



## Computational Analysis of Protein-Protein Interactions of G Protein Coupled Receptors

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### Abstract

**Background & Objective:** Chemokine (C-C motif) receptor 7 (CCR7) is a class A subtype G-Protein Coupled Receptor. It contributes to the migration and activation of dendritic cells, T cells, eosinophils, B cells and different cancer cells. CCR7 signalling system is involved in T cell activation, immune tolerance, inflammatory response and cancer metastasis. CCL19 and CCL21 are the two CCR7 ligands and are differential in their signalling through CCR7. **Methodology:** The sequence of the ligand and the receptor has been extracted from GPCR database to understand the signalling system. The interacting 3D structure of CCR7-CCL19 and CCR7-CCL21 has been studied with 3did the database of three dimensional interacting domains (3did) to know the domain interaction. The interaction networks of GPCR and the ligands have been studied with STRING, an interaction database. The GPCR and the ligands have been modelled using Swiss Model. The network of the differential signalling of CCL19 and CCL21 with CCR7 has been visualized with the help of NetSlim. The modelled CCR7 were further docked with CCL19 and CCR7 in Hex. **Results:** The network was obtained for CCR7 pathway and the maximum score for the interaction between CCR7 and CCL19 was found to be 0.999 and the maximum score for interaction between CCR7 and CCL21 was found to be 0.999. The interaction between CCR7 and CCL19 is between chain A and chain C of 7tm\_1 (CCR7) and IL8 (CCL19). The modelled CCR7 was docked against the two ligands CCL19 and CCL21 and the energy for the interaction between CCR7 and CCL19 was 477 units where as the energy for interaction between CCR7 and CCL21 was 452.38 units. This shows that the two ligands bind differentially to CCR7. showed significant inhibition with minimum binding energy, when compare to standard drug ciprofloxacin and amphotericin B. **Conclusion:** This study clearly showed that the amentoflavone used as broad spectrum of antimicrobial drug.

**Keywords:** CCR7, CCL19, CCL2, Hex, amentoflavone

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### 1. Introduction

Protein-protein interactions (PPIs) are of interest in biology because they regulate roughly all cellular processes, including metabolic cycles, DNA transcription and replication, different signalling cascades (Ref). Through recent rapid advances in high-throughput technologies, massive protein-protein interaction data of various organisms have become available and are currently stored in several databases like HUPO-PSI (Human Proteome Organization Proteomics Standards Initiative), IMEx (International Molecular Exchange), MIMIX (minimum information about a molecular interaction experiment), Bio GRID, DIP ( Database of

Interacting Proteins), BIND (The Bimolecular Interaction Network Database), MINT (Molecular Interaction database), HPRD (Human Protein Reference Database), InAct [1]. These databases are a source of protein and genetic interactions from major model organisms. The available methods for predicting protein-protein interaction are based on genomic context and structural information, network topology to predict protein-protein interaction, text mining and literature mining (or database search) and machine learning algorithms utilizing heterogeneous genomic/proteomic features. Since then genomic context has major drawbacks it can be successfully used in case of eukaryotes, better methods have to be used for protein-protein interaction. The network topology uses Graph theory and probability theory for forming the network and our selected research paper has used the same method for determining the protein-protein interaction (ref). There are tools available for building these networks like Graphcrunch2. Text mining and algorithm

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based methods work on identifying the proteins and uses mathematical interpretation respectively. Finally the visualization and analysis of the networks is done through tools like Cytoscape, Medusa (for visualizing large scale biological networks in 2D) and NAViGaTOR. This study was to design a network CC-chemokine receptor 7 and understand the role of CCR7 using in silico tools.

## 2. Materials and methods

### Collection of Dara

CCR7 information was obtained from the GPCR DB. The sequence and amino acid arrangement of CCR7.

String is a biological database for known and predicted protein-protein interactions using data from numerous resources, including experimental data, computational prediction methods and public text collections. With the help of this database, we have tried to analyse the protein-protein interaction networks of G-Protein Coupled Receptors and the differential ligands. By understanding this, we can determine the role played by these interactions and will be used for further studies. 3DID is a Three-dimensional interacting domain database which is involved in the collection and classification of all structural models of domain-domain interaction and used in protein-protein interaction. It uses Pfam to define the position of protein domains in the protein structures. It is a source of information that can be used as a reference for the structural characterization of protein interaction networks. Using this database, we have tried to analyse the possible interaction between CCR7-CCL19 and CCR7-CCL21. This successfully helps us to analyse the protein-protein interaction between the ligands and the G-protein coupled receptors. NetPath hosts 20 signaling pathways including 10 pathways with a major role in the regulation of immune system and 10 pathways with relevance to regulation of cancer.

