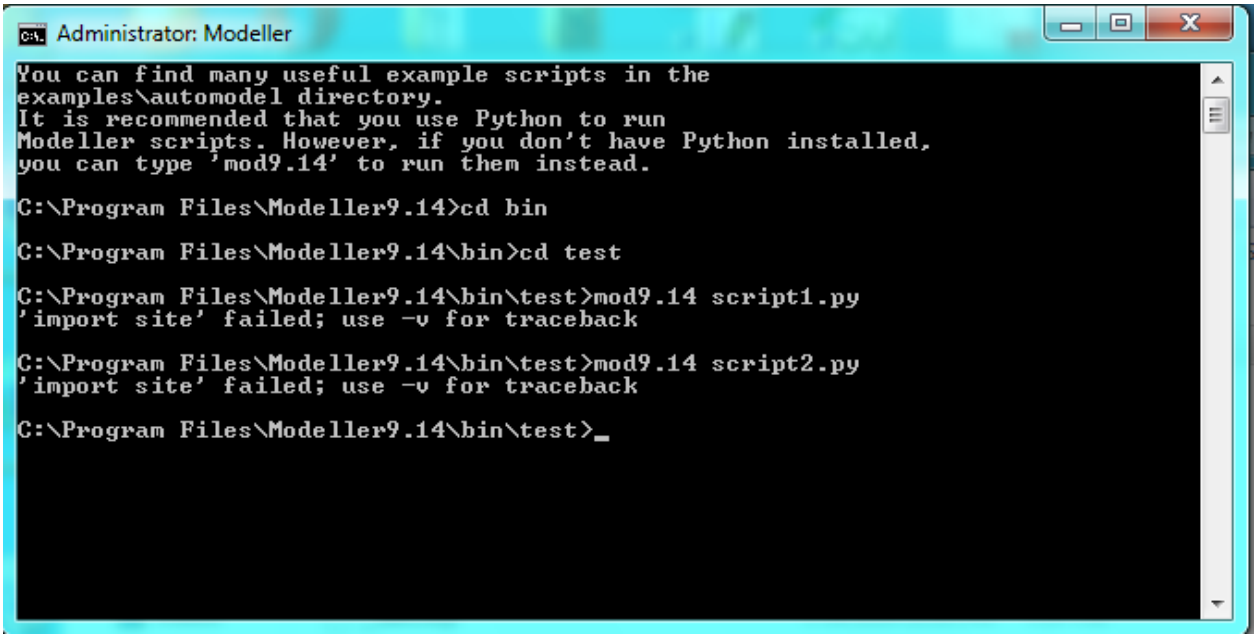


Fig 5 :Modeller showing the execution of scripts

- The following commands are used:
  - cd bin
  - cd test
  - mod9.14 script1.py
  - mod9.14 script2.py
  - mod9.14 script3.py
  - mod9.14 script4.py
  - mod9.14 script5.py

- After running script 4 , the text file was opened for script 4 and the structure with best DOPE score is picked.

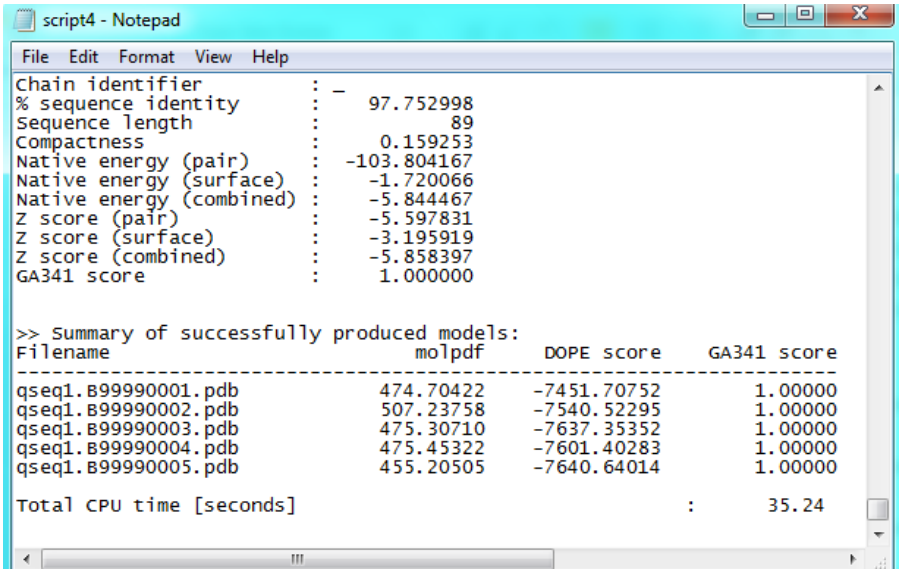


Fig 6: text file obtained after execution of script 4.

- The structure with lowest DOPE score is copied in the script5.py file and thereafter the 5th script is executed.
- Finally the structure with lowest DOPE score is the final modelled structure.

4.2 Ligand Preparation:

For inhibitory receptor KIR the classical MHC supertypes were used as ligands. For KIR2DL1 , HLA-C supertypes HLA-cw2 , cw4, cw5 , for KIR2DL2 , HLA-cw1 , cw3, cw7 and for KIR3DL2 , HLA-a3 , a11 were used.
For NKG2A receptor , the non classical MHC HLA-E and HLA-G were used.
All the classical MHC supertypes including HLA-cw1 , cw3,cw7 ,cw2 , cw 4 , cw5 , a3 and a11 are predicted by the same method mentioned above using modeller.
The non-classical MHC molecules that has shown interaction with NKG2A receptors that include HLA-E , HLA-G. The crystallized structure of HLA-E and HLA-G are available in RCSB Protein Data Bank (Berman *et al.*,2000) with PDB ID's 1KTL nad 1YDP (Strong , RK *et al*.,2003).
For the non-classical receptors , HLA-E and HLA-G , since multiple chains are present and for docking only single chain is required both for ligand and receptor as well . So the ligand molecule in this case is edited in viewerlite and all its chains are separated.

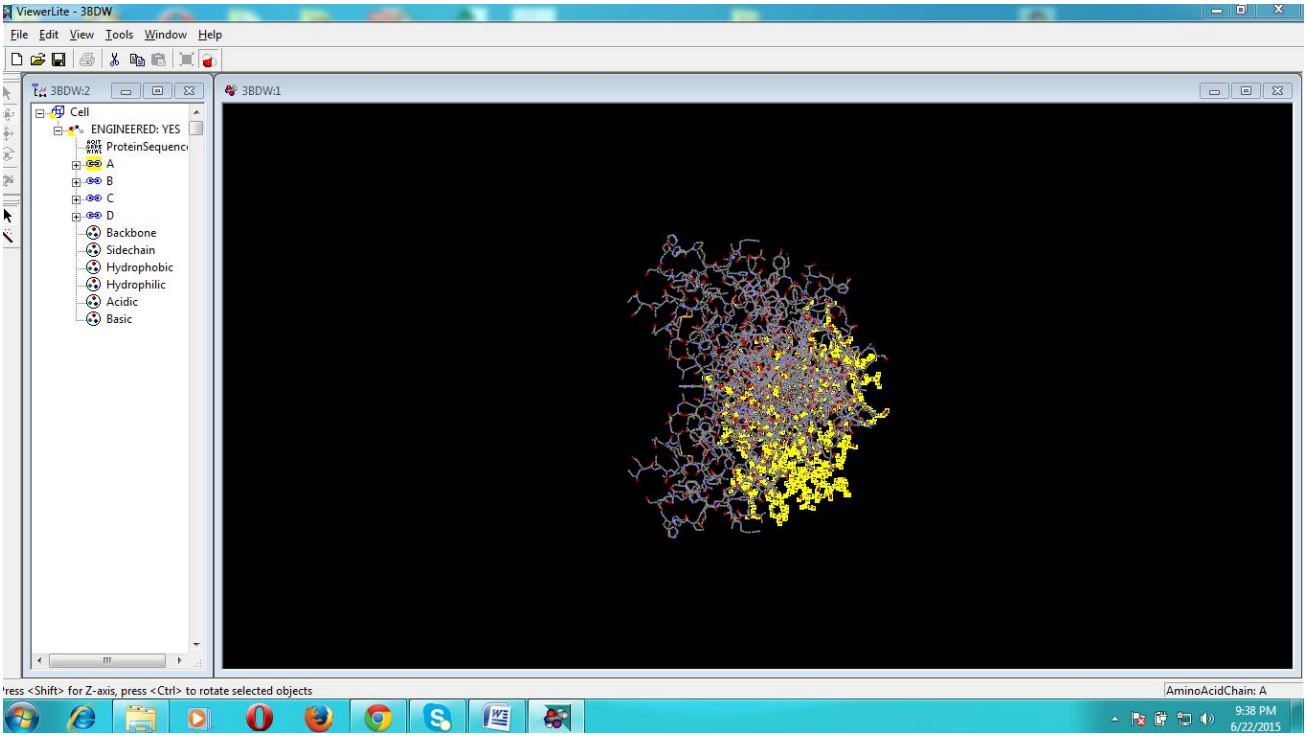


Fig 7: Molecule editing in viewerlite

4.3 Molecular Docking using PATCHDOCK(an automatic server for molecular docking) :

PatchDock is an algorithm used for molecular docking.Here , the input is two molecules of any type eg., proteins, DNA, drugs. The outputobtained is a list of potential complexes that are sorted by the shape complementarity criteria. The PatchDock algorithm has been inspired by image segmentation and object recognition techniques that are used in Computer Vision. Docking could be compared to assembling a jigsaw puzzle as when solving the puzzle we first try to match two pieces, by picking one of the piece and searching for the other complementary one. Webasically concentrate on the patterns which are unique for puzzle element and then look for the matching patterns in rest of the pieces. PatchDock server employs a similar technique. If two molecules are given, then their surfaces are then divided into patches depending upon the surface shape. These patches corresponds to the patterns that distinguish between the puzzle pieces. Once these patches are identified, thenthey can be superimposed using the shape matching algorithms. The algorithm has three main stages:

- Molecular Shape Representation
- Surface Patch Matching
- Filtering and Scoring

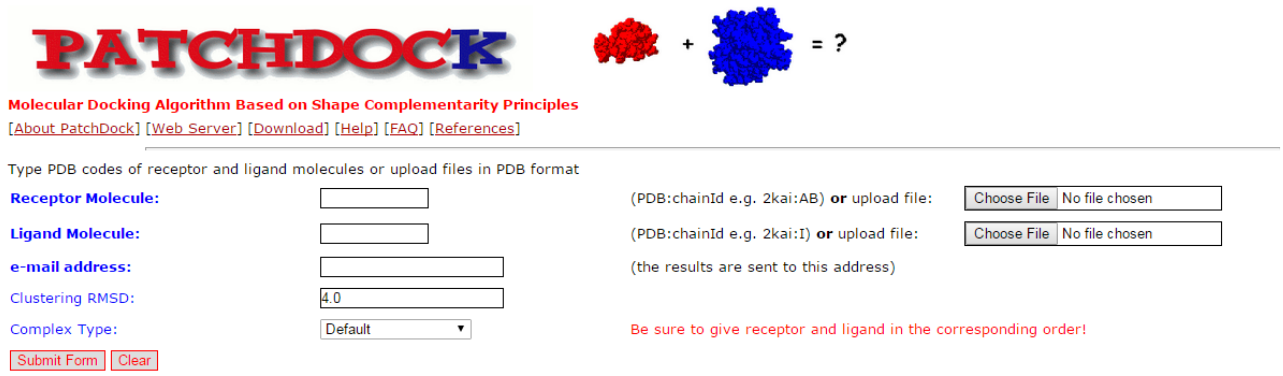
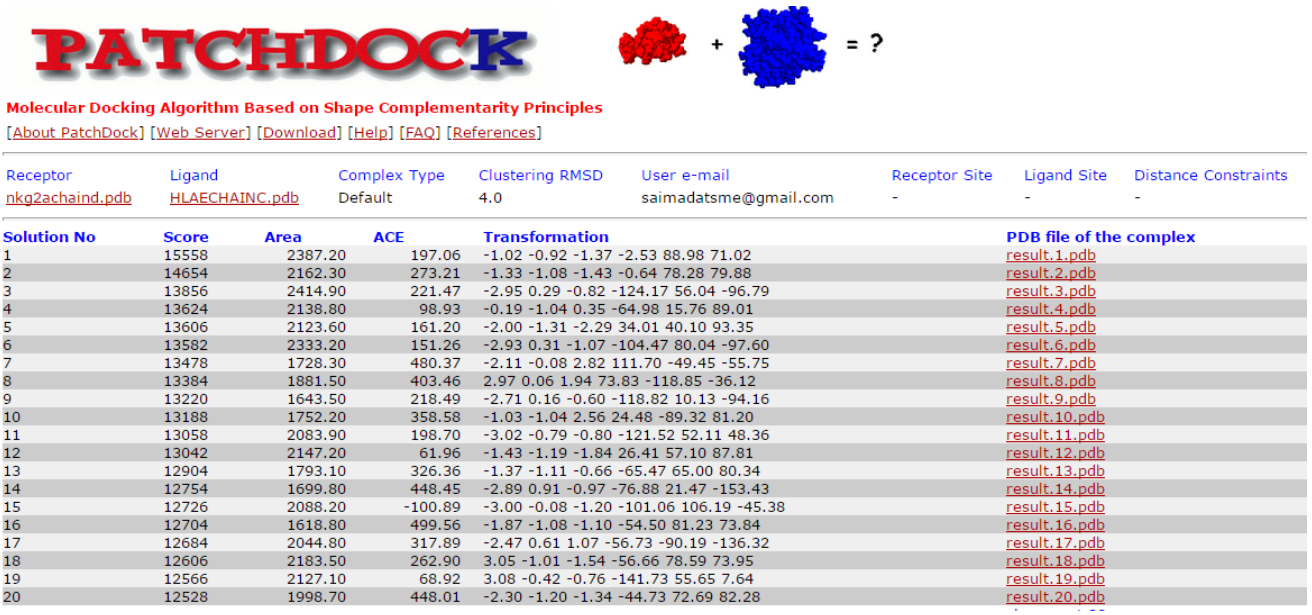


Fig 8 : Screenshot of the PATCHDOCK server

The receptor molecule along with its ligand molecule was uploaded in the PATCHDOCK server , the respective e-mail id was entered and then the server gives the results.



Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
nkg2achaind.pdb	HLAECHAINC.pdb	Default	4.0	saimadatsme@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	15558	2387.20	197.06	-1.02 -0.92 -1.37 -2.53 88.98 71.02	result.1.pdb
2	14654	2162.30	273.21	-1.33 -1.08 -1.43 -0.64 78.28 79.88	result.2.pdb
3	13856	2414.90	221.47	-2.95 0.29 -0.82 -124.17 56.04 -96.79	result.3.pdb
4	13624	2138.80	98.93	-0.19 -1.04 0.35 -64.98 15.76 89.01	result.4.pdb
5	13606	2123.60	161.20	-2.00 -1.31 -2.29 34.01 40.10 93.35	result.5.pdb
6	13582	2333.20	151.26	-2.93 0.31 -1.07 -104.47 80.04 -97.60	result.6.pdb
7	13478	1728.30	480.37	-2.11 -0.08 2.82 111.70 -49.45 -55.75	result.7.pdb
8	13384	1881.50	403.46	2.97 0.06 1.94 73.83 -118.85 -36.12	result.8.pdb
9	13220	1643.50	218.49	-2.71 0.16 -0.60 -118.82 10.13 -94.16	result.9.pdb
10	13188	1752.20	358.58	-1.03 -1.04 2.56 24.48 -89.32 81.20	result.10.pdb
11	13058	2083.90	198.70	-3.02 -0.79 -0.80 -121.52 52.11 48.36	result.11.pdb
12	13042	2147.20	61.96	-1.43 -1.19 -1.84 26.41 57.10 87.81	result.12.pdb
13	12904	1793.10	326.36	-1.37 -1.11 -0.66 -65.47 65.00 80.34	result.13.pdb
14	12754	1699.80	448.45	-2.89 0.91 -0.97 -76.88 21.47 -153.43	result.14.pdb
15	12726	2088.20	-100.89	-3.00 -0.08 -1.20 -101.06 106.19 -45.38	result.15.pdb
16	12704	1618.80	499.56	-1.87 -1.08 -1.10 -54.50 81.23 73.84	result.16.pdb
17	12684	2044.80	317.89	-2.47 0.61 1.07 -56.73 -90.19 -136.32	result.17.pdb
18	12606	2183.50	262.90	3.05 -1.01 -1.54 -56.66 78.59 73.95	result.18.pdb
19	12566	2127.10	68.92	3.08 -0.42 -0.76 -141.73 55.65 7.64	result.19.pdb
20	12528	1998.70	448.01	-2.30 -1.20 -1.34 -44.73 72.69 82.28	result.20.pdb

Fig 9 :Screenshot of results obtained from PATCHDOCK

PATCHDOCK then returns the result in the form of docked files i.e. the PDB files of the complex which is the predicted complex structure , a number a complexes were obtained along with the score , area that is the approximate interface area of the complex and the ACE (atomic contact energy).

4.4 Refining models by FIREDOCK :

After running PATCHDOCK , the top 10 results were refined by using FIREDOCK.The FireDockserver then addresses refinement problem of the protein-protein docking solutions. This method simultaneously targets problem of flexibility alongwith the scoring of solutions that are produced by fast rigid-body docking algorithms. Given upto a set of 1000potential docking candidates, it can refine and score them based on an energy function.

This is the first webserver that allows scoring of docking solutions and performing large-scale flexible refinement online.

FireDock

[Web Server] [About] [Download] [FAQ] [Help] [References]

Receptor	Ligand
nkg2achainc.pdb	HLAGCHAINA.pdb

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE	HB	Structure show/hide
1	5	-25.81	-29.51	9.55	4.10	-2.84	☑
2	7	-5.23	-26.09	8.89	10.06	-2.22	☐
3	8	-4.21	-21.10	12.20	2.07	-2.48	☐
4	9	-1.97	-23.38	9.39	11.05	-3.82	☐
5	1	1.36	-31.97	16.51	13.37	-7.65	☐
6	2	15.67	-7.54	9.11	3.44	-1.50	☐
7	6	16.20	-10.80	26.14	6.68	-2.11	☐
8	4	18.00	-21.02	20.47	9.20	-2.14	☐
9	3	23.60	-33.37	30.85	6.94	-3.33	☐
10	10	251.06	-40.33	397.60	-9.46	-4.86	☐

Fig10: Screenshot of results obtained with FireDock

FireDock then results the best structures in the form of zip folder along with the Global energy which is the binding energy of the solution , the attractive and repulsiveVdW forces , the contribution of atomic contact energy to global binding energy and the contribution of hydrogen bonds to global binding energy.

The downloaded structures were then unzipped and checked for the correct format and chains , if the separate chains are not present for ligand and receptor then chains are added.

To observe the hydrogen bonds and the non-bonded interactions , modeller was used , the LIGPLOT file containg the required scripts was saved in the C drive , and the solutions obtained from FireDock are then saved into ligplot file and the following commands are used in modeller:

- C:\ Program Files\Modeller9.14>
- C:\ Program Files\Modeller9.14>cd..
- C:\ Program Files>cd..
- C:\>cd Ligplot
- C:\ Ligplot>dimplot docking res.pdb X Y
- Exit

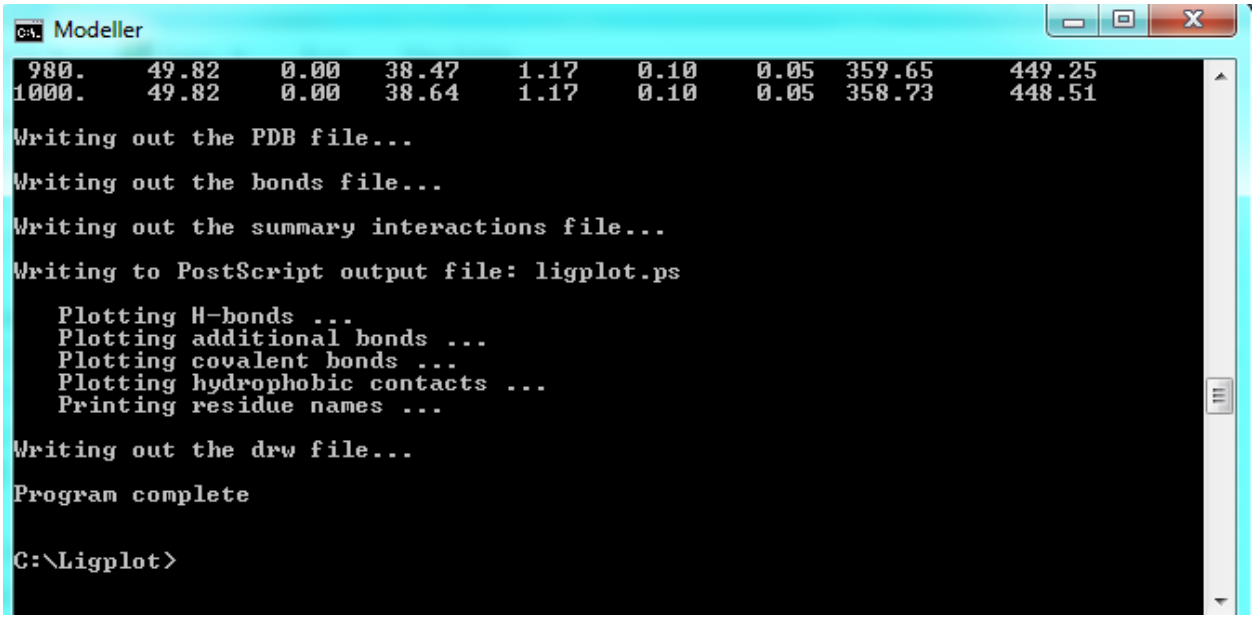


Fig 11: modeller showing the calculation of different bonds

This results into the solutions with hydrogen bonds , the non bonded interactions and the ligplot obtained here was in postscript format so PDFill PDF tool was used to convert postscript file into PDF.

The DCOMPLEX predicts the binding affinity of the protein complex , determining the energy in kcal/mol.

5. RESULTS

5.1 3D structure of NK inhibitory receptor KIR2DL1 and KIR3DL2 :

The 3D structure of KIR2DL1 and KIR3DL2 is not available at PDB , so it was predicted using modeller9.14.MODELLER isbeen used for comparative or homology modeling of protein 3-D structures (N.Eswar et al.,2006). User provides alignment of the sequence that is to be modeled with the known related structures and then MODELLER calculates a model which contains all the non-hydrogen atoms. MODELLER implement a comparative structure modeling for protein by the satisfaction of spatial restraints (A.Sali et al.,1993), and also it can perform many additional tasks that includes optimization of the various model of protein structure ,de novo modelling of loops in protein structure , also multiple alignment of protein sequences and structures, clustering, comparison of the protein structures. The sequences of KIR2DL1 with accession number AJI81019.1 and KIR3DL2 with accession number ABZ02151.1 were obtained from NCBI and their structures were modelledwith modeller9.14.

The predicted structure of KIR2DL1 and KIR3DL2:

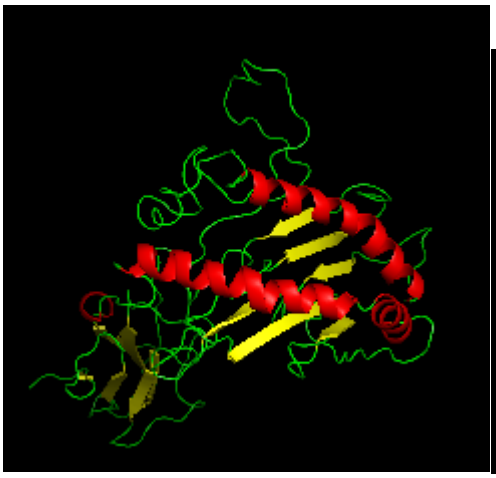


Fig 12: Modelled3D structure of KIR2DL1

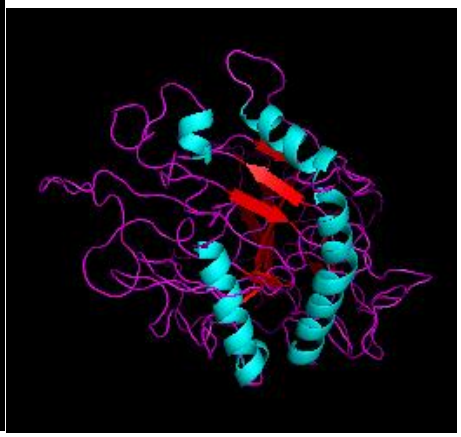


Fig 13 :Modelled3D structure of KIR3DL2

5.23Dstructure of classical MHC class-I HLA supertypes:

For all the classical MHC supertypes including HLA-cw1 , cw3,cw7 ,cw2 , cw 4 , cw5 , a3 and a11 , the structures were not present at PDB. So the structure were also predicted with modeller.

5.2.1 KIR2DL1- HLA-cw2,cw4,cw5 :

The HLA –C supertypes HLA-cw2,cw4 and cw5 structures were not available at PDB so the sequences were obtained from NCBI . Sequence of HLA-cw2 with accession number AAA59702.1 , HLA-cw4 with accession number P30504.1 and HLA-cw5 with accession number BAA19534.1 were obtained from NCBI in FASTA format and their structures were modelled. The predicted structures obtained are as mentioned below:

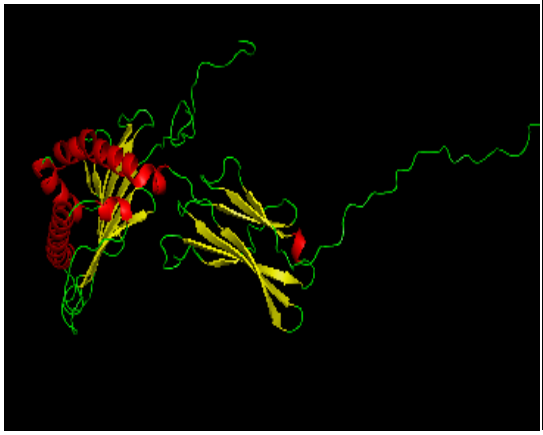


Fig 14: Modelled3D structure of HLA-cw2

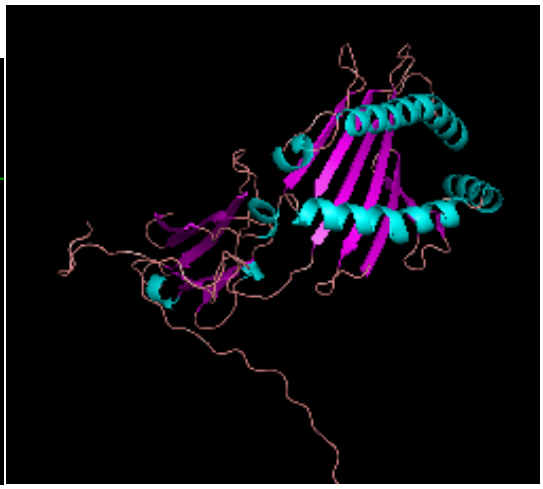


Fig 15:Modelled 3D structure of HLA-cw4

Fig 16: Modelled3D structure of HLA-cw5

5.2.2KIR2DL2-HLA-cw1,cw3,cw7 :

The HLA –C supertypes HLA-cw1 ,cw3 and cw7, which are potential ligands for KIR2DL2 are not available at PDB so the sequences were obtained from NCBI . Sequence of HLA-cw1 with accession number CAA86839.1 , HLA-cw3 with accession number AAB02773.1 and HLA-cw7 with accession number AAA50217.1 were obtained from NCBI in FASTA format and their structures were modelled. The predicted structures obtained are as mentioned below:

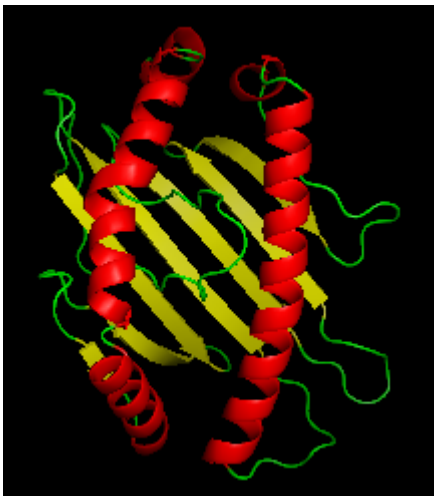


Fig 17:Modelled3D structure of HLA-cw1

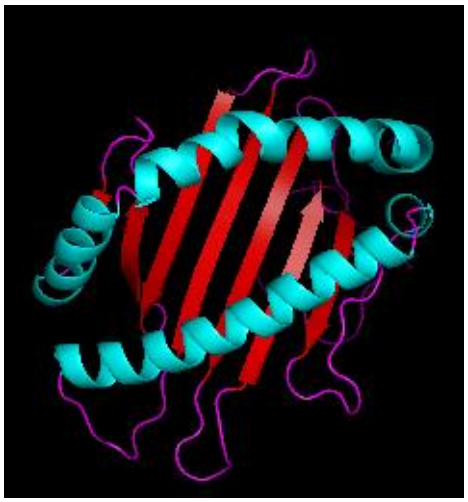


Fig 18 :Modelled 3D structure of HLA-cw3

Fig 19 :Modelled3D structure of HLA-cw7

5.2.3 KIR3DL2-HLA-a3,a11:

The HLA –A supertypes HLA-a3 and a11, which are potential ligands for KIR3DL2 are not available at PDB so the sequences were obtained from NCBI . Sequence of HLA-a3 with accession number AAA59839.1 and HLA-a11 with accession number AAA76607.1 were

obtained from NCBI in FASTA format and their structures were modelled. The predicted structures obtained are as mentioned below:

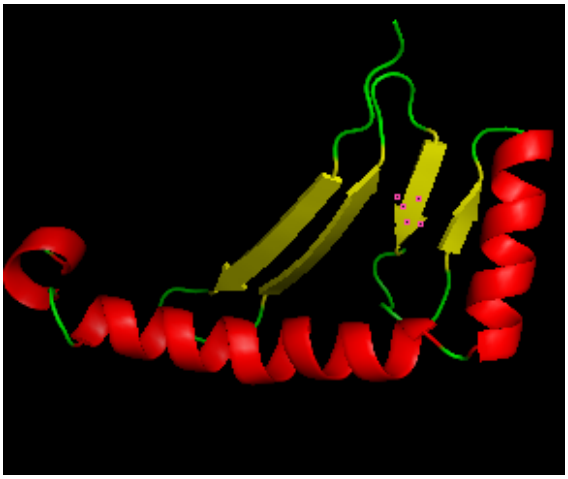


Fig 20 : Modelled3D structure of HLA-a3

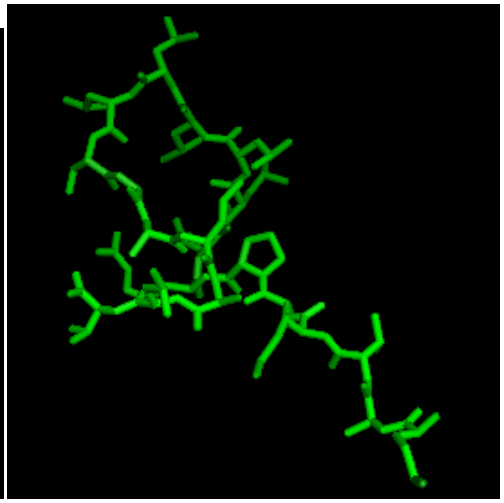


Fig 21: Modelled3D structure of HLA-a11

5.3 Molecular Docking with PATCHDOCK :

Molecular Docking with PATCHDOCK is done and the output obtained here is a list of potential complexes that are sorted by the shape complementarity criteria. Docking predicts the orientation of a molecule to another when bound to one another forming a stable complex (Lengauer T et al.,1996).

