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In-Silico Approach to Study Affinities of NK Cell Inhibitory Receptor Interaction with Classical and Non -Classical MHC Ligands

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Abstract

***Objective**: Different classes of inhibitory receptors are present in each human NK cell. It has been seenthat NK inhibitory receptor and non-classical MHC interaction results into better inhibition of NK cell cytotoxicity than with classical MHC molecule, but the basis for this difference in inhibition potential of NK inhibitory receptors binding to classical and non-classical MHC proteins has remained unexplored. Since each NK cell expresses a multitude of receptors; it is difficult to study binding affinities and residues involved in receptor-ligands interaction through experimental studies.*

***Methods**: The present work aims to investigate binding affinity of different inhibitory receptors with classical and non-classical MHC molecules and to explore important structural residues involved in the interactions through computational approach.*

***Results:** Our results showed that there is high diversity of bonding in case of non-classical MHC binding to inhibitory receptors. Also, number of interface residues involved in interactions and presence of different chains were numerous in case of non-classical MHC molecules. A significantly higher number of H-bonds were observed in case of non-classical MHC interaction with inhibitory receptors.*

***Conclusion**: Comparison between the affinities of inhibitory receptor with classical vs non-classical MHC molecules would give insights to correlate their influence on risk of cancer, therefore will help in devising promising NK- based immunotherapy.*

Keywords: *NK cell receptors, classical MHC, non-classical MHC, KIRs*

Introduction

Natural Killer (NK) cells are large granular lymphocytes of the innate immune system. They do not require prior sensitization with an antigen and play important role in host defence by killing broad range of virus-infected and tumor transformed cells. NK cytotoxicity is regulated by a fine balance between activation and inhibition signals by a multitude of receptors stochastically expressed on each NK cell. Inhibiting NK cell receptors recognize major histocompatibility complex (MHC) class I proteins and tumor antigens with varying degrees of peptide specificity.Both NK receptor and HLA are highly polymorphic and HLA proteins contain motifs that mediate recognition by the receptors.

NK cells receptors may belong to either Ig-superfamily of type I membrane receptor superfamily or C-type lectin family of type II membrane protein.NKp46, NKp30, and NKp44 (collectively called natural cytotoxicity receptors; NCRs), all belongs to the Ig superfamily [1].Among Ig superfamily, there are also killer immunoglobulin-like receptors (KIRs) and ligands for these receptors are amino acid epitopes contained in HLA molecules. C-type lectin-like receptors includes NKG2D, recognized by the stress inducible MHC class-I related chain A/B (MICA/B)

and ULBP proteins.Inhibitory KIRs are known to bind various HLA-A, HLA-B and HLA-C alleles [2]. Also, CD94/NKG2A inhibitory receptors recognized by non-classical HLA-E and HLA-G [3]. It has been seen that NK inhibitory receptor and non-classical MHC interaction results in better inhibition of NK cell cytotoxicity than with classical MHC molecule. It has been reported that extravilloustrophoblasts (EVT) cells lack HLA-A/B expression and express an unusual combination of classical MHC class I molecule HLA-C and non-classical class I molecules HLA-E and HLA-G, which protects foetal tissues from maternal immune system [4]. Moreover, non-small-cell lung cancer (NSCLC) tumor showed higher expression of non- classical HLA-E and HLA-G, protecting them from NK cytotoxicity and resulted into tumor progression [5]. Therefore, it is important to understand the multifarious recognition pattern of classical and non-classical MHC proteins by NK receptors. A better understanding about the binding potential of the residues involved in interactions is needed for exploring the structural basis for NK receptor mediated inhibition by non-classical MHC molecules. This may further be correlated to the predisposition of certain MHC haplotype, to tumor susceptibility or susceptibility to infections.

The present study aims to explore the comparative study of binding affinities of the classical and non-classical MHC molecules with their inhibitory receptors. Investigation of differential binding affinity of variousinhibitory receptors and cognate ligand interactions may give in-sights to better understanding ofcancer susceptibility or resistance.

Materials and Methods

3D Structure Prediction

NK inhibitory receptor KIR (KIR2DL1, KIR2DL2) and NKG2A/CD94 were studied. Putative ligands for different receptors are mentioned in Table 1. The structures of NK receptors and NK receptor complex and HLA ligand were retrieved from RCSB Protein Data Bank (Table 2) while structure of NK receptor KIR2DL1, classical HLA molecules were not available so they were predicted through *de novo* method using IterativeThreadingASSEmblyRefinement(I-TASSER).I-TASSER is web-based protein 3D structure prediction tool based on threading approach. It simulates the generated structure and defines its *C*-score, TM-score, and active sites. *C*-score refers to a confidence level of predicted structure of a given protein sequences where a high *C*-score (range from −5 to 2) indicates an absolute precise quality of the predicted structure. TM-score is structural assessment parameter in which, a smaller distance between the structures is weighted high. TM-score defines the topology of the structure, and it shows that a score more than 0.5 ensures a model of absolute topology [12].

Generated 3D structures were further refined by using 3Drefine, a webserver that involves optimization of hydrogen bonding network combined with atomic-level energy minimization of optimized model [13] and GalaxyRefineserver which rebuilds all side-chain conformations and repeatedly relaxes the structure by short molecular dynamics simulations [14]. Structures with lowest RMSD value were selected for protein –protein interactions.

Molecular Docking of NK Receptor – Ligand

Interaction analysis of NK receptors and their putative ligand were predicted by ClusPro protein–protein molecular docking program. ClusPro server is a rigid docking program based on fast Fourier trans-formation, which clusters the interaction complexes with low energy and identifies the stability of the interaction clusters using the medium-range optimization algorithm. Resulting top docking score NK receptor-ligand were selected, and its docked complex wereanalysed to determine the molecular interactions.

Furthermore, NK receptor-ligand complexes were evaluated for its stereo-chemical properties through Ramachandran plot usingPDBsum.Ramachandran plot determines the dihedral angles [phi (Φ) and psi (ψ)], and the number of residues lying in favoured, allowed, and outlier regions of the protein structure[15].