The following immune signaling pathways are hosted by Netpath: B cell receptor pathway, T cell receptor pathway, Interleukin-1 pathway, Interleukin-2 pathway, Interleukin-3 pathway, Interleukin-4 pathway, Interleukin-5 pathway, Interleukin-6 pathway, Interleukin-7 pathway, Interleukin-9 pathway.

The major cancer signaling pathways include Epidermal growth factor receptor Pathway, Transforming growth factor beta receptor pathway, Tumor necrosis factor alpha pathway, Alpha6 Beta4 Integrin pathway, Inhibitor of DNA binding pathway, Hedgehog pathway, Notch pathway, Wnt pathway, Androgen receptor pathway, Kit receptor pathway. A network simulation and network emulation tool used for designing PPI networks. Various technologies such as Cognitive Radio, Wireless Sensor Networks, Wireless LAN, Wi Max, TCP, IP are covered in NetSim.

### Homology Modelling

There was not much structural information available for CCL7 in protein structure database (PDB). There as a template CC- chemokine receptor 19 (CCL19) and CC-chemokine receptor 21 (CCL21) were obtained from protein data base. In SWISS MODEL WORSPACE programs and databases required for protein model are integrated accessible via the ExPasy server and used for homology modelling. Homology modelling is the

construction of a model of a target protein using its amino acid sequence using homologous sequences. The sequence alignment and template structure are then used to produce a structural model of the target. The quality of the homology model is dependent on the quality of the sequence alignment and template structure.

HEX Molecular docking is performed for CCR7-CCL19 and CCR7-CCL21 separately and the energy values are tabulated to check for the probable poses and energy values. The results are then analysed for the probable interactions between the ligand and the receptor.

## 3. Results and discussion

The amino acid sequence of CCR7, CCL19 and CCL21 were retrieved. The network was obtained for CCR7 and gives information regarding evidence for the network. A legend shows information on the edges, nodes and node colour for the network. The highest score between CCL19 and CCR7 interaction was found out to be 0.999. Information regarding CCR7 and CCL19 and their involvement in cell signalling in the immune system was retrieved. CCL19 and CCR7 have been modelled using homology modelling. Two models have been developed so far for each of these proteins. The selected models were verified using WHAT\_IF. WHAT IF is a versatile molecular modelling package that is specialized on working with proteins and the molecules in their environment like water, ligands, and nucleic acids [4]. CCR7-CCL19 specifies the highest score when compared with the other query proteins. Similarly, Network between CCR7- CCL21 was retrieved. This gave information regarding the network connecting CCR7 and CCL21. It also specified the evidence for developing the network between the required ligand-receptor. Their combined score was found to be 0.999. The scores of CCR7-CCL19 and CCR7-CCL21 were equal which provided evidence for the differential ligand binding to the GPCR. [Fig 1].

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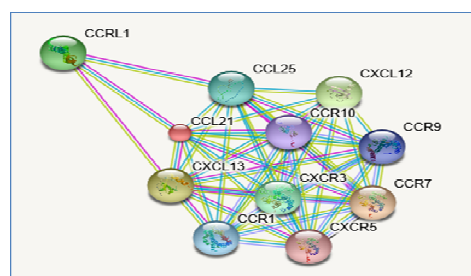


Fig 1: The interaction network of CCR7 and CCL19/CCL21

The interacting domain of the receptor and the ligand were considered and their 3D structure was

identified. 3did takes Pfam id and fetch UniProt ID of desired protein. The interactions were found to be 7tm\_1 and IL\_8 Pfam ID's of CCR7 and CCL19 respectively. The signalling network between IL8 and 7tm\_1 was retrieved which is that of G-Protein Coupled Receptor Signalling. This also gives information regarding the interacting domains of the proteins interacting and the 3D structure. The interacting proteins with highest score are shown in the 3D view. The interaction is between

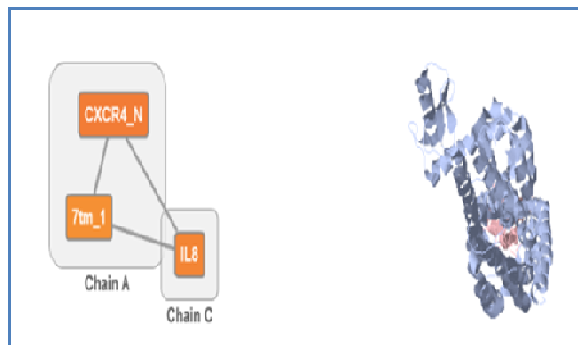


Fig 2: The interaction is between chain A and chain C of CCR7 (7tm\_1) and CCL19 (IL8)

chain A and chain C of 7tm\_1 and IL8. [Fig ]. The CCR7 pathway was obtained from NetSlim in order to study the various reactions and interactions between CCR7 and CCL19 and between CCR7 and CCL21. The pathway was further analysed to predict the differential binding of CCL19 and CCL21 with CCR7. [Fig 3]