5.4NK inhibitory receptor interaction with classical MHC supertypes:

KIR2DL1-HLA- cw2,4,5:

PATCHDOCK was used to perform molecular docking KIRs with the classical MHC supertypes of HLA. PATCHDOCK determines a ligplot which shows the hydrogen bonds , hydrophobic interactions , ACE (atomic contact energy),non-bonded interactions and the D-complex energies. The top 10 complexes were observed , for each of the complex , the d-complex energy and the interactions between them are observed .

5.4.1 KIR2DL1-HLA-cw2 :

The results obtained after the docking of receptor KIR2DL1 with HLA-cw2 were observed. For each complex obtained , following data was observed.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
6	17050	2691.30	-22.39	11.73	-3.49	5	89	16	-6.596533 Kcal/mol
8	16674	2436.70	-17.15	9.42	-3.79	3	166	21	-5.779720 Kcal/mol
3	17708	2454.10	2.07	10.91	-3.32	3	112	14	**-8.511224 Kcal/mol**
9	16282	2670.00	8.28	2.74	0	5	440	21	9.712703 Kcal/mol
10	16256	3165.50	14.41	2.73	-0.99	2	63	6	-2.643966 Kcal/mol
7	16808	2392.80	60.15	14.73	-3.27	1	73	17	-6.981052 Kcal/mol
4	17310	2840.10	272.40	2.69	-4.88	0	89	16	-6.074229 Kcal/mol
2	18344	3037.80	2565.26	-2.20	-6.83	13	1141	37	29.323216 Kcal/mol
5	16282	2670.00	2662.19	5.78	-14.55	6	115	16	-4.894935 Kcal/mol
1	19780	2745.80	3028.94	15.96	-8.76	0	3	0	-4.648359 Kcal/mol

Table 3 : KIR2DL1-HLA-cw2 interaction energies

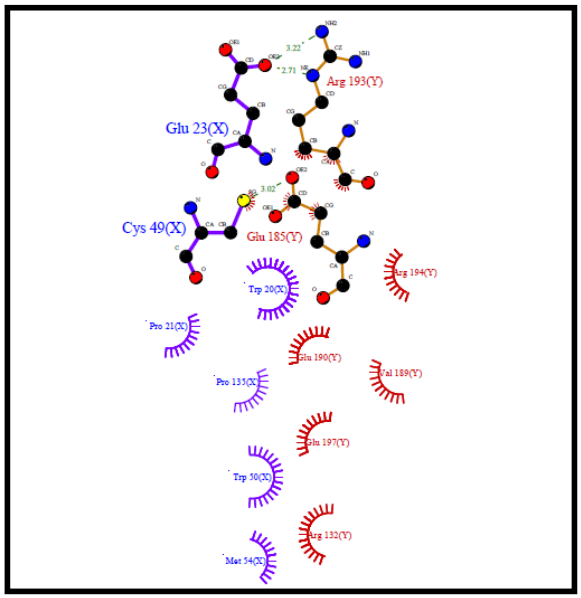


Fig 22: ligplot for KIR2DL1-HLA-cw2

Here , the complex with minimum energy is is the third one with D-complex energy -8.11224 Kcal/mol and above is the LigPlot obtained for the 3rd complex . With the binding energies , a mean was calculated to compare the overall binding affinities of NK inhibitory receptor with its different supertypes.

Here 8th complex shows high positive energy because of large number of non-bonded interactions so it was considered as an outlier and the mean of the remaining binding affinities is -3.97.

5.4.2 KIR2DL1-HLA-cw4 :

The D –complex and various interactions observed for this receptor ligand complex is mentioned in table.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
2	20254	3036.00	3.22	5.31	-1.91	4	140	23	-5.131172 kcal/mol
10	17560	2512.20	4.01	4.73	-0.62	1	49	5	-4.596647 kcal/mol
8	18320	3186.10	14.59	5.30	0.00	3	129	24	-8.593745 kcal/mol
3	19822	3446.50	61.13	-0.65	-3.83	5	191	30	**-11.915498 kcal/mol**
7	18644	2704.90	193.96	-17.02	-4.20	6	236	34	-5.929207 kcal/mol
6	18928	2482.10	570.61	-12.86	-5.70	3	250	22	-3.725424 kcal/mol
5	19232	3108.00	2410.15	-28.30	-7.95	7	394	35	-7.768958 kcal/mol
9	17654	2950.60	2470.39	1.29	-14.89	5	379	27	3.655929 kcal/mol
1	23102	3578.80	5988.58	-20.11	-14.41	3	425	39	-5.604556 kcal/mol
4	19238	3069.80	11965.00	-16.09	-17.82	27	3772	110	127.765051 kcal/mol

Table 4 : KIR2DL1-HLA-cw4interaction energies

Here , the receptor ligand complex with minimum energy is 4th complex with energy -11.915498 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

Here , the 10th complex shows high positive energy so was considered as an outlier and the mean comes out to be -5.51.

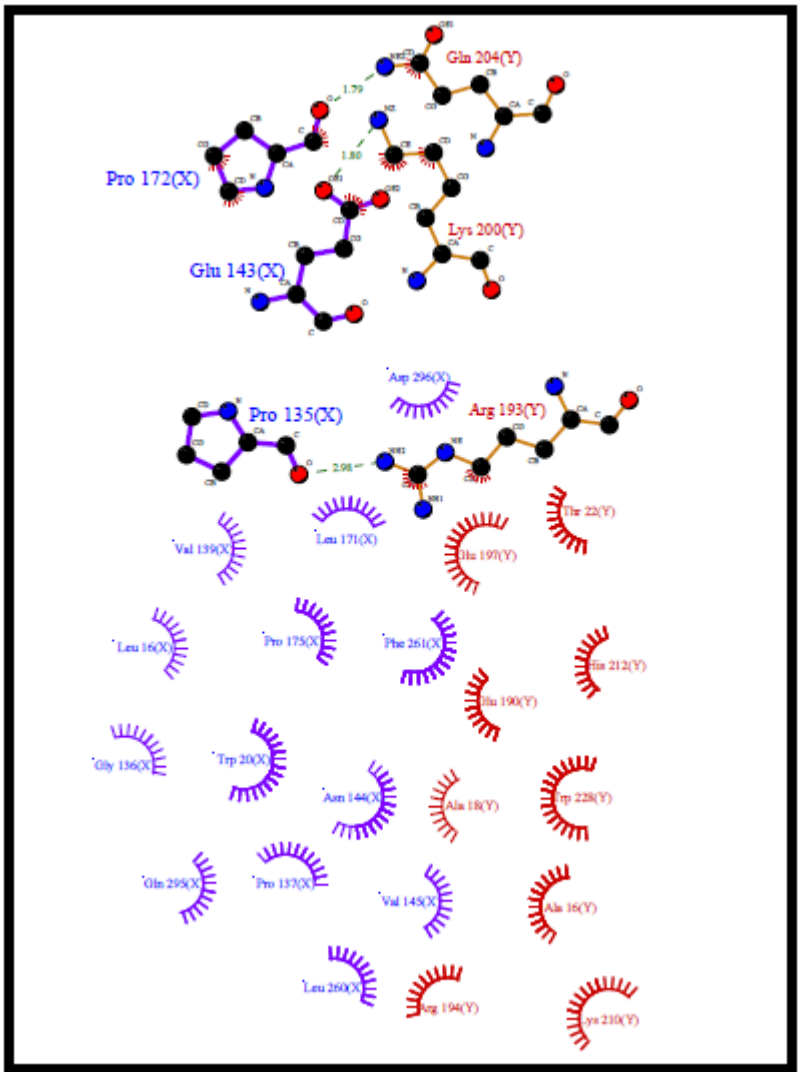


Fig 23 : LIGPLOT of KIR2DL1-HLA-cw4

5.4.3 KIR2DL1-HLA-cw5 :

The D –complex and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 1st complex with energy -9.543037 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

Here , the 5thcomplex shows high positive energy so was considered as an outlier and the mean comes out to be -4.47.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
5	17620	2199.60	-26.87	2.81	-2.80	3	195	36	**-9.543073 kcal/mol**
7	17282	2545.50	-7.63	11.75	-2.12	2	123	19	-6.518122 kcal/mol
4	17626	3232.30	-6.81	2.79	-2.23	1	87	18	-4.523109 kcal/mol
8	17130	3031.50	0.31	-4.05	-10.22	6	251	21	-1.563377 kcal/mol
6	17592	3198.30	3.07	1.24	0.00	42	5057	120	211.65502 kcal/mol
10	16960	2626.10	3.29	5.07	-0.47	6	84	9	-6.278315 kcal/mol
2	19134	2647.90	3.75	3.38	-0.41	3	165	10	1.808574 kcal/mol
3	17770	2920.80	4.48	-0.00	0.00	2	25	10	-4.575456 kcal/mol
1	20690	3020.80	10.35	-0.95	0.00	0	27	7	-4.535223 kcal/mol
9	17046	2534.90	53.70	8.08	-1.95	6	155	22	-4.570753 kcal/mol

Table 5 : KIR2DL1-HLA-cw5interaction energies

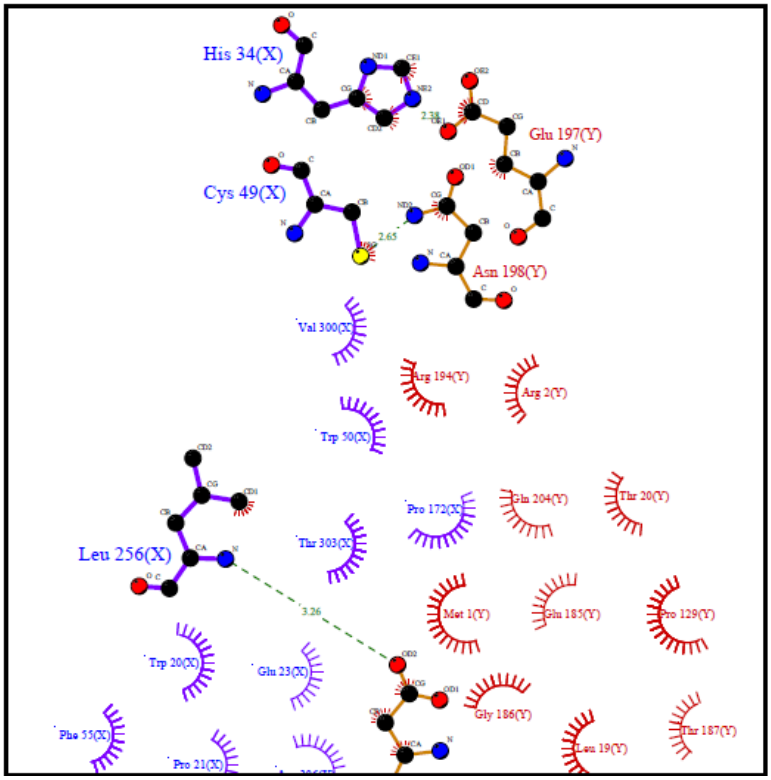


Fig 24 : LIGPLOT of KIR2DL1-HLA-cw5

KIR2DL2 –HLA-cw1,3,7 :

5.4.4 KIR2DL2 –HLA-cw1 :

The structure of KIR2DL2 was obtained from PDB with PDB id 2DL2. This receptor was docked with HLA-C supertype cw1 and 10 complexes were obtained with the binding energies and interaction energies mentioned in the table.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
4	14220	1990.20	-23.58	3.03	-6.49	2	151	19	-3.164051 kcal/mol
7	13906	1865.00	-5.65	2.97	0.00	8	99	19	-8.792201 kcal/mol
3	14318	1934.70	4.44	8.38	-1.23	4	91	18	-9.377776 kcal/mol
8	13720	1782.90	4.55	3.01	-4.59	1	144	23	-6.978940 kcal/mol
6	13996	2366.40	6.97	6.12	-2.73	3	74	9	-5.139285 kcal/mol
2	14396	1793.40	18.22	3.42	-0.27	1	81	11	-7.514706 kcal/mol
10	13632	2105.80	43.55	3.75	-2.58	8	116	16	**-11.161303 kcal/mol**
1	15198	2392.00	263.45	3.81	-3.83	3	64	8	-4.715805 kcal/mol
5	14010	2250.30	1153.04	4.28	-1.94	0	19	3	-4.195892 kcal/mol
9	13636	2294.50	3600.61	1.78	-7.47	0	27	3	-5.083462 kcal/mol

Table 6 : KIR2DL2-HLA-cw1interaction energies

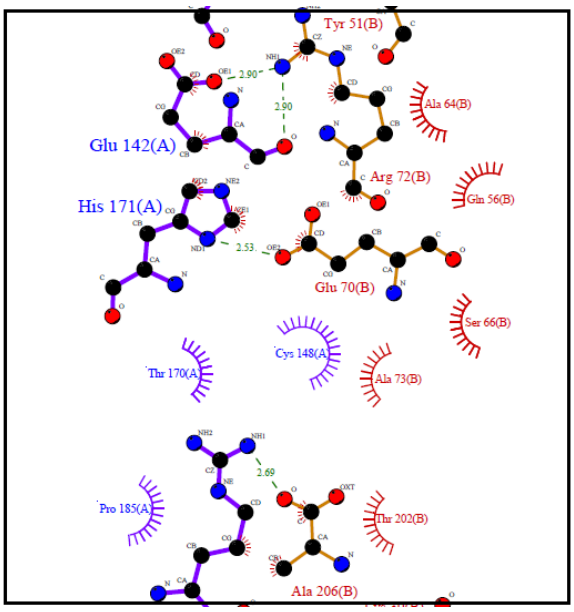


Fig 25 : LIGPLOT of KIR2D2-HLA-cw1

For this docked complexes obtained here , the receptor ligand complex with minimum energy is 7st complex with energy -11.161303 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
The mean of the following binding energies is -6.191.