Table 1. *Ligands for different inhibitory receptors on NK cells*

Receptors	Ligands
KIR2DL1	HLA-Cw2, HLA-Cw4, HLA-Cw5
KIR2DL2	HLA-Cw1, HLA-Cw3, HLA-Cw7
NKG2A/CD94	HLA-G, HLA-E

Table 2. *List of NK receptors, ligand and complex structures available on PDB*

Receptors	Resolution (Å)	PDB entry	Reference
KIR2DL2	2.9	2DL2	[6]
NKG2A/CD94	2.5	3BDW	[7]
Ligands			
HLA-G	1.9	1YDP	[8]
NK receptors- HLA complex.			
NKG2A/CD94-HLA-E	2.5	3CDG	[9]
KIR2DL2/HLA-Cw3	3.0	1EFX	[10]
KIR2DL1/HLA-Cw4	2.8	1IM9	[11]

Results

3D Structure Prediction

In the absence of crystal structures for various NK cell receptor and ligand, the 3D structure was generated. Amino acid sequences were searched for the identification of suitable template through Basic Local Alignment Search Tool (BLAST). Due to unavailability of a template with higher query coverage and with good identity, 3D structure was predicted using I-TASSER. The sequences of KIR2DL1 (accession number ABF13296.1), HLA-Cw2 (accession number AAA59702.1), and HLA-Cw5 (accession number BAA19534.1), HLA-Cw1 (accession number CAA86839.1) and HLA-Cw7 (accession number AAA50217.1) were obtained from NCBI in FASTA format.I-TASSER generated five models of each structure, which were analysed for their properties. Each model was refined by 3Drefine and GalaxyRefine

Analysis of Interaction of Classical and Non-Classical MHC with Cognate Receptor

Among these models, the models with high C-score, TM-score, and RMSD value were selected for molecular docking (data not shown) using ClusPro and interactions were studied through PDBsum server, data has been summarized in Table 3. It was observed that the receptor interactions with classical MHC as well as non-classical MHC complexes were found to have high-rank conformation with a low energy score. However, numbers of residues involved in receptor ligand interactions, hydrogen bonds (H-bonds), No. of salt bridges in contact, No. of non-bonded contacts were significantly more in receptor interaction with non-classical MHC complex NKG2A-HLAE. Also, we observed sulphide bonds interaction in case of NK receptor and non-classical MHC molecules (supplementary data). A significantly higher number of H-bonds contributing to stability of interaction were observed in case of inhibitory receptor interaction with non-classical MHC.It was observed that more number of chains was involved in receptor-ligand interactions, thus more of residues participated in interactions, which contribute to stronger binding affinity of NK receptor with non-classical MHC molecules. Thestereochemical properties were analyzed using PDBsum.It showed that 99.2% and 100% residues of NKG2A/CD94-HLAE and NKG2A/CD94-HLAGcomplexesrespectively were in the allowed region, and 0.8% and 0.0% residues respectively were in the outlier region (Table 3).On the other hand, on an average 97% of residues lies in allowed regions and 3% residues comes in outlier region (Fig.1 and 2). This shows more stability and stronger affinity of NK receptor and non-classical MHC molecules in comparison to NK receptor and classical MHC molecules.

Table 3. Molecular interactions between NK inhibitory receptors with classical and non-classical MHC molecules

Receptor ligand complex	Energy	No. of interface residues chain:chain	Interface Area (A^2)	No. of salt bridges	No. of sulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
NK receptor- classical MHC molecules							
KIR2DL1-HLACW2	-1042.5	29:28 A:B	1630:1768	1	-	10	175
KIR2DL1-HLACW4	-1235.3	32:23 A:B	1234:1384	2	-	14	166
		30:23 E:F	1230:1360	3	-	14	165
		12:15 A:D	699:652	4	-	8	77
		3:3 A:E	183:193		-	2	8
		4:3 B:E	151:156	1	-		14
KIR2DL1-HLACW5	-1094.0	25:27 A:B	1409:1334	2	-	9	149
KIR2DL2-HLACW1	-799.9	14:16 A:B	903:932	3	-	9	82
KIR2DL2-HLACW3	-877.7	31:21 A:B	1220:1330	5	-	13	168
		12:14 A:D	752:666	5	-	8	85
		5:7 D:E	380:322	-	-	1	32
KIR2DL2-HLACW7	-972.6	19:25 A:B	1159:1082	-	-	8	141
NK receptor- non-classical MHC molecules							
NKG2A-HLAE	-896.9	30:23 A:B	1237:1366	4	-	15	185
		30:22 C: D	1245:1347	3	-	15	182
		15:16 A:C	890:893	-	-	7	82
		2:2 A: D	77:86	2	-		3
		1:1 A:E	63:79	-	-		5
		1:1 C:J	38:51	-	-		5
		9:12 A:J	586:580	2	-	3	44
		10:13 C:E	588:541	2	-	4	53
		6:10 A:K	422:374	1	-		29
		2:2 B:C	85:75	2	-		2
		5:5 B: D	239:241	-	-	5	34
		6:6 C:F	315:295	2	-	1	19
		14:16 E:F	704:714	1	1	6	55
		15:12 J:K	683:694	1	1	4	43
NKG2A-HLAG	-718.4	18 :19 A:B	766:746	2	1	11	97
		18:18 C: D	757:741	2	1	11	89
		2: 2 B: D	168:168	-	-	-	4