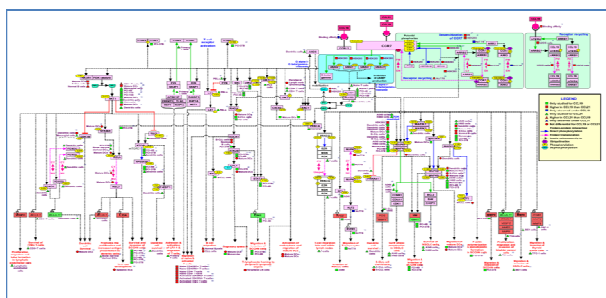


Fig 3: Pathway network for the signalling network of CCR7 obtained from NetSlim and the pathway for further prediction to know the interactions of CCR7 with the various ligand molecules.

**CCR7 modelling and docking studies**

Since there was no existing model available for CCR7 in PDB, CCR7 was modelled using Swiss Model and was viewed using Rasmol. [Fig 4a, 4b].

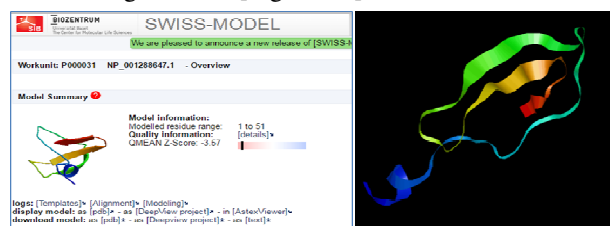


Fig 4a, 4b: CCR7 model using homology modelling

The modelled CCR7 was docked against the two ligands CCL19 and CCL21 to understand their differential

binding. The energy for the interaction between CCR7 and CCL19 was 477 units where as the energy for interaction between CCR7 and CCL21 was 452.38 units. This shows that the two ligands bind differentially to CCR7.

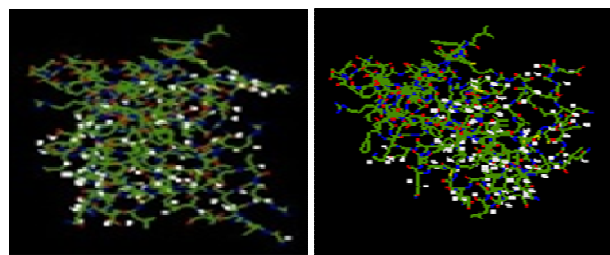


Fig 5a, 5b: The two proteins before and after docking. The docking was conducted once for CCR7-CCL19 and once for CCR7-CCL21

The energy for each of the interaction was high and a less RMS value indicated that the interaction of ligand with CCR7 was relatively strong. The energy for the interaction between CCR7 and CCL19 was 477 units where as the energy for interaction between CCR7 and CCL21 was 452.38 units.

**Discussion**

CCL19 and CCL21 bind cell surface receptors CCR7 and ACKR4. Their modulation of cellular functions has been identified to be mediated through the CCR7. The differential signaling between CCL19 and CCL21 through CCR7 activates these GPCRS kinases, receptor phosphorylation, arrestin recruitment and desensitization of CCR7. A key feature of the immune system is its ability to induce protective immunity against pathogens while maintaining tolerance towards self and innocuous environmental antigens. Recent evidence suggests that by guiding cells to and within lymphoid organs, CC-chemokine receptor 7 (CCR7) essentially contributes to both immunity and tolerance. This receptor is involved in organizing thymic architecture and function, lymph-node homing of naive and regulatory T cells via high endothelial venules, as well as steady state and inflammation-induced lymph-node-bound migration of dendritic cells via afferent lymphatics. Here, we focus on the cellular and molecular mechanisms that enable CCR7 and its two ligands, CCL19 and CCL21, to balance immunity and tolerance. It specifically induces the activation G proteins leading to the inhibition of adenylate cyclase and cyclic AMP production. The migration of different cell types is mediated by cytoskeleton reorganization by Rho/Rac/Cdc42 pathway in concert with JAK/STAT and also MAPKs such as ERK, p38MAPK and JNK (12-31). The Gai/o and βγ subunits induce the activation of p85 alpha and the catalytic subunits of PI3K to mediate the activation MTOR signaling pathway and NFκB pathway and the inhibition of GSK3-beta and FOXO1/3 leading to the cell survival .NFκB pathway is also activated by the PLC/ PKC pathway. The CCR7 signaling also mediates integrin signaling, T cell receptor signaling and also the VEGF signaling system among many others. The

differential signaling between the two ligands is represented in the pathway image.

### **Acknowledgement**

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### **Conflict of interest**

The author's declares none.

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