5.4.5 KIR2DL2-HLA-cw3 :

The D –complex and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is3rd complex with energy -9.636099 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
The mean of the following binding energies is -6.210.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
10	13254	2024.10	-8.27	1.08	-0.89	6	108	15	-7.198477 kcal/mol
6	13816	2052.10	-6.87	-3.17	-0.77	1	87	14	-8.158289 kcal/mol
3	14328	1834.90	-3.41	6.79	-3.22	13	146	30	**-9.636099 kcal/mol**
7	13498	1809.90	0.36	1.29	-0.86	1	19	3	-5.486737 kcal/mol
5	13956	2186.50	10.01	1.13	0.00	0	19	3	-4.613120 kcal/mol
9	13304	1970.50	19.05	1.89	0.00	1	17	6	-6.200383 kcal/mol
1	15248	1869.50	32.20	16.90	-1.39	7	133	18	-7.649282 kcal/mol
8	13340	2118.20	35.58	4.25	-2.17	2	127	13	-4.281970 kcal/mol
2	14998	2126.00	108.88	12.36	-3.46	8	124	12	-5.022979 kcal/mol
4	14042	2289.20	1943.19	9.30	-6.87	5	85	14	-3.866371 kcal/mol

Table 7: KIR2DL2-HLA-cw3interaction energies

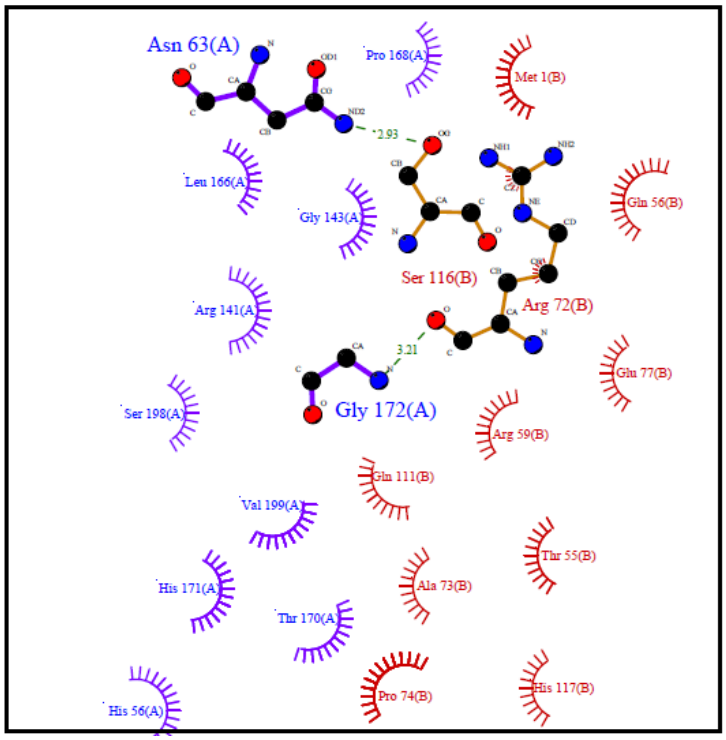


Fig 26 : LIGPLOT of KIR2D2-HLA-cw3

5.4.6 KIR2DL2-HLA-cw7 :

The D –complex and various interactions observed for this receptor ligand complex is mentioned in table:

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
5	16896	2310.10	-4.64	10.66	-2.10	4	96	12	-7.201163 kcal/mol
4	16932	2575.40	0.51	0.87	-0.55	4	5	1	-4.700000 kcal/mol
6	16736	2275.70	2.10	15.18	-3.21	18	122	12	**-8.914885 kcal/mol**
10	15098	2466.30	5.53	-0.62	0.00	3	29	3	-5.615619 kcal/mol
7	15662	2486.20	13.91	0.04	-0.58	11	659	27	20.934747 kcal/mol
1	17438	3050.40	16.26	-0.00	0.00	48	5505	108	196.657515 kcal/mol
9	15100	2544.70	74.25	21.96	-6.40	5	239	27	-8.585541 kcal/mol
3	16960	2461.40	116.67	16.69	-5.59	7	159	13	-5.591105 kcal/mol
2	17116	2843.10	853.09	-1.91	-3.65	3	308	35	-2.360526 kcal/mol
8	15276	2518.90	1264.35	13.21	-8.18	2	99	15	-5.276175 kcal/mol

Table 8 : KIR2DL2-HLA-cw7interaction energies

For the docked complexes obtained here , the receptor ligand complex with minimum energy is3rd complex with energy -8.914885 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

Here , the 5thand 6thcomplex shows high positive energy so was considered as an outlier and the mean comes out to be -6.02.

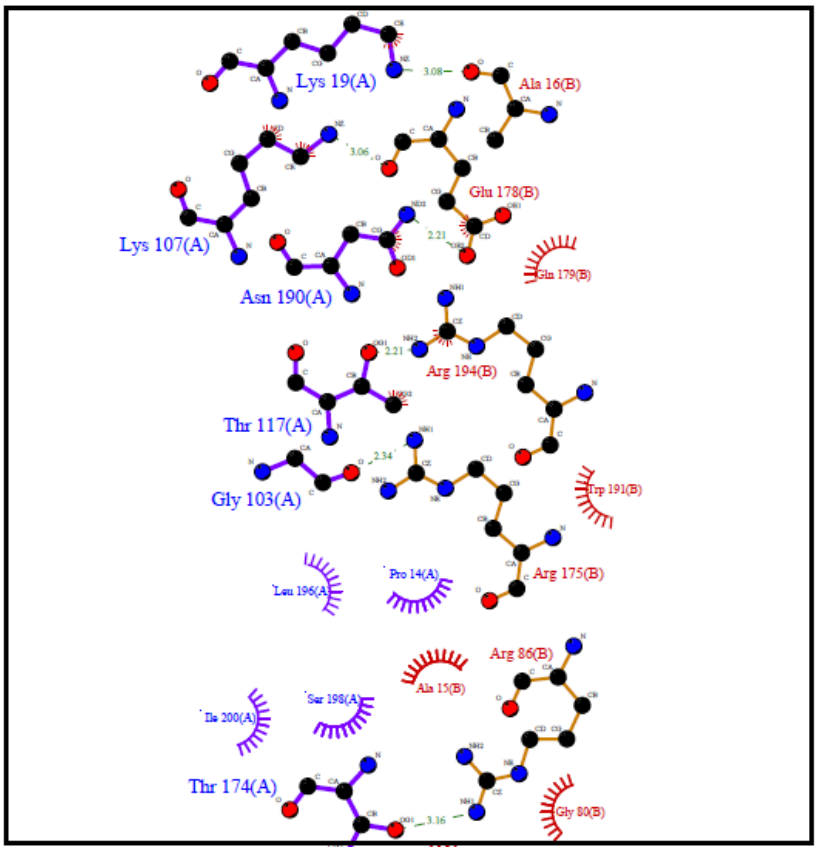


Fig 27 : LIGPLOT of KIR2D2-HLA-cw7

KIR3DL2-HLA-a3,a11 :

5.4.7 KIR3DL2-HLA-a3:

The structure of KIR3DL2 was not available at PDB so was predicted with modeller. Also the structure of HLA-A supertypes was predicted.This KIR3DL2 receptor was docked with HLA-A supertype a3 and 10 complexes were obtained with the binding energies and interaction energies mentioned in the table.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
1	18364	2495.40	-8.56	2.98	-1.54	10	43	462	**-7.684858 Kcal/mol**
6	16800	2166.80	-6.32	6.68	-3.95	5	49	579	2.977184 Kcal/mol
7	16582	2548.00	7.01	5.41	0.00	5	46	546	5.381015 Kcal/mol
9	16392	3108.50	21.94	-1.55	-2.01	2	43	508	-0.232939 Kcal/mol
8	16398	2598.70	75.37	3.69	-6.28	7	47	422	-6.768826 Kcal/mol
4	17180	2479.10	213.74	23.96	-9.17	12	34	401	-2.912748 Kcal/mol
5	16990	2293.50	224.97	8.12	-4.75	6	53	539	1.321023 Kcal/mol
3	17564	2436.60	344.69	5.56	-6.26	9	41	775	12.404016 Kcal/mol
2	18256	2530.10	607.04	6.69	-4.03	10	64	729	3.923209 Kcal/mol
10	16360	2235.70	767.99	6.78	-3.21	12	29	448	2.834522 Kcal/mol

Table 9: KIR3DL2-HLA-a3interaction energies

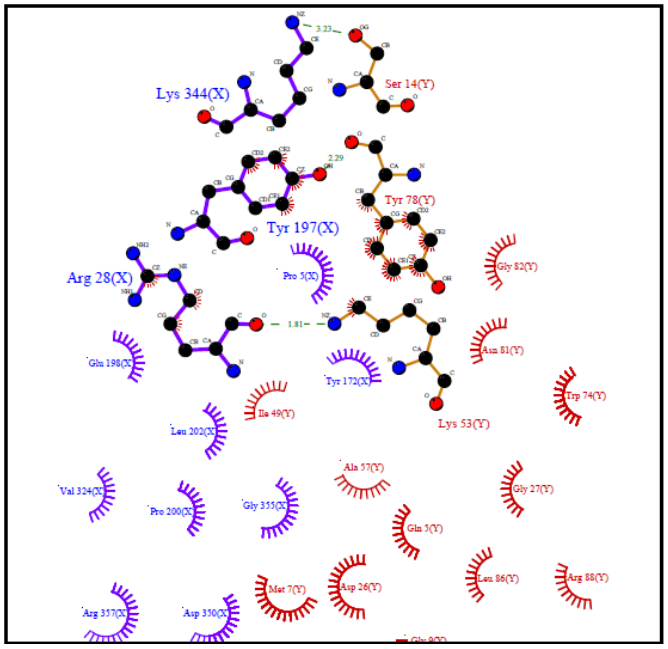


Fig 28 : LIGPLOT of KIR3D2-HLA-a3

For the docked complexes obtained here , the receptor ligand complex with minimum energy is1st complex with energy -7.684858 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

The mean comes out to be +1.12.

5.4.8 KIR3DL2-HLA-a11 :

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is9th complex with energy -12.229871 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
The mean comes out to be +4.7.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
4	11580	1520.30	-54.62	-10.39	-1.79	3	35	429	4.909619 Kcal/mol
9	10504	1376.90	-32.13	-10.02	0	8	23	258	**-10.307178 Kcal/mol**
8	10522	1335.00	-18.54	-9.04	0	1	42	478	9.487103 Kcal/mol
1	12830	1806.20	-15.19	-4.06	0	5	22	330	1.236972 Kcal/mol
10	10324	1481.50	-1.11	0.94	-0.69	9	29	798	30.279410 Kcal/mol
2	11658	1583.40	116.64	-26.03	-4.66	6	35	581	15.931448 Kcal/mol
7	10706	1511.10	1218.43	-13.74	-6.03	8	24	419	8.540869 Kcal/mol
3	11606	1904.10	1257.58	-34.59	-3.70	1	32	254	-0.967363 Kcal/mol
6	10792	1845.90	3279.70	-39.42	-10.78	5	22	240	-12.229871 Kcal/mol
5	10948	1924.60	6736.58	-21.98	-13.59	1	30	331	0.117231 Kcal/mol

Table 10: KIR3DL2-HLA-a11interaction energies

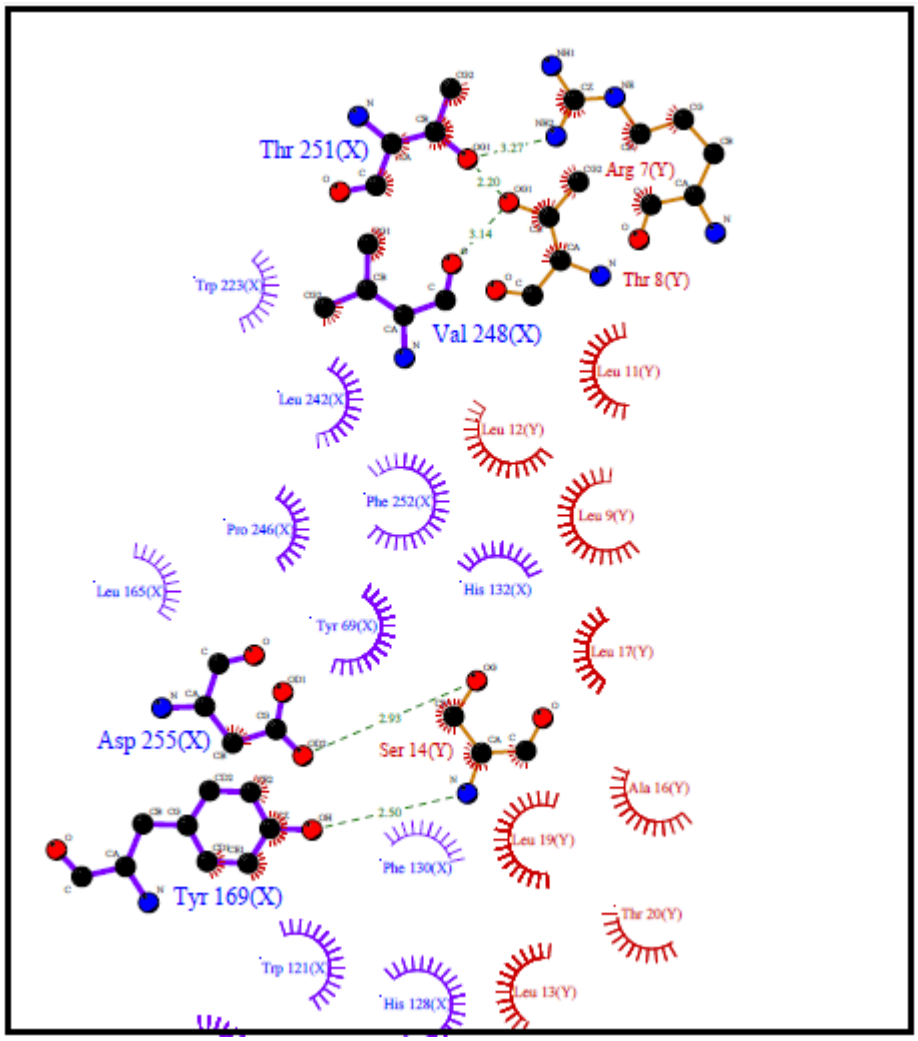


Fig 29 : LIGPLOT of KIR3D2-HLA-a11

Classical MHC supertypes have shown significant interactions with their respective NK inhibitory receptors . The binding energies obtained with A supertype and KIR3DL2 was negative in only one of the transformation The results of docking represented number of solutions which are sorted on the basis of score and approximate area of complex covered. The PDB file of different complexes ,based on different transformations of ligand and receptor is obtained , some of the complexes showed better energies than the other.Mean of all the complexes was calculated to compare the binding affinities exempting the ouliers.The mean for HLA A supertype and KIR3DL2 was positive that means no significant interaction occurs while for KIR2DL1-HLA-cw2,4,5 , mean is in range of -3 to -5 and in KIR2DL2-HLA-cw 1,5,7 , mean is in range of -6 to -7. So here the interaction with KIR2DL2 and HLA-cw 1,5,7 was found to best with more number of interactions.Comparatively below are the interactions of the non classical MHC molecules with their respective inhibitory receptors.