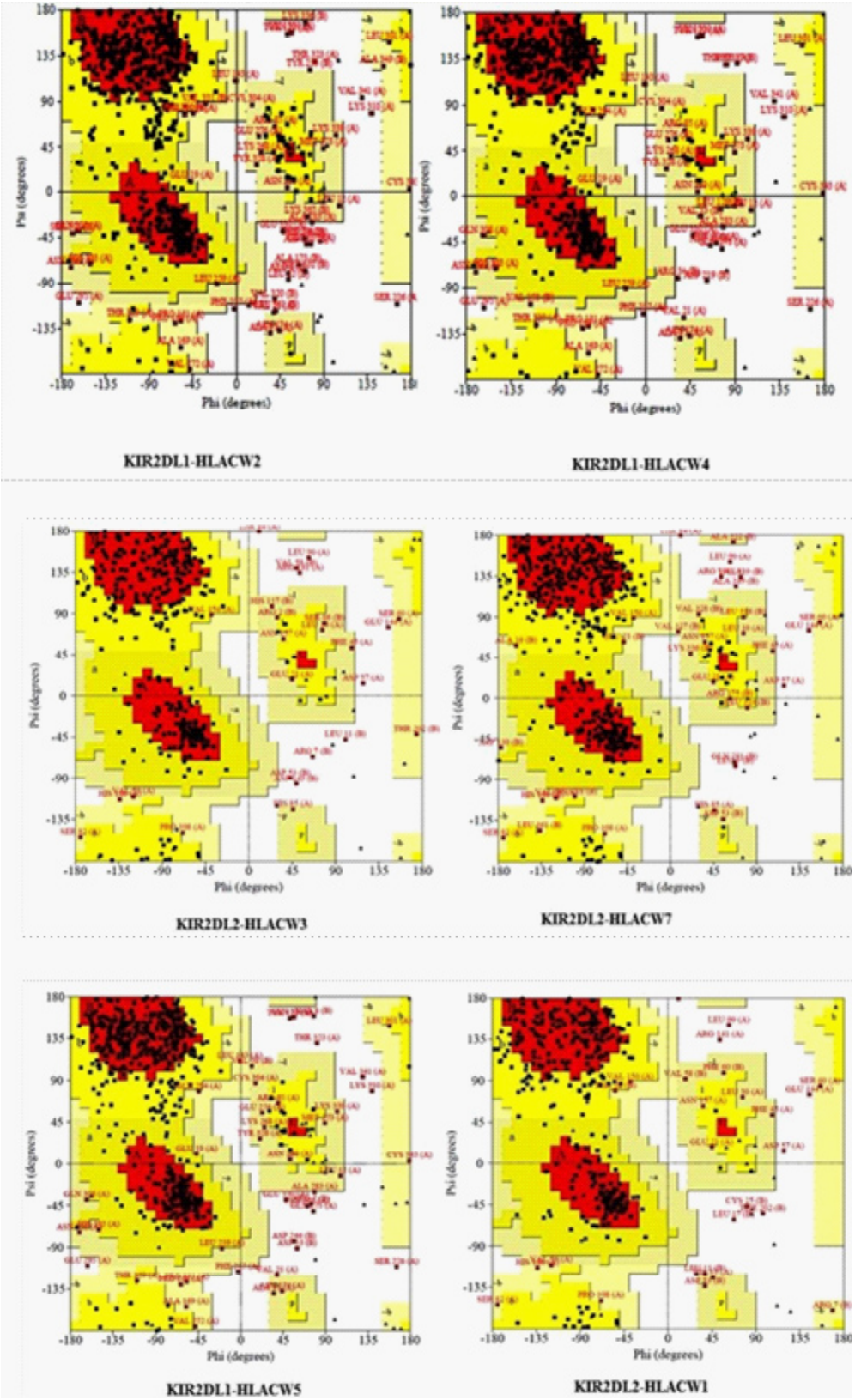

Fig 1. *Ramachandran plot of NK receptors interactions with classical MHC molecules*

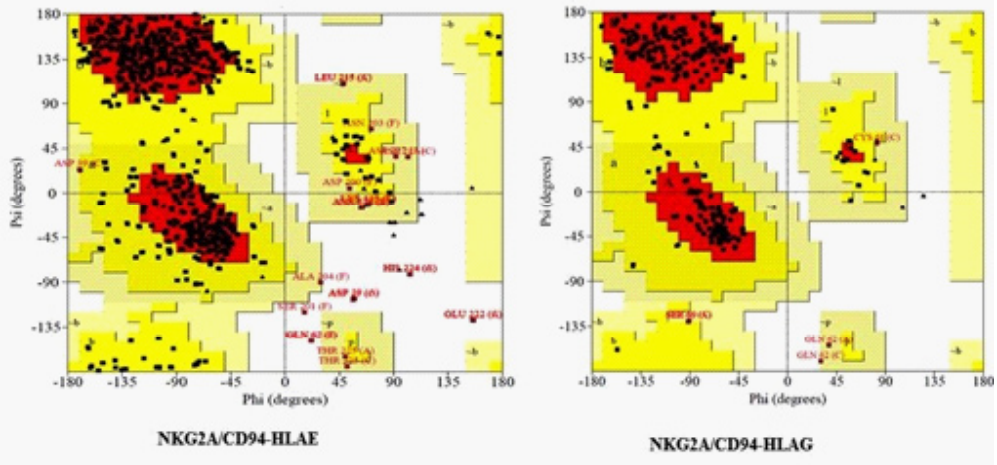

Fig 2. *Ramachandran plot of NK receptors interactions with non-classical MHC molecules*

Discussion

Human NK cells express receptors for MHC class I encoded by the Killer cell Inhibitory Receptor (KIR) gene family present on chromosome 19q13.4. The KIR molecules are monomeric type I glycoproteins that contain either 2 or 3 Ig-like domains in their extracellular region, designated KIR2D and KIR3D, respectively. KIRs recognize classical MHC molecule. In addition to KIR, another type of receptor is responsible for recognition of MHC class I. This receptor, designated CD94/NKG2, is a heterodimer, composed of a CD94 glycoprotein that is disulfide-bonded to NKG2A subunit. CD94/NKG2A receptors bind to the non-classical (HLA-E and HLA-G) MHC molecules [16].

We have shown greater inhibition of non classical MHC molecules interaction with NKG2A as opposed to classical MHC binding to KIRs [17]. Surface expression of HLA-E was enough to protect target cells from lysis by CD94/NKG2A+ NK-cell clones.It has also been established that inhibition of degranulation confered resistance to NK-cell-mediated lysis[17]. .

Despite the structural differences between the KIRs and CD94/NKG2A,both inhibitory receptors appear to usea common strategy to inhibit NK and T cell activation. The unifying characteristicis the presence of ITIM sequences in the cytoplasmic domain of thesereceptors that upon tyrosine phosphorylation are able to recruit SHP-1, and SHP-2, to mediate the inhibitory function. Thus, downstream signalling events are similar, so the difference in inhibition potential is probably at the receptor –ligand interaction. So, we explored the bindingaffinities of NK cell receptor with classical MHC vs non-classical MHC ligands to understand the binding patterns required for the inhibition of NK cell activity. Our results showed that there is high diversity of bonding in case of non-classical MHC binding to inhibitory receptors. Also, number of interface residues involved in interactions and presence of different chains were numerous in case of non-classical MHC molecules. A significantly higher number of H-bonds were observed in case of non-classical MHC interaction with inhibitory receptors.These binding pattern of NK receptors with their cognate ligand may be responsible for stronger affinity of NK receptors towards non-classical MHC molecules. Our study provides the further expalantion to the fact that NK cells in the uterus remain non-toxic to the fetal derived trophoblasts at the fetal-maternal interface by overexpression of non-classical HLA. In case of some tumor cells there is a increased expression of non-classical MHC molecules and in such tumor microenvironment NK cell receptor interaction with non-classical MHC molecules results inimpairment of NK cell activity and thereby promotes cancer progression.

Conclusion

Receptor-ligand combination is highly correlated with NK cytolytic potential. Our study provides insights in understanding the difference in inhibition potential of individual NK receptors with their cognate ligands. Knowledge of binding affinities of NK receptor with

classical and non-classical MHC would facilitate the elucidation of the nature of receptor-ligand recognition, modulation of expression and thus influence on NK activity. These findings can be correlated withtheir impact on cancer susceptibility or resistancewhich will help to design novel therapeutic strategies for cancer.

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Author's contribution: All authors have contributed significantly to the manuscript.

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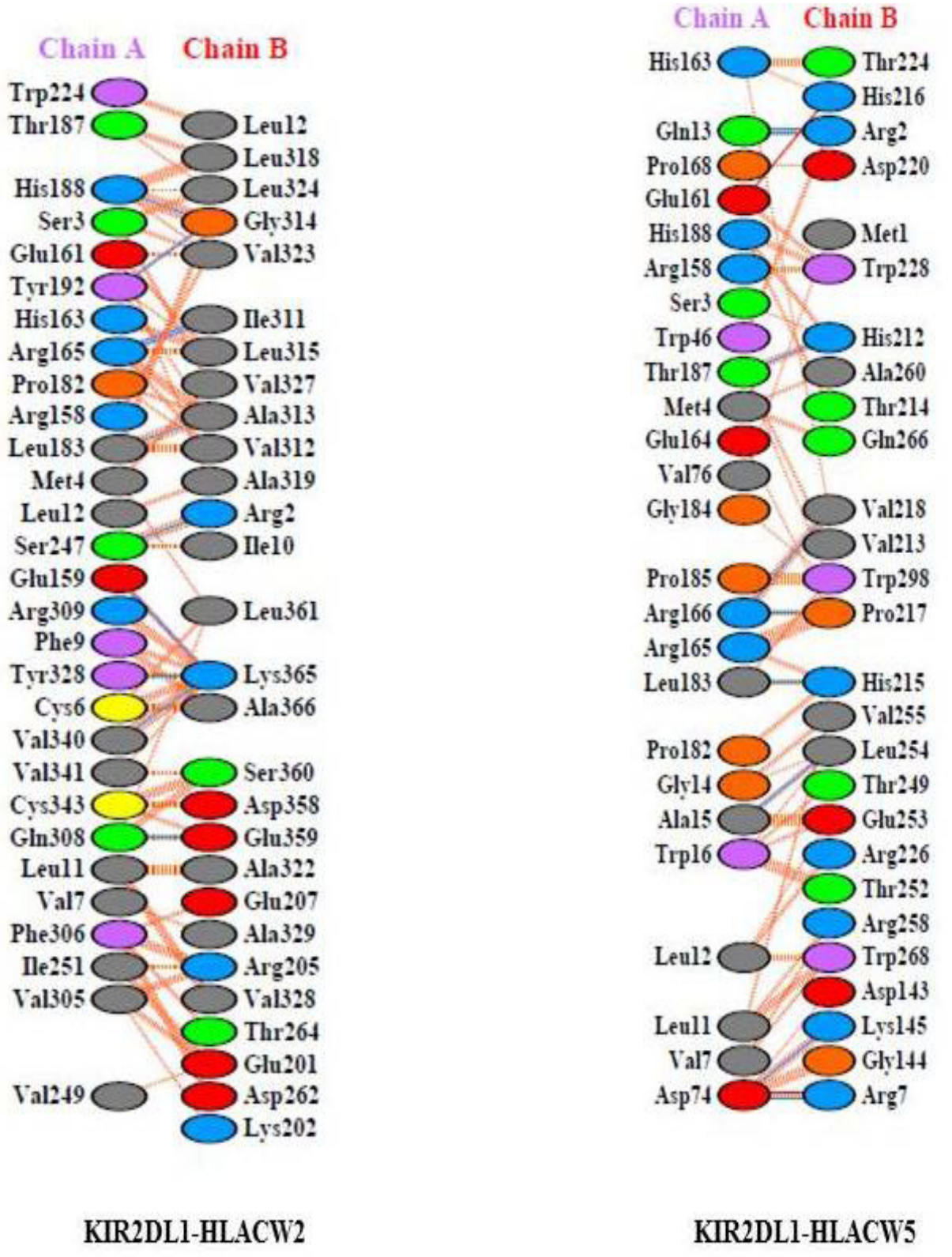
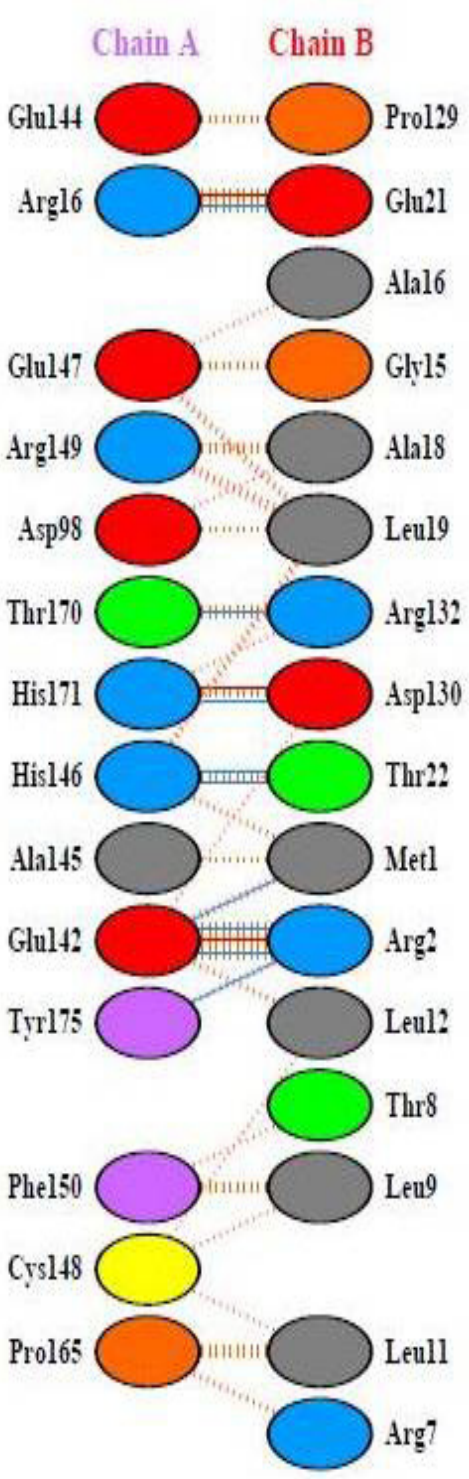
Supplementary Data