5.5 NK inhibitory receptor interaction with non- classical MHC supertypes:

NKG2A-HLA-G :

NK inhibitory receptor NKG2A was downloaded from PDB with PDB id 3BDW.This NKG2A receptor is present in heterodimer form with CD94. CD94/NKG2A heterodimer has four chains A,B,C and D in its structure and since docking requires single chain both for ligand and receptor the the four chains of this receptor molecule was separated in viewerlite.

The ligand molecule HLA-G that is the non classical MHC is present in single chain A was obtained from PDB with PDB ID 1YDP.

Each chain of the recetopr molecule was docked against the ligand molecule to cover all the sites and to determine to which particular transformation does the ligand HLA-G show better binding affinity to the NKG2A receptor.

5.5.1 NKG2A(CHAIN –A) – HLA-G(CHAIN-A) :

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table:

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
7	13804	1822.70	-20.88	8.72	-3.94	9	175	18	**-11.696746 kcal/mol**
6	13860	2457.00	-2.40	4.41	-4.17	4	139	15	-5.3644 kcal/mol
3	14778	2039.10	5.78	3.73	-1.18	5	102	9	-8.117427 kcal/mol
1	15286	2003.10	10.55	7.13	-2.51	5	130	18	-9.426056 kcal/mol
4	14756	1895.70	12.24	5.63	-1.26	3	125	13	-5.49358 kcal/mol
8	13786	1899.70	15.84	0.61	0.00	3	31	2	-6.459208 kcal/mol
2	14850	2362.90	43.79	15.64	-5.09	7	129	14	-4.397150 kcal/mol
10	13560	1872.90	46.22	-0.46	-2.22	1	83	15	-8.207142 kcal/mol
5	14704	2027.50	411.92	10.18	-13.08	3	80	12	-4.566867 kcal/mol
9	13728	2006.90	7184.80	13.25	-12.87	3	173	19	-3.445480 kcal/mol

Table11 : NKG2A(A)-HLA-G(A)interaction energies

For this docked complexes obtained here , the receptor ligand complex with minimum energy is1st complex with energy -11.696746 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

The mean calculated for all the complexes is -6.7.

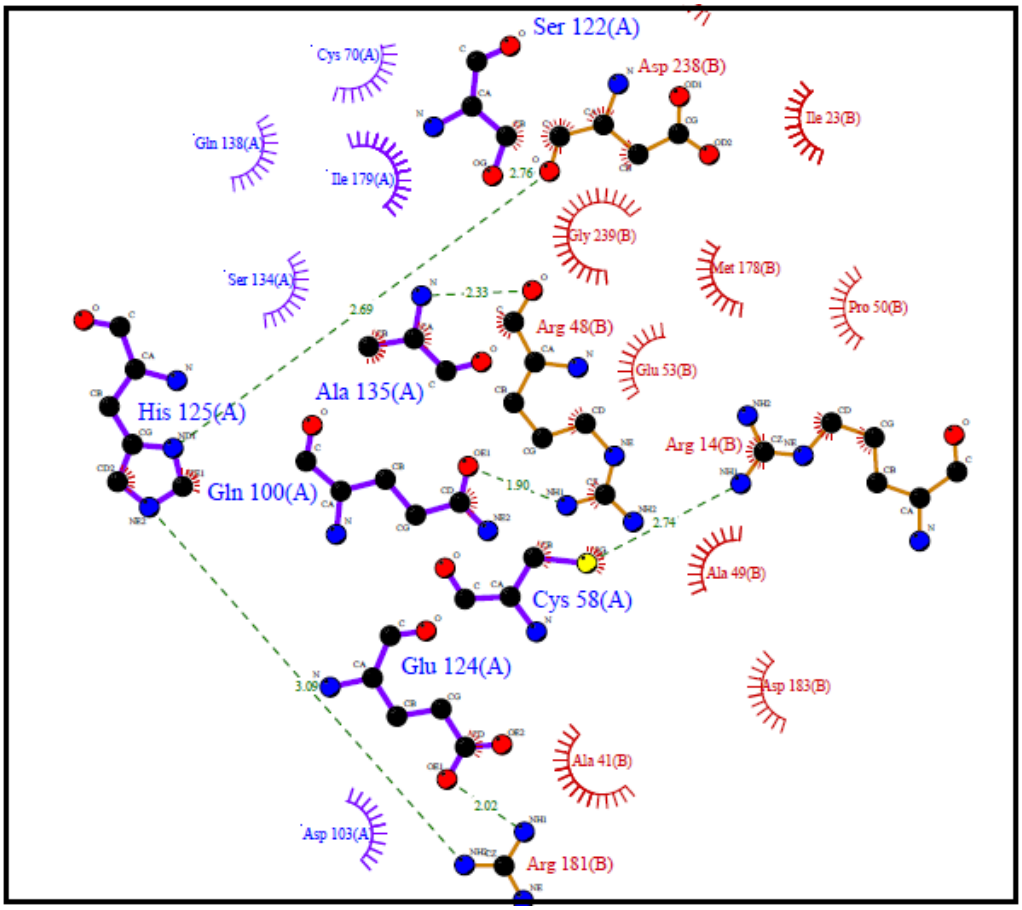


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Fig 30 : LIGPLOT of NKG2A(A)-HLA-G(A)

5.5.2 NKG2A (CHAIN-B) – HLA-G (CHAIN-A):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 3rd complex with energy -9.879062 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

The mean calculated is-5.92. , 2nd and 7th complex were taken as outlier because of high positive energy due to large number of non bonded interactions.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
2	14798	2421.00	-12.44	6.32	-1.49	3	106	17	-8.247068 kcal/mol
1	15000	1958.80	-3.65	1.03	-0.68	8	954	27	37.498 kcal/mol
7	13588	1916.30	-2.88	11.77	-3.42	4	117	22	**-9.879062 kcal/mol**
5	13728	2046.30	4.67	3.02	0.00	2	76	5	-3.638696 kcal/mol
3	13972	2154.80	5.15	7.15	0.00	7	101	7	-7.344359 kcal/mol
4	13908	1782.30	9.72	17.25	-3.31	2	174	26	-8.3596 kcal/mol
10	13372	2250.70	10.91	-0.47	0.00	6	482	14	17.8436 kcal/mol
8	13580	2047.40	15.56	18.09	-1.87	0	18	4	-5.0537 kcal/mol
9	13580	2349.30	110.65	13.96	-2.51	6	139	15	-4.1546 kcal/mol
6	13618	1857.90	1665.92	23.61	-6.56	2	299	29	-0.826450 kcal/mol

Table 12: NKG2A(B)-HLA-G(A)interaction energies

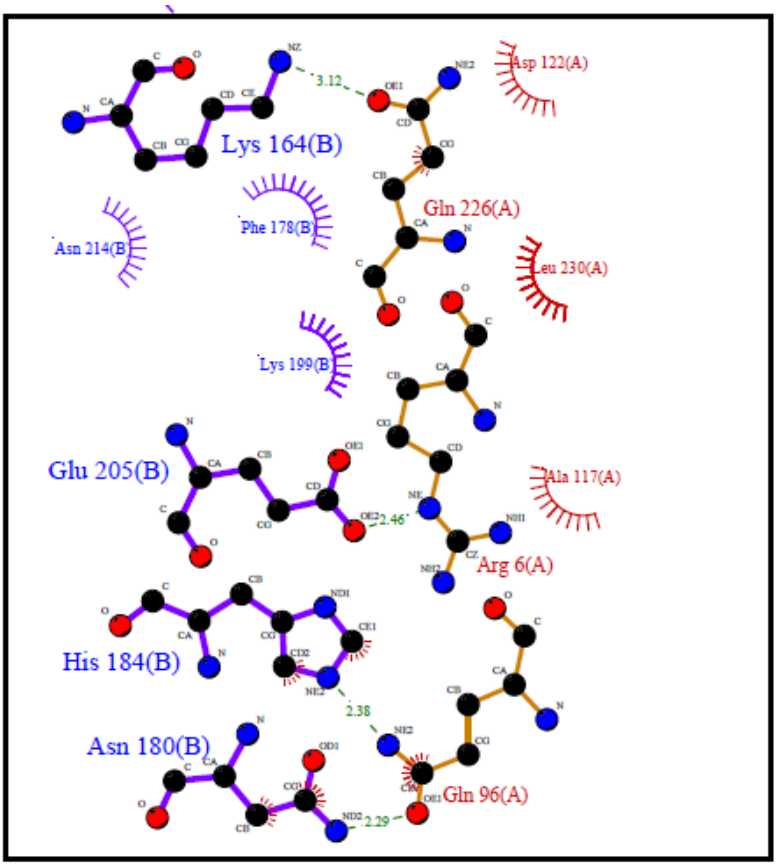


Fig 31 : LIGPLOT of NKG2A(B)-HLA-G(A)

5.5.3 NKG2A(CHAIN-C)-HLA-G(CHAIN-A) **:** The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
5	13692	1663.10	-25.81	4.10	-2.84	5	146	17	-10.5529 kcal/mol
7	13550	1748.10	-5.23	10.06	-2.22	1	123	17	-5.4899 kcal/mol
8	13194	2584.20	-4.21	2.07	-2.48	12	187	16	**-13.9717 kcal/mol**
9	13170	1966.20	-1.97	11.05	-3.82	2	128	15	-6.7682 kcal/mol
1	15222	1960.70	1.36	13.37	-7.62	4	163	21	-11.4608 kcal/mol
2	14382	1820.50	15.67	3.44	-1.50	7	143	12	-7.5320 kcal/mol
6	13688	2056.60	16.20	6.68	-2.11	1	42	5	-5.5099 kcal/mol
4	13692	1921.40	18.00	9.20	-2.14	3	130	20	-9.8425 kcal/mol
3	13952	2091.20	23.60	6.94	-3.33	3	173	25	--8.5788 kcal/mol
0	13122	1903.80	251.06	-9.46	-4.89	0	50	3	-2.1767 kcal/mol

Table 13: NKG2A(C)-HLA-G(A)interaction energies

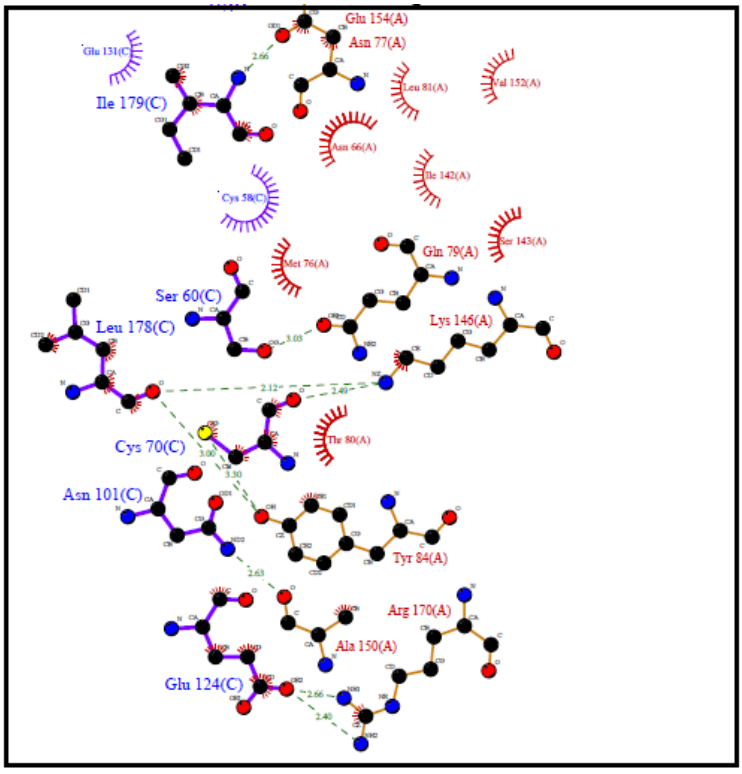


Fig 32 : LIGPLOT of NKG2A(C)-HLA-G(A)

For this docked complexes obtained here , the receptor ligand complex with minimum energy is 3rd complex with energy -13. 9717 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
Mean comes out to be -8.1.

5.5.4 NKG2A(CHAIN-D)-HLA-G(CHAIN-A):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	NON BONDED INTERACTIONS	HYDROPHOBIC INTERACTIONS	D COMPLEX (kcal/mol)
1	15106	1984.20	-37.20	6.76	-4.17	4	154	23	-9.1620 kcal/mol
4	13588	1832.60	-22.00	5.60	-1.57	2	112	15	-9.4364 kcal/mol
10	13298	1808.50	-20.48	8.10	-3.85	0	74	15	-7.88113 kcal/mol
8	13318	2045.20	-14.61	10.84	-5.30	8	133	10	-6.1328 kcal/mol
2	13888	1839.60	-3.14	15.30	-3.95	5	178	24	-3.4162 kcal/mol
3	13666	1712.70	-2.54	18.19	-3.08	3	165	29	-7.8990 kcal/mol
9	13314	1993.90	7.12	18.23	-9.02	7	187	17	**-10.9938** kcal/mol
7	13364	2312.30	12.08	2.54	-2.18	3	82	6	-4.1204 kcal/mol
6	13480	1883.60	22.46	5.39	-1.62	18	2399	69	93.535 kcal/mol
5	13542	1845.00	1819.50	11.22	-5.45	15	2657	71	122.4826 kcal/mol

Table 14: NKG2A(D)-HLA-G(A)interaction energies

For this docked complexes obtained here , the receptor ligand complex with minimum energy is 7th complex with energy -10.9938 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
Exempting the outliers i.e. 9th and 10th complex , mean comes out to be -7.375.

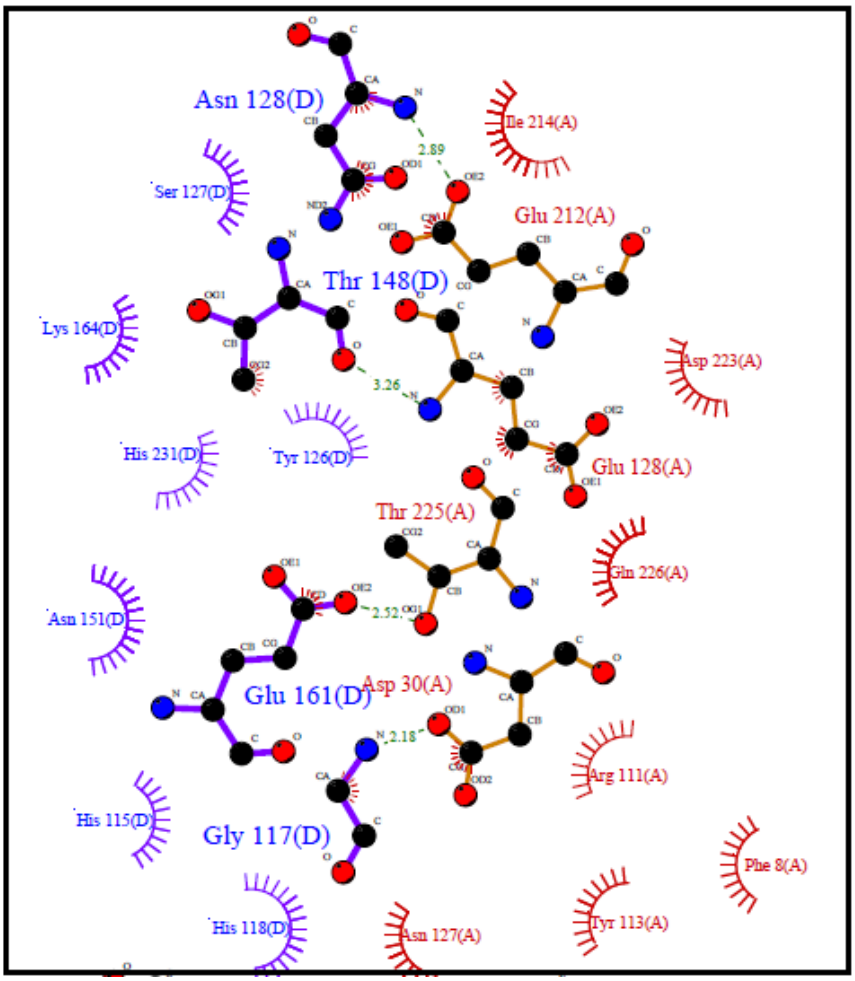


Fig 33 : LIGPLOT of NKG2A(D)-HLA-G(A)

NKG2A-HLA-E:

The ligand molecule HLA-E with PDB ID 1KTL was downloaded from PDB . The ligand molecule was found to have two chains A,C and since the receptor molecule is having four chains A,B,C,D. So a combination of chains was formed to cover all the sites to determine the complexes that show best binding affinity.