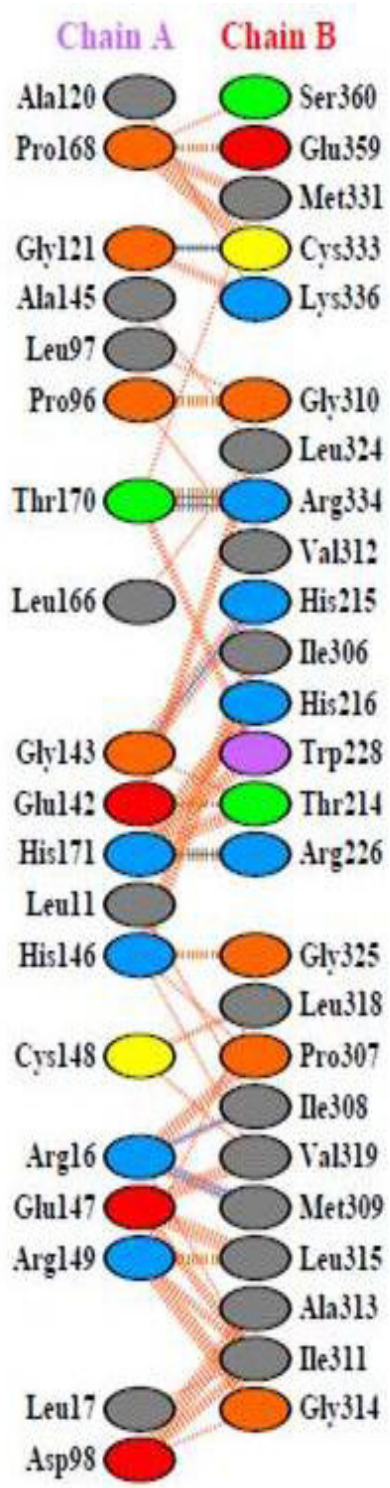
Residues involved in interactions between NK receptor and classical MHC molecules



Residues involved in interactions between NK receptor and classical MHC molecules

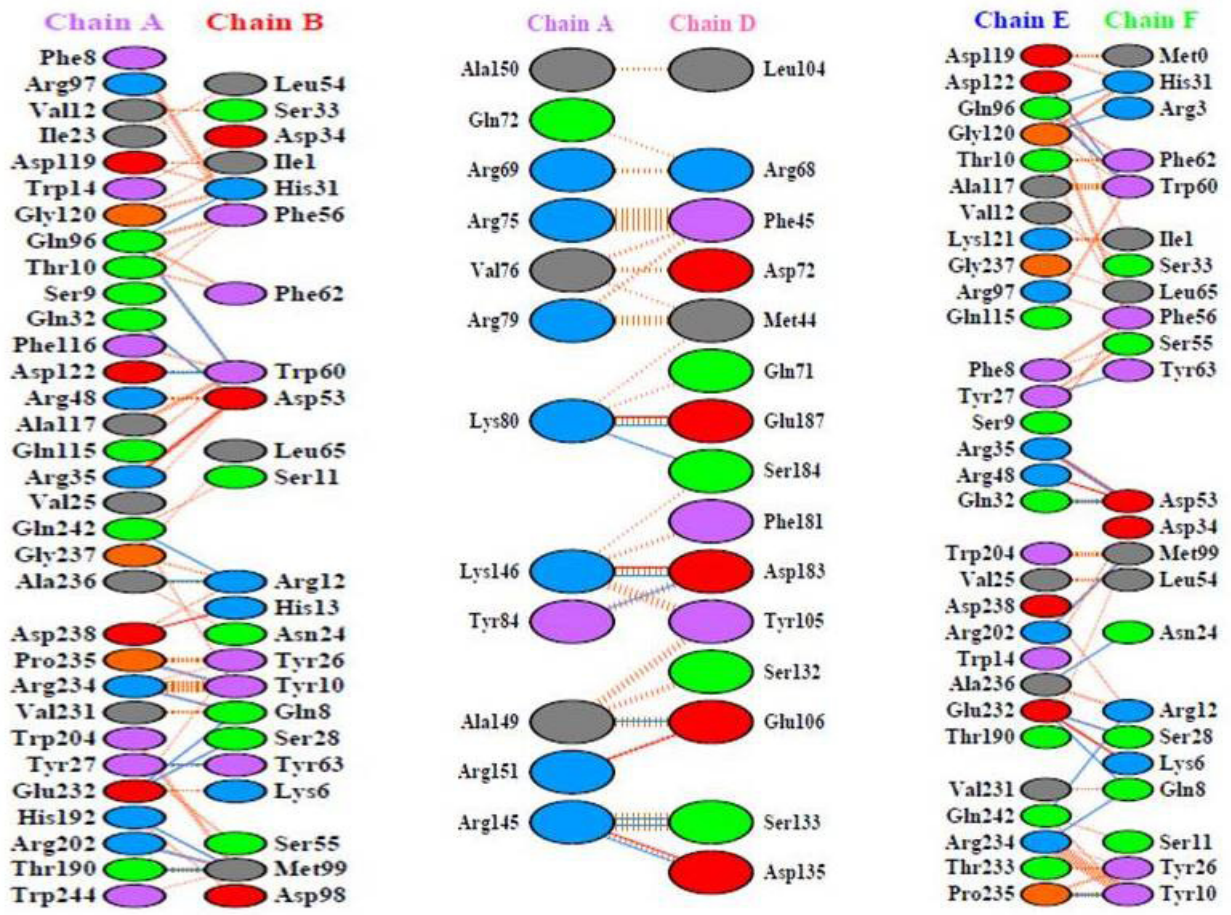


KIR2DL2-HLACW1

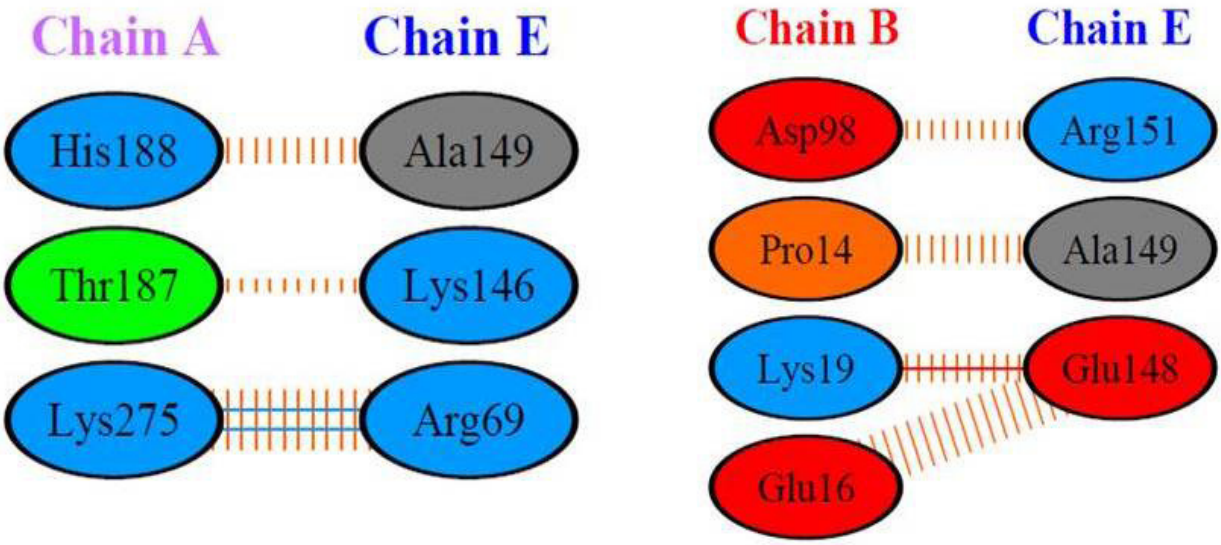


KIR2DL2-HLACW7

Residues involved in interactions between NK receptor and classical MHC molecules