5.5.5 NKG2A(CHAIN –A) – HLA-E(CHAIN-A) :

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 9th complex with energy -10.3281 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

The 3rd complex shows high positive energy because of large number of non-bonded interactions so was considered as an outlier and the mean comes out to be -7.271 .

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
7	13804	1822.70	-20.88	8.72	-3.94	9	24	247	-8.8355 Kcal/mol
6	13860	2457.00	-2.40	4.41	-4.17	7	20	216	-9.8845 Kcal/mol
3	14778	2039.10	5.78	3.73	-1.18	38	82	4171	162.717 Kcal/mol
1	15286	2003.10	10.55	7.13	-2.51	2	12	100	-6.188 Kcal/mol
4	14756	1895.70	12.24	5.63	-1.26	3	6	82	-5.1028 Kcal/mol
8	13786	1899.70	15.84	0.61	0.00	6	18	137	-10.0590 Kcal/mol
2	14850	2362.90	43.79	15.64	-5.09	1	4	35	-5.2320 Kcal/mol
10	13560	1872.90	46.22	-0.46	-2.22	0	9	72	-2.4335 Kcal/mol
5	14704	2027.50	411.92	10.18	-13.08	4	20	181	**-10.3281 Kcal/mol**
9	13728	2006.90	7184.80	13.25	-12.87	2	17	77	-7.3966 Kcal/mol

Table 15: NKG2A(A)-HLA-E(A)interaction energies

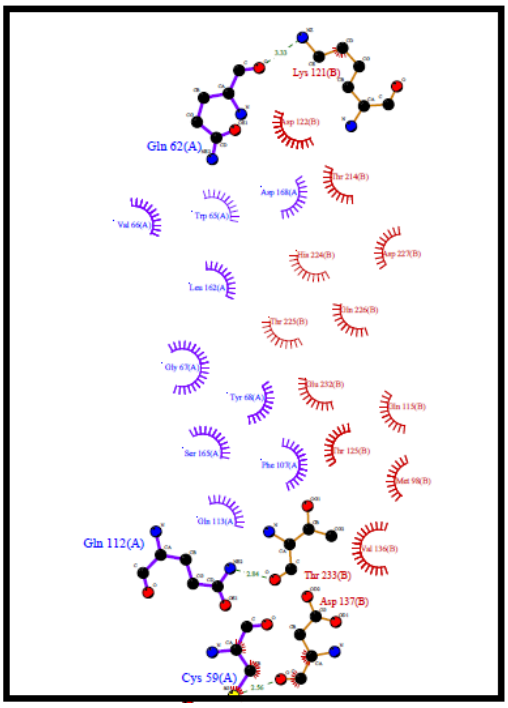


Fig 34 : LIGPLOT of NKG2A(A)-HLA-E(A)

5.5.6 NKG2A(CHAIN –A) – HLA-E(CHAIN-C):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex

with minimum energy is 1st complex with energy -11.1733 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined
Mean comes out to be -6.1.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
3	14578	2048.80	-20.65	4.79	-3.30	11	18	162	**-11.1733 Kcal/mol**
8	13810	1623.60	-20.39	10.75	-3.19	2	27	190	-9.7928 Kcal/mol
1	15220	2257.70	3.18	13.29	-4.38	5	23	139	-6.984 Kcal/mol
9	13662	2422.60	6.01	1.22	0.00	5	13	207	1.0770 Kcal/mol
6	13996	2060.40	10.95	0.61	-0.64	1	3	38	-4.8804 Kcal/mol
7	13988	1811.40	14.68	5.04	-1.40	4	1	56	-3.5563 Kcal/mol
4	14290	2047.70	15.83	14.53	-1.42	6	21	141	-8.0243 Kcal/mol
5	14244	1842.70	217.80	9.36	-6.40	1	21	110	-9.1640 Kcal/mol
10	13652	2254.70	952.38	13.27	-8.64	0	7	70	-3.0178 Kcal/mol
2	14734	2021.60	1541.55	19.61	-5.25	2	5	44	-5.9860 Kcal/mol

Table16: NKG2A(A)-HLA-E(C)interaction energies

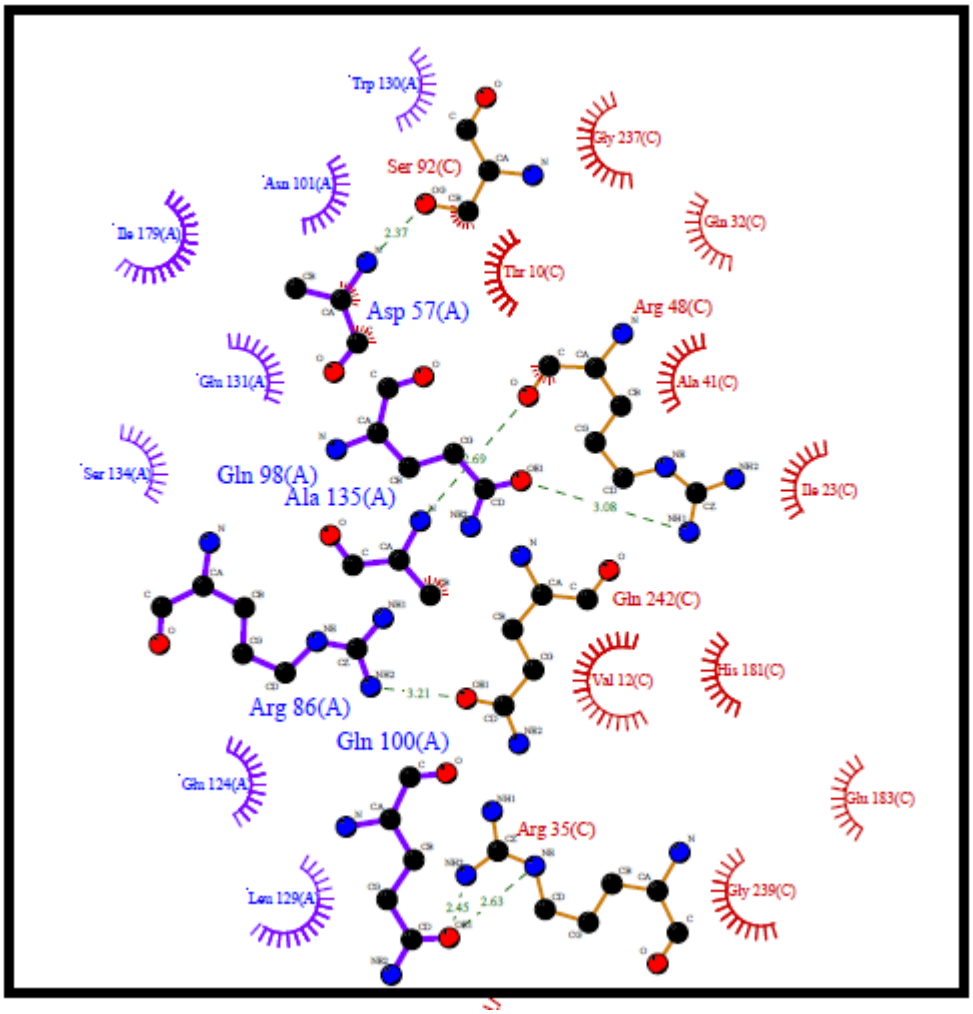


Fig 35 : LIGPLOT of NKG2A(A)-HLA-E(C)

5.5.7 NKG2A(CHAIN –B) – HLA-E(CHAIN-A):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 5thcomplex with energy -9.9827 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.

Mean comes out to be -6.1 , exempting the outliers with high positive energy.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
8	13770	1791.90	-11.65	0.79	-1.64	6	16	137	--5.3137 Kcal/mol
7	14106	1787.60	-8.96	7.42	-1.94	3	16	136	-6.8396 Kcal/mol
1	15370	2280.40	-6.39	-2.59	-0.57	5	8	113	-7.2750 Kcal/mol
3	14866	1993.90	2.17	1.35	-0.37	0	19	131	-6.5620 Kcal/mol
5	14492	1868.00	4.69	9.03	-4.24	4	20	146	**-9.9827 Kcal/mol**
10	13368	1878.10	17.53	14.40	-3.83	5	16	127	-6.4069 Kcal/mol
2	15058	2139.70	21.98	3.35	-0.70	0	0	15	-4.9959 Kcal/mol
9	13432	1851.70	63.24	14.07	-4.83	5	16	171	-7.6644 Kcal/mol
6	14208	2024.40	595.57	7.87	-1.84	3	15	313	9.1527 Kcal/mol
4	14556	2209.80	2188.51	8.31	-9.62	1	15	59	-6.9598 Kcal/mol

Table 17: NKG2A(B)-HLA-E(A)interaction energies

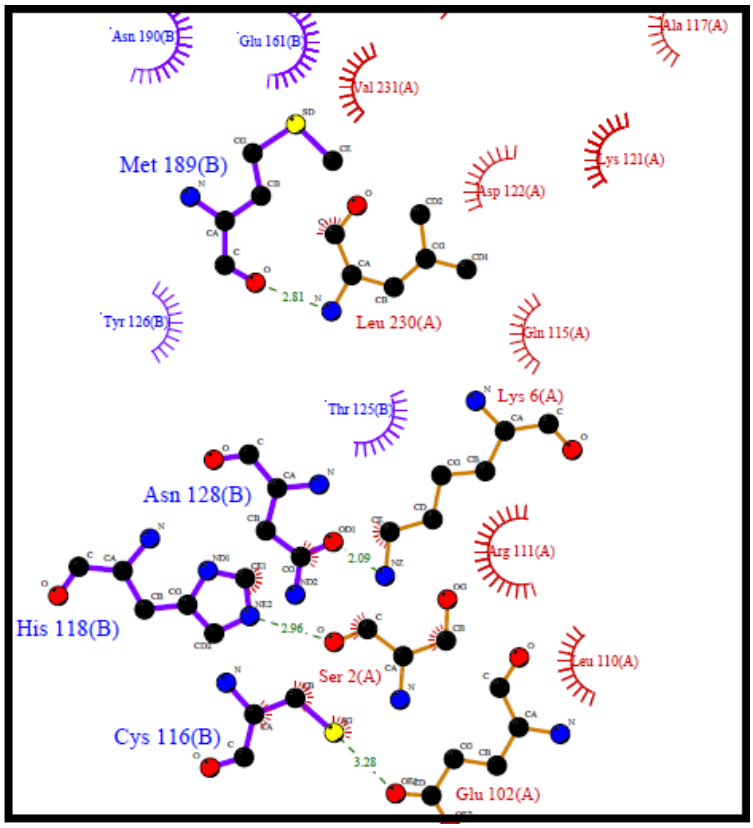


Fig 36 : LIGPLOT of NKG2A(B)-HLA-E(A)

5.5.8 NKG2A(CHAIN –B) – HLA-E(CHAIN-C):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 8thcomplex with energy -9.7447 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
Exempting the outliers , Mean comes out to be -6.279

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
1	14040	2162.70	-8.59	1.61	-2.09	2	17	129	-7.5599 Kcal/mol
9	13132	1888.80	6.99	-0.70	0.00	4	17	435	13.4970 Kcal/mol
7	13196	1670.30	7.43	11.82	-3.32	3	16	161	-6.9328 Kcal/mol
2	13880	1999.30	33.44	4.99	-2.86	3	16	152	-5.4194 Kcal/mol
10	13072	1701.40	51.17	18.29	-3.30	4	20	146	-5.8829 Kcal/mol
6	13296	1865.80	52.29	22.27	-10.20	7	17	202	-3.5038 Kcal/mol
3	13514	1799.80	61.39	18.92	-6.57	6	17	168	-6.8141 Kcal/mol
5	13366	2001.70	663.26	13.40	-6.90	9	11	158	**-9.7447 Kcal/mol**
4	13376	2468.00	3119.05	20.42	-14.33	1	3	16	-3.9808 Kcal/mol
8	13142	2261.20	7895.30	14.99	-14.44	4	8	74	-6.6933 Kcal/mol

Table 18 : NKG2A(B)-HLA-E(C)interaction energies

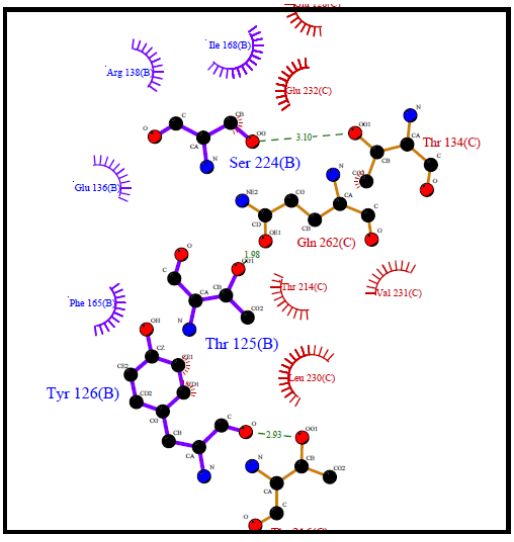


Fig 37 : LIGPLOT of NKG2A(B)-HLA-E(C)

5.5.9NKG2A(CHAIN –C) – HLA-E(CHAIN-A):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 1st complex with energy -12.1526 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
Mean comes out to be -7.86.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
5	14834	1972.40	-44.96	9.06	-7.63	8	20	195	**-12.1526 Kcal/mol**
10	14166	1788.30	-42.02	10.50	-8.61	9	24	213	-11.6530 Kcal/mol
9	14210	1989.00	-24.19	3.14	-1.96	5	14	121	-10.6647 Kcal/mol
1	16174	2287.50	-4.21	7.17	-2.55	1	15	119	-5.8086 Kcal/mol
2	15604	2123.60	-4.17	-1.90	0.00	6	9	167	-5.9711 Kcal/mol
6	14524	1987.40	8.05	1.13	-0.76	1	6	79	-3.6611 Kcal/mol
3	15078	2120.40	16.79	1.85	-1.37	5	23	142	-8.0766 Kcal/mol
4	14872	2201.00	92.02	19.04	-3.09	3	20	127	-9.1119 Kcal/mol
8	14290	1976.60	2353.12	1.24	-7.28	5	23	191	-5.2149 Kcal/mol
7	14362	2330.50	6848.37	11.08	-11.34	4	6	54	-6.3623 Kcal/mol