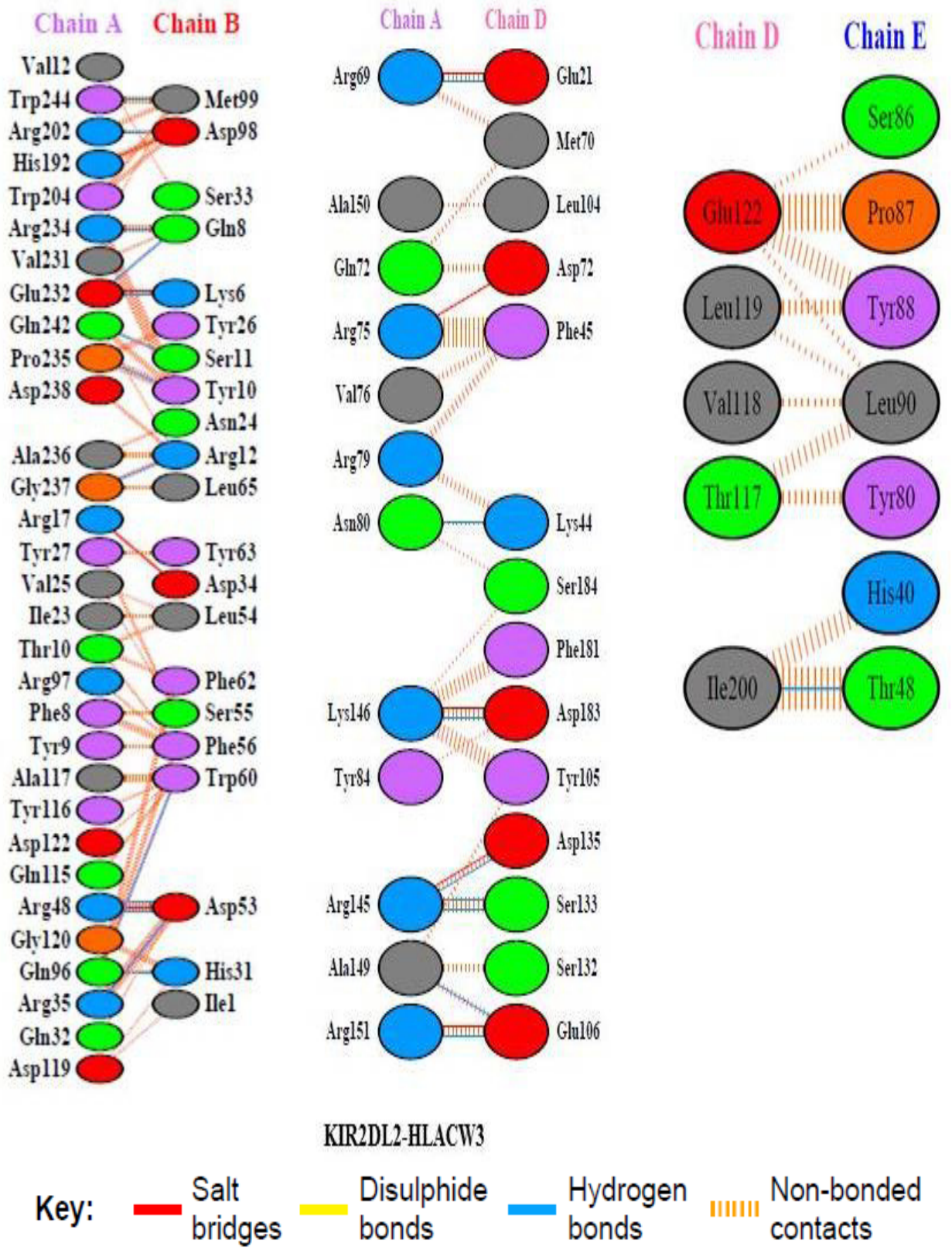


KIR2DL1-HLACW4

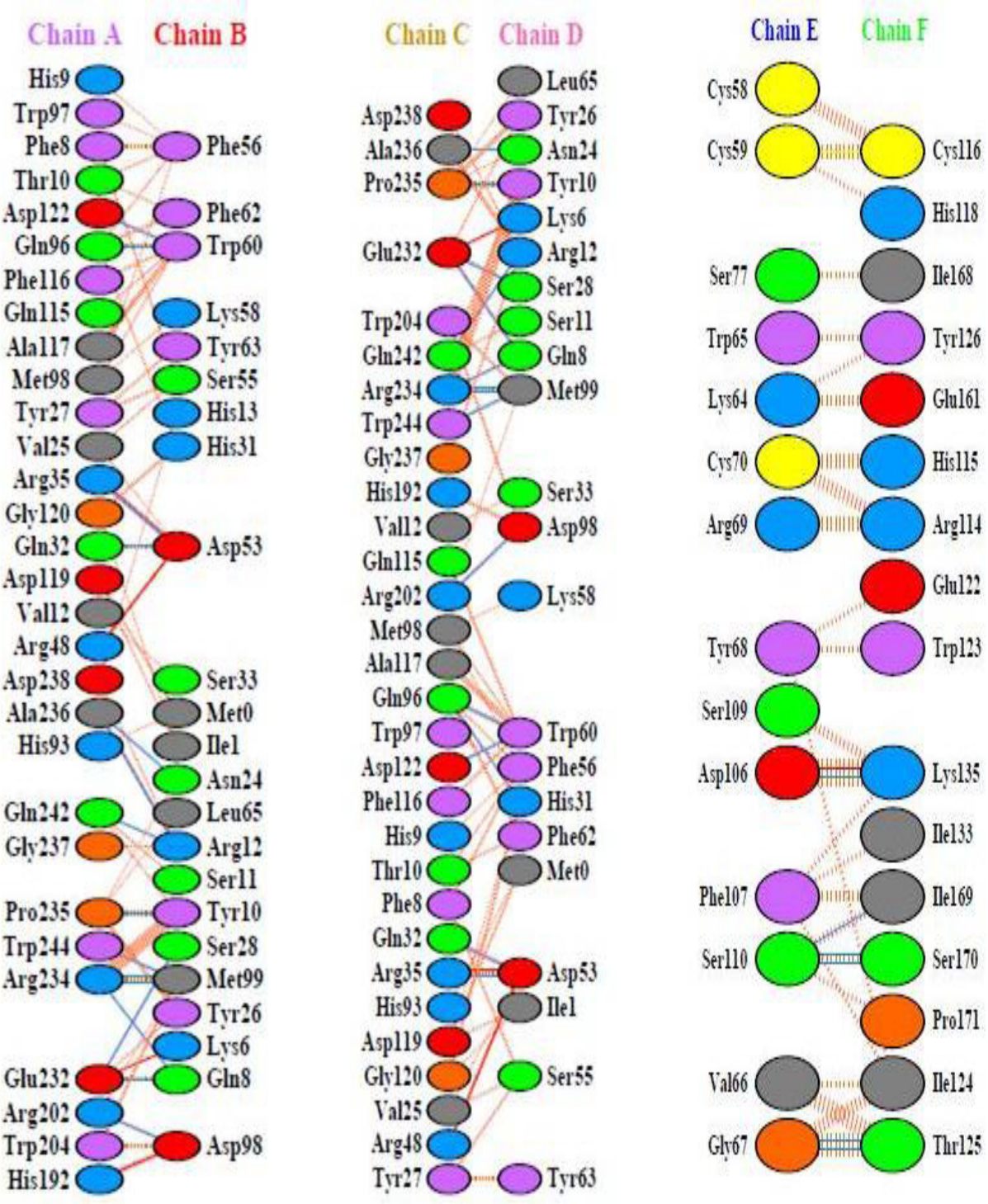


KIR2DL1-HLACW4

Residues involved in interactions between NK receptor and classical MHC molecules

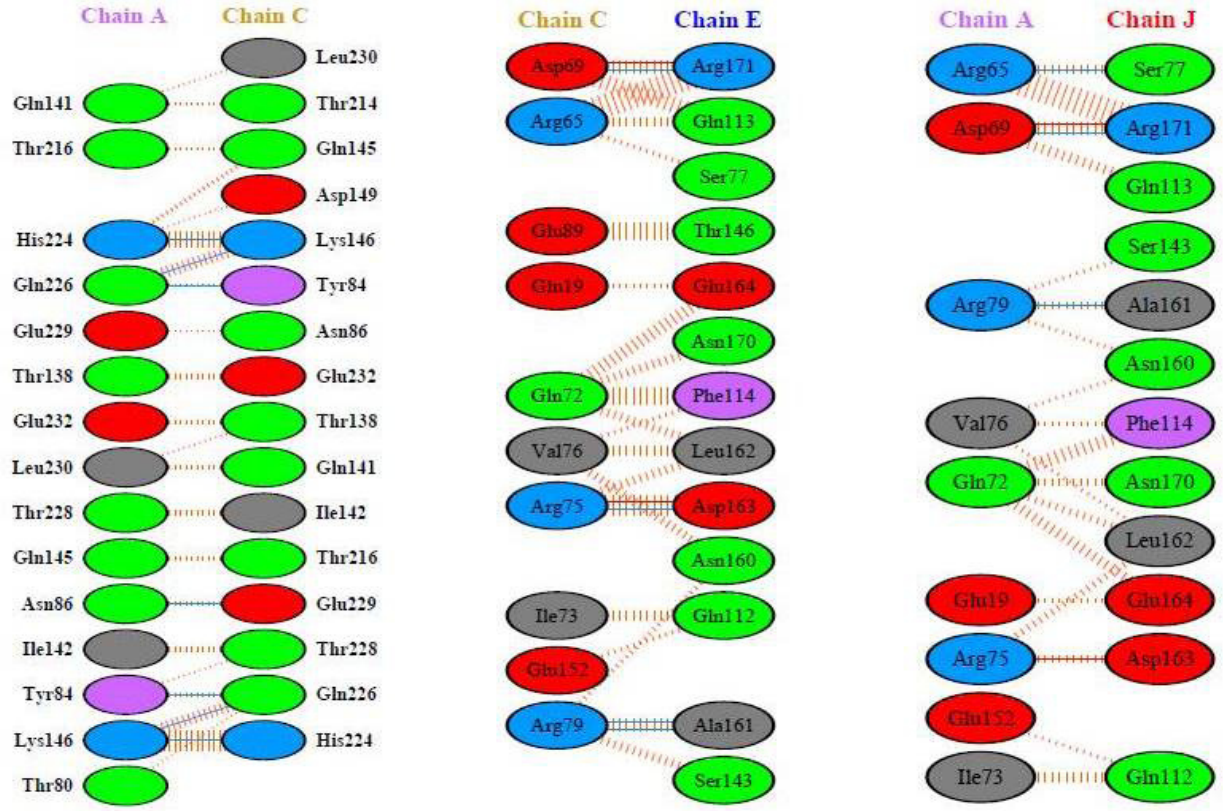


Residues involved in interactions between NK receptor and non-classical MHC molecules