Table 19: NKG2A(C)-HLA-E(A)interaction energies

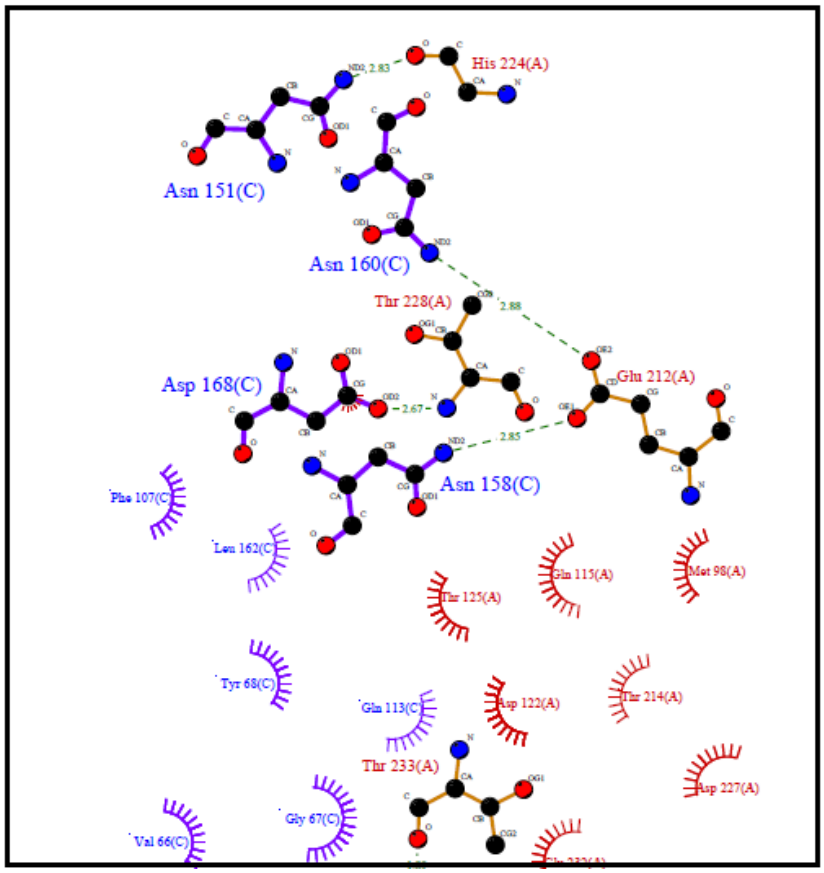


Fig 38 : LIGPLOT of NKG2A(C)-HLA-E(A)

5.5.10 NKG2A(CHAIN –D) – HLA-E(CHAIN-A):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 1st complex with energy -10.5579 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
Mean comes out to be -6.81.

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
8	13534	1823.20	-6.28	3.90	-1.61	3	21	133	**-10.5579 Kcal/mol**
3	14314	1936.40	3.59	3.80	0.00	2	12	111	-5.7362 Kcal/mol
5	14006	2328.80	7.56	-0.54	0.00	3	7	44	-8.1261 Kcal/mol
7	13648	2188.30	8.82	7.28	-1.05	4	18	135	-9.0918 Kcal/mol
2	14444	2504.20	34.76	13.85	-4.93	7	8	89	-5.9566 Kcal/mol
10	13296	2059.20	82.78	3.17	-4.41	5	19	185	--6.3253 Kcal/mol
9	13426	2143.50	109.89	0.76	0.00	0	13	85	-5.1746 Kcal/mol
1	15786	2206.20	120.20	11.94	-4.20	5	10	120	-5.2837 Kcal/mol
4	14036	2150.70	2699.89	11.76	-10.35	0	5	20	-4.9135 Kcal/mol
6	13696	2431.30	5190.63	26.09	-8.81	3	15	84	-7.0515 Kcal/mol

Table 20: NKG2A(D)-HLA-E(A)interaction energies

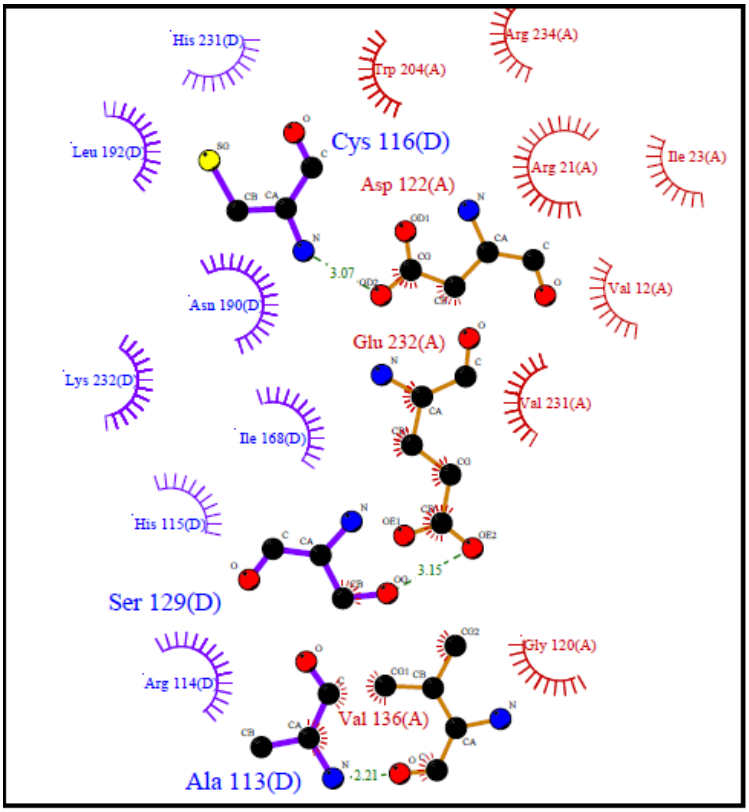


Fig 39 : LIGPLOT of NKG2A(D)-HLA-E(A)

5.5.11 NKG2A(CHAIN –D) – HLA-E(CHAIN-C):

The D –complex energy and various interactions observed for this receptor ligand complex is mentioned in table.For this docked complexes obtained here , the receptor ligand complex with minimum energy is 5th complex with energy -8.3845 kcal/mol. The ligplot for this complex that depicts all the hydrogen bond interactions along with hydrophobic interactions and non bonded interactions is determined.
The mean comes out to be -6.1

S NO. (rank)	PATCHDOCK SCORE	AREA	GLOBAL ENERGY	ACE	HB	NO. OF H BONDS	HYDROPHOBIC INTERACTIONS	NON BONDED INTERACTIONS	D COMPLEX (kcal/mol)
2	14654	2162.30	-15.93	12.70	-8.25	7	20	212	-8.0186 Kcal/mol
9	13220	1643.50	-3.74	6.38	-0.31	2	20	110	-6.3715 Kcal/mol
7	13478	1728.30	4.92	6.64	-1.53	3	16	152	-5.5150 Kcal/mol
8	13384	1881.50	15.90	13.32	-2.99	5	13	121	-5.4646 Kcal/mol
10	13188	1752.20	18.60	15.99	-4.75	5	16	159	**-8.3845 Kcal/mol**
4	13624	2138.80	148.95	6.54	-4.67	4	14	161	-5.5002 Kcal/mol
5	13606	2123.60	329.18	7.32	-2.97	2	15	119	-6.3483 Kcal/mol
1	15558	2387.20	339.44	4.92	-3.95	5	7	105	-6.8772 Kcal/mol
3	13856	2414.90	1139.81	12.34	-2.32	1	11	112	-3.7869 Kcal/mol
6	13582	2333.20	6573.07	12.98	-13.19	4	24	289	-4.8517 Kcal/mol

Table 21 : NKG2A(D)-HLA-E(C)interaction energies

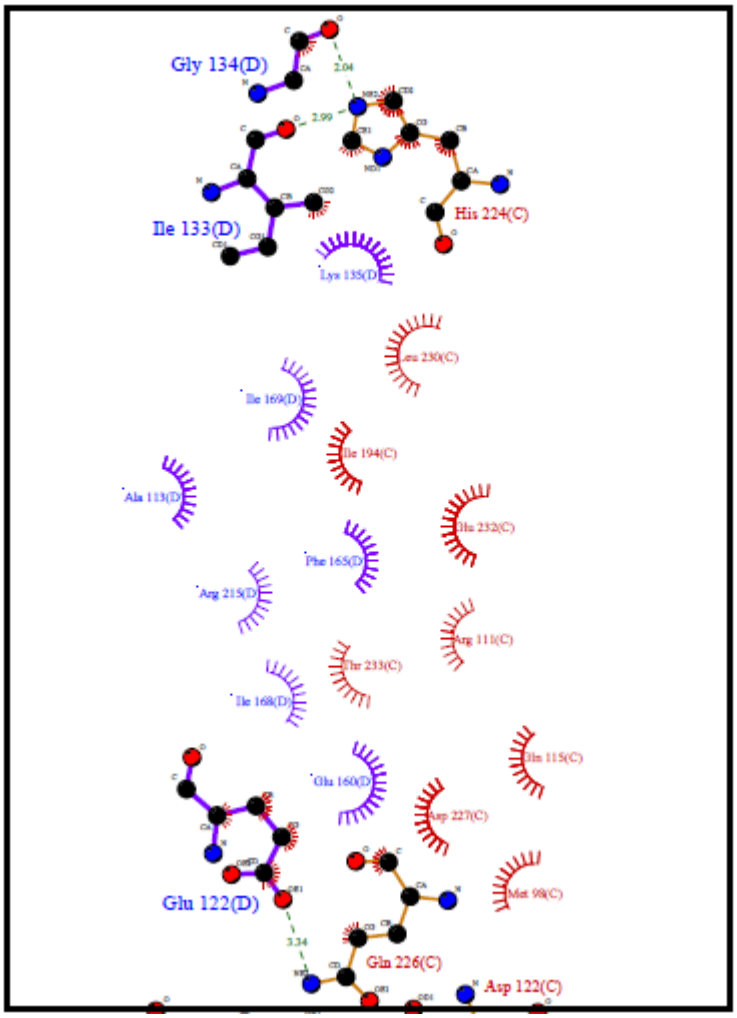
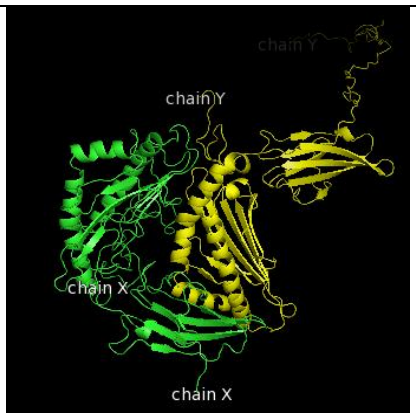
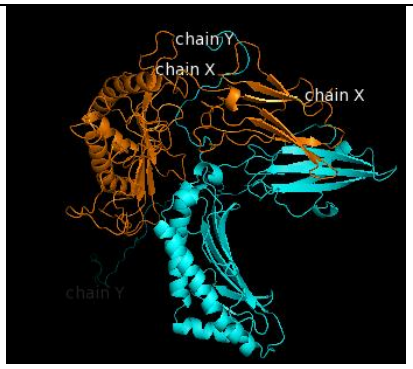
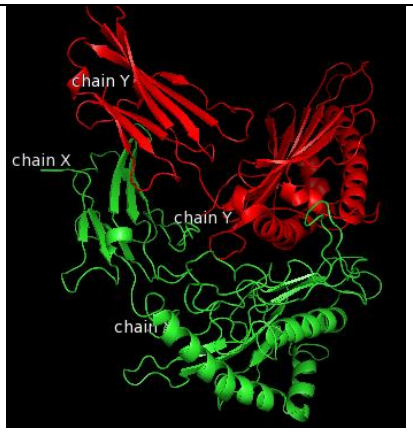
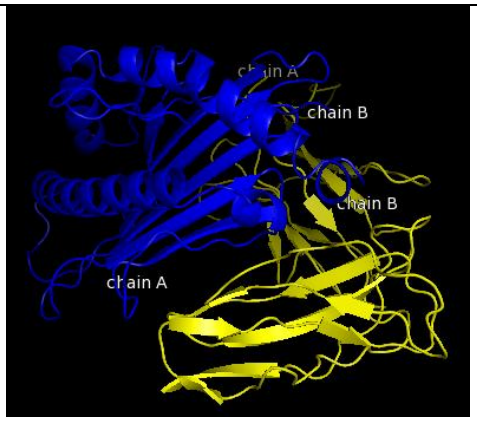
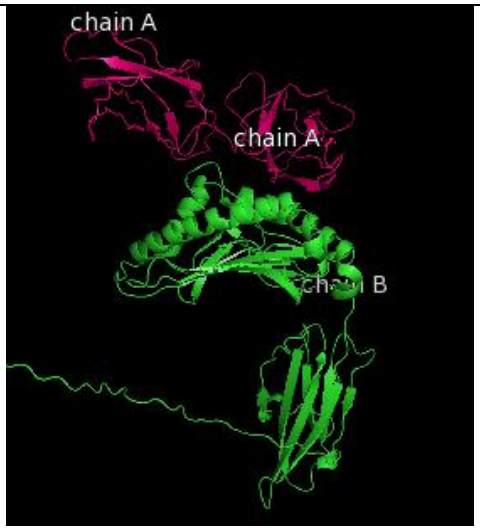


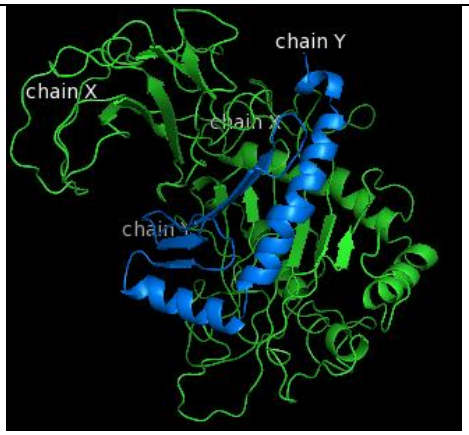
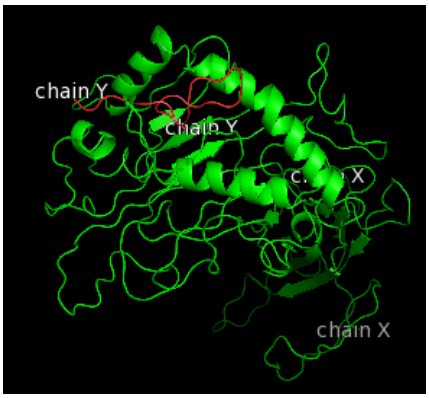
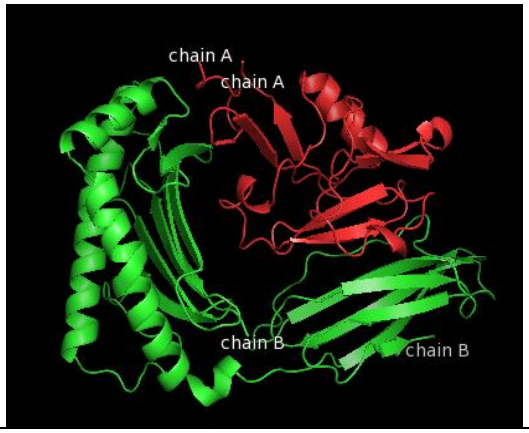
Fig 40 : LIGPLOT of NKG2A(D)-HLA-E(C)

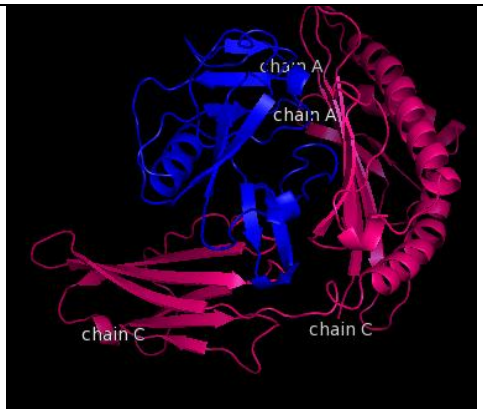
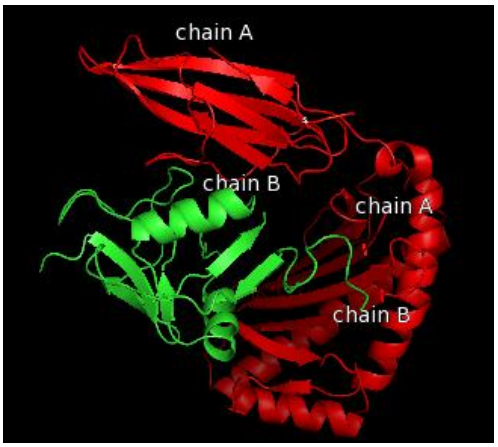
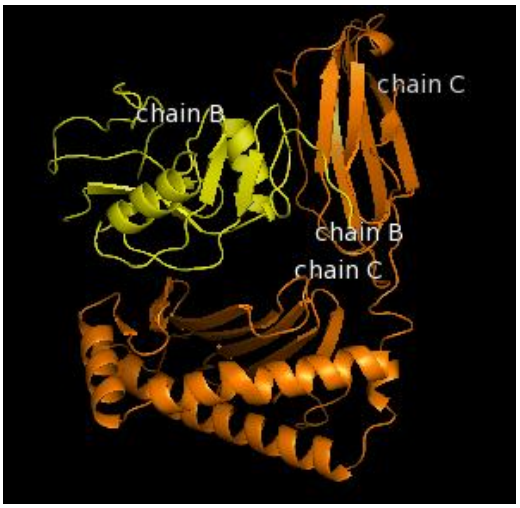
The LIGPLOT clearly depicts the 2D represntation of the interactions , hydrogen bonds and the hydrophobic contacts between the protein and its bound ligand. In case of non-classical molecules , HLA-G and HLA-E , interaction with the inhibitory receptor NKG2A is studied . Compared to the classical molecules they have shown better binding energies in more transformations . Below is a table showing comparative analysis of both the classical and non-classical MHC's along with the docked complex obtained for the structure with best energy.

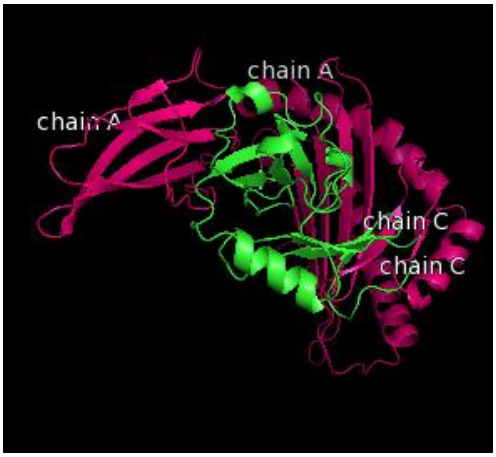
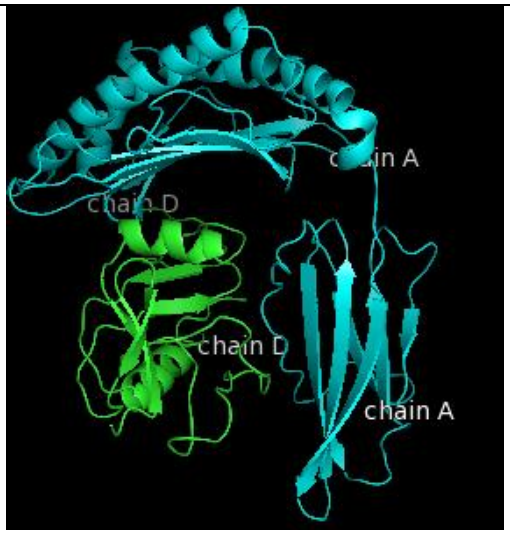
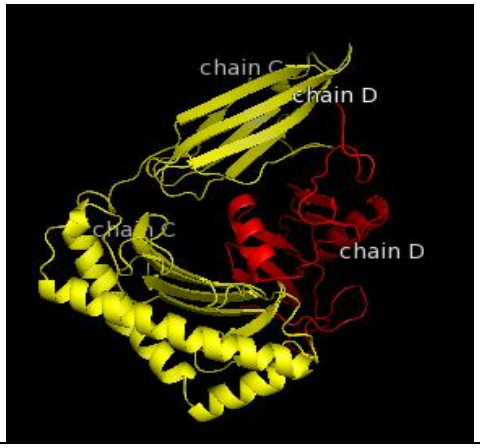
Below is the table representing the docked receptor ligand complex with the best binding energy for both the classical and non-classical MHC.

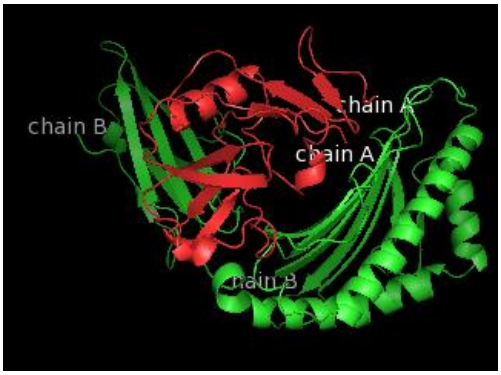
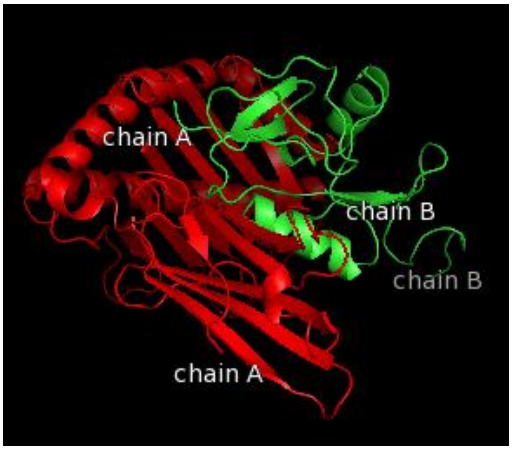
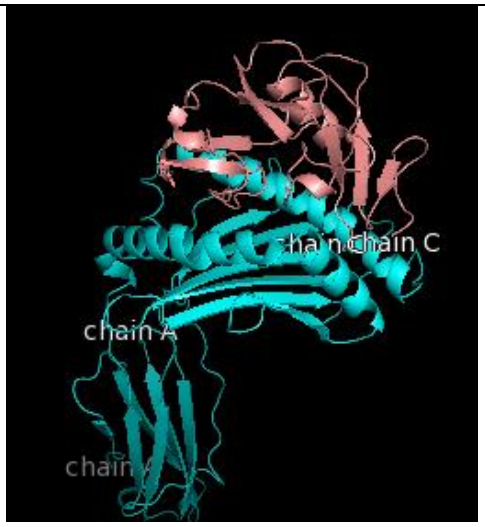
S.no	Receptor	Ligand (classical MHC)	Docked Complex	D-complex energy
1.	KIR2DL1	HLA -cw 2		**-8.511224 Kcal/mol**
		HLA-cw4		**-11.915498 kcal/mol**
		HLA-cw5		**-9.543073 kcal/mol**
2.	KIR2DL2	HLA-CW1		**-11.161303 kcal/mol**

				
		HLA-CW3		**-9.636099 kcal/mol**
		HLA-CW7		**-8.914885 kcal/mol**
3.	KIR3DL2	HLA-A3		**-7.684858 Kcal/mol**

				
		HLA-A11		**-10.307178 Kcal/mol**
4.	NKG2A CHAIN – A , B , C , D	(non-classical) HLA-E CHAIN A , C		
		CHAIN A-A		**--10.328101 Kcal/mol**
	NKG2A CHAIN – A , B , C , D	(non-classical) HLA-E CHAIN A , C	Docked complex	D-complex energy

		CHAIN A- C		**-11.173326 Kcal/mol**
		CHAIN B-A		**-9.982788 Kcal/mol**
		CHAIN B-C		**-9.744729 Kcal/mol**

	NKG2A CHAIN – A , B , C , D	(non-classical) HLA-E CHAIN A , C	Docked complex	**D-complex energy**
		CHAIN C-A		**-12.152668 Kcal/mol**
		CHAIN D-A		**-10.557935 Kcal/mol**
		CHAIN D-C		**-8.384561 Kcal/mol**

5.	NKG2A	HLA – G (CHAIN A)		D-complex energy
		CHAIN A-A		**-11.696746 Kcal/mol**
		CHAIN B-A		**-8.395685 Kcal/mol**
		CHAIN C-A		**-13.971720 Kcal/mol**

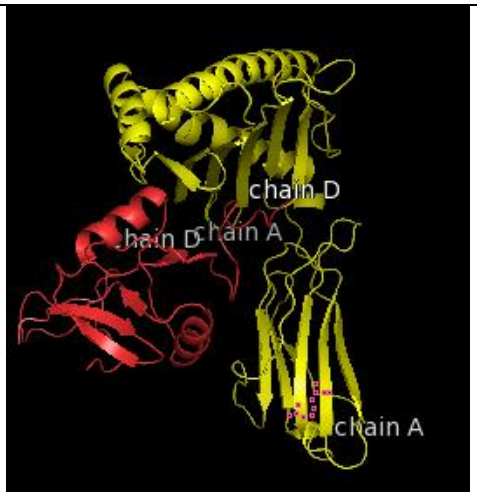
		CHAIN D-A		**-10.993817 Kcal/mol**

Table 22 :Docked complexes of receptor ligand with minimum energy

The results depict that the binding affinities in case of non-classical was found to be better , as mean of the total binding affinities was calculated for all the transformations of both classical and non-classical to compare their binding affinities with their respective NK inhibitor. For classical MHC supertypes , mean of the binding affinity was positive for HLA-A ans for HLA-C supertypes it lied in range of -3 to -6 .

In case of non-classical MHC , HLA-E and HLA-G , the mean calculated with binding affinities for all the receptor ligand complexes lied in range of -5.5 to -8.

This data represents that non classical MHC binding to its inhibitory receptor is better compared to the classical MHC binding.

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6. CONCLUSION

Inhibitory receptor present on NK cell recognise MHC molecule and upon engagement with these receptors , block the ability of NK cells to attack the target cells, if the inhibitory ligand is not present in the target cell , NK cell will cause lysis of the target cell.

The results obtainedshow that the binding affinities obtained with docking of non-classical MHC with their inhibitory receptor NKG2A is found to be better with better signaling strength in almost all transformations of the chains.Comparatively with classical MHC molecules, binding affinities calculated for their inhibitory KIR receptors particularly , HLA-A supertypes,A3 and A11 showed negative binding affinity in only one or two transformationsand the overall mean calculated with the binding affinities of their all ten transformations comes out to be positive , HLA-A3 is found to be associated with the self peptides and is expected to prevent the NK cell lysis of autologous healthy cells by interacting with KIR3DL2, also both A3 and A11 interactions with KIR3DL2 are known to be relevant during EBV infections.It is known that HLA-C molecules play role in NK cell activation through binding with KIR , here HLA-C supertypes have shown good interactions with the KIR's , their mean values lied in the range of

-3 to -6 , so it can be concluded that HLA-C binding to its inhibitory receptor is better than HLA-A , further because of their good interactions with KIR's, their relevance in diseases can be understood.

On the other hand , in case of HLA-E and HLA-G , the docked complexes obtained represent good binding affinities with their receptor NKG2A.NKG2A recognises self proteins that are poorly expressed by normal cells but in case of diseased cells , such as infected cells or tumour it is found in elevated levels. Mean was calculated for all the results obtained after docking of all chains of HLA-E and HLA-G with NKG2A , and it was found to be in range of -5.5 to -8 which represents that non-classical binding to its receptor is way better.

HLA-E favorsthe activating immune response to colorectal carcinoma. They are known to provide evidences in humans that tumor cells entertain extensive negotiation with the immune system until a compromise between recognition and escape is reached. Expression of the non-classical HLA-G class I antigen is physiologically restricted to a limited number of tissues including thetrophoblasts, and is known to play a role in establishing tolerance of the fetus by the maternal immune system. So with the understanding of binding energies , the role of the receptor ligand interactions in these mechanism can be studied.

7. DISCUSSION AND FUTURE PROSPECTS

Inhibitory KIR's and NKG2A receptors bind to the classical and non classical MHC class respectively (Rajagopalan and Long ,2012) . Different classes of inhibitory receptors are present in each human NK cell.The downstream signaling is similar for both the classical and non-classical MHC .Several in vitro studies have proved that the interaction between inhibitory receptor and non classical MHC like HLA-E / HLA-G is stronger as compared to classical MHC (Das and Long ,2010). In the present work also , it was proved that non-classical binding to its receptor is better as mean of binding energies for all receptor ligand complexes was calculated , in some of the transformation , high positive energy was obtained which may be due to large number of non bonded interactions so such energies were considered as outlier and were exempted from mean calculations , the results represented that binding energies obtained with non-classical MHC are relatively strong .Since structural basis for the difference in NK activity has not yet been explored , so in order to determine better immunotherapeutic strategies for cancer treatment , we need to explore the structural basis of the different inhibitory receptors with their ligands and see how the expression level of receptors is altered in NK cells in different organs. NK cell employs analog signaling where a repertoire of inhibitory and activating receptors binds to their cognate ligands and the net outcome is the result of quantitative effect produced by the binding of the receptors to their ligands. In past years , major progresses have been done in understanding the molecular mechanisms that are involved in NK cell function , number of receptors with opposing functions and even many of their ligands are identified and are molecularly characterized. However there are still major issues that need to be clarified in order to have a better understanding of NK cell physiology and thus exploiting them in immunotherapy.

NK inhibitory receptors and non classical MHC interactions has been shown to have better inhibition of NK cell cytoxicity than the classical MHC molecule . The diverse recognition pattern of classical and non classical MHC proteins by NK receptors and every accessible surface involved in this binding event should be explored .The knowledge of affinity of NK cell receptor with MHC I ligands will help in understanding the binding patterns required for the inhibition of NK cell activity.

By understanding the structure of HLA supertypes in human population all over the world and the epidemiological studies of their existence in different geographical regions can correlate with the existence of certain cancers and viral diseases in those geographical locations. A better understanding of these molecular mechanism that are involved in the NK cell function provides insight to exploit them in therapies , particularly in cancer, infectious diseases , and BM transplantation.

8. REFERENCES

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