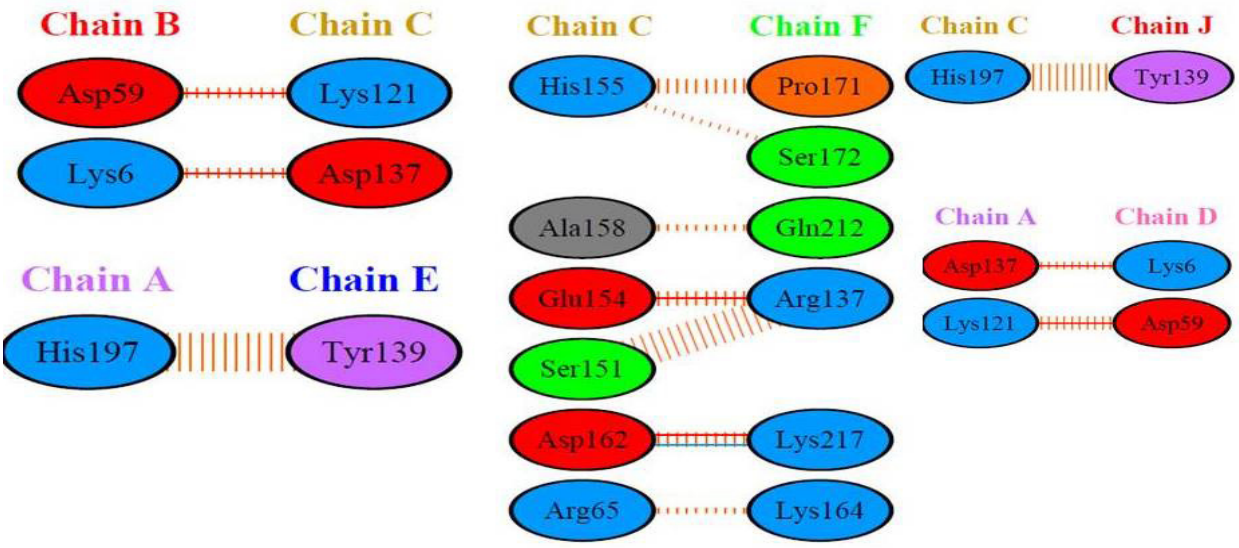


NKG2A/CD94-HLA-E

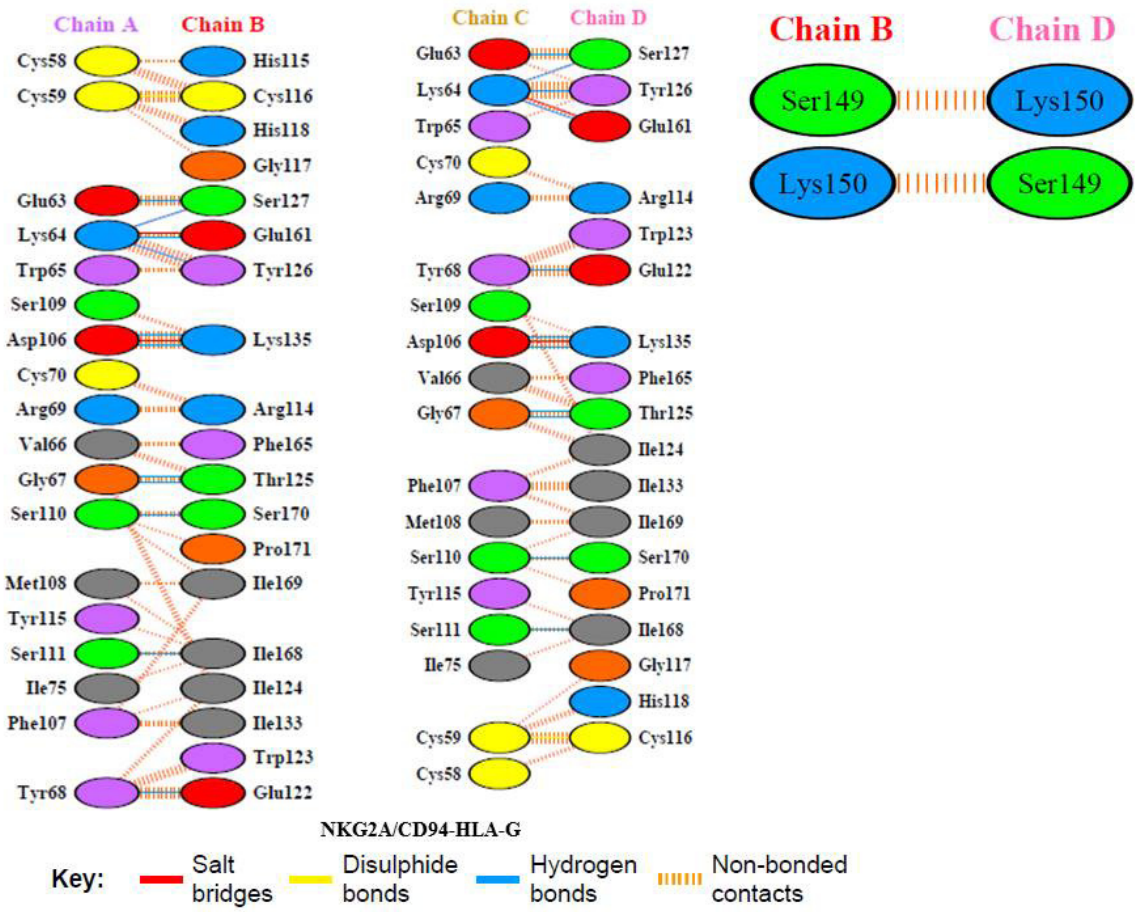
Residues involved in interactions between NK receptor and non-classical MHC molecules



NKG2A/CD94-HLA-E



NKG2A/CD94-HLA-E



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