Exploring Potential Intracellular Allosteric Modulation of CCR4 Protein Using MD Simulations

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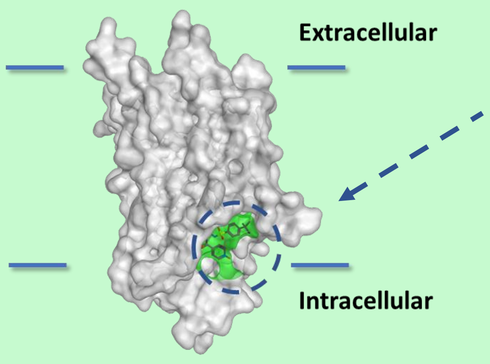
Introduction

- CCR4 implicated in T-cell-mediated inflammatory diseases such as asthma, atopic dermatitis, and certain T-cell lymphomas (Solari & Pease, 2015).
- Plays a role in the immune system's control of tumour immunity by regulatory T cells (Ishida & Ueda, 2006).
- Current treatment options for diseases involving CCR4, such as certain cancers and inflammatory disorders, often include chemotherapy, immunotherapy, and steroids. These treatments can be associated with significant side effects, including immunosuppression and toxicity (Yoshie, 2021)
- Inhibition of CCR4 appears an attractive option for treatment of theses diseases. Currently, the only FDA approved CCR4 inhibitor is the monoclonal antibody Mogamulizumab with absence of any FDA approved small organic molecule for CCR4 inhibition (Miao et al., 2020).
- An intracellular allosteric binding site (IABS) has been reported in a number of chemokine receptor as CCR2, CCR9, CCR7 and CXCR2.
- Allosteric ligands interacts with this IABC that overlaps with G-protein binding site, altering the shape of the receptor and affecting its activity indirectly (Allegretti et al., 2016).
- Allosteric ligands offers a number of advantages compared to orthosteric ones including more specific targeting and reduced side effects (Nussinov & Tsai, 2012).



Advantages of targeting allosteric pocket:
- Absence of endogenous competition
- More compact
- Less conservation

Figure 1. Representation of IABC of CCR2 bound to CCR2RA

Aims

- To evaluate the presence of an intracellular allosteric binding site (IABS) in CCR4 protein.
- Identify the key determinants for binding in the IABS of CCR4 protein.

Methodology

1. Template Selection

The 3D structure of the inactive state of CCR4 was obtained from GPCR database. The structure was aligned on the 4 available crystal structures of chemokine proteins bound to allosteric inhibitors: CCR2 (PDB code:4MBS), CCR7 (PDB code:3EAW), CCR9 (PDB code:6L6Z) and CXCR2 (PDB code:2LNL)

2. Molecular Docking

Docking of the co-crystallized ligand of the chosen template in the 3D coordinates of CCR4 protein using GOLD.

Three different scoring methods (ChemPLP, ChemScore, Goldscore) were evaluated with regards to: binding mode predictions. The best method was used to dock compound 1, a pyrazinyl sulfonamide compound previously reported to inhibit CCR4.

3. Molecular Dynamics (MD)

CCR4-compound 1 complex was inserted into a lipid bilayer membrane using CHARMM-GUI and simulated for 100 ns. The simulations were performed under isothermal and isobaric conditions, and the trajectories were analyzed using XMgrace and VMD software.

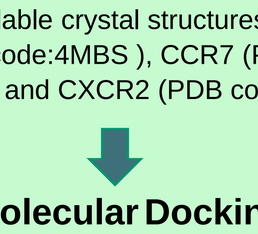


Figure 2. Potential CCR4 allosteric modulator (compound1)

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Results

Template Selection:

Highest sequence and structural similarity was observed between the binding pocket of CCR4 and CCR2.

Table1. RMSD values for the template selection

Protein	CCR2	CCR7	CCR9	CXCR2
RMSD	0.97	2.97	1.66	2.95
Differences	21.05%	29.03%	33.33%	44.44%
%Identity	78.94%	70.96%	66.66%	55.55%

Molecular Docking:

Chemscore showed the best performance in binding mode predictions with an RMSD of **1.983 Å** upon docking CCR2-RA-[R]. Docking of compound 1 showed good occupancy of CCR4 IABS.

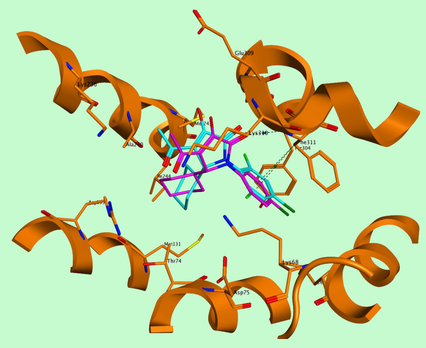

Figure3. Overlay of the docked pose of CCR2-RA-[R] (magenta, sticks) and its crystallized coordinates (cyan, sticks) in the allosteric pocket of CCR4 (orange, cartoon).

Molecular Dynamics (MD):

Compound 1 showed stable binding in the IABS of CCR4 protein throughout the 100 ns simulation.

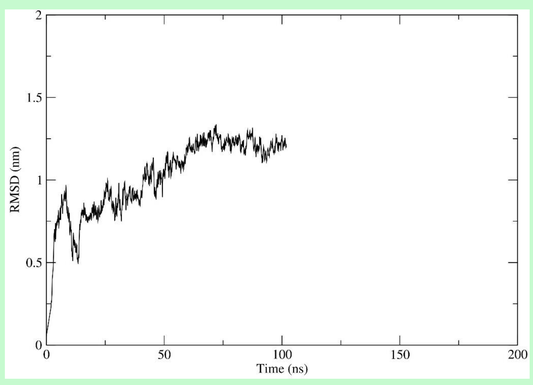

Figure.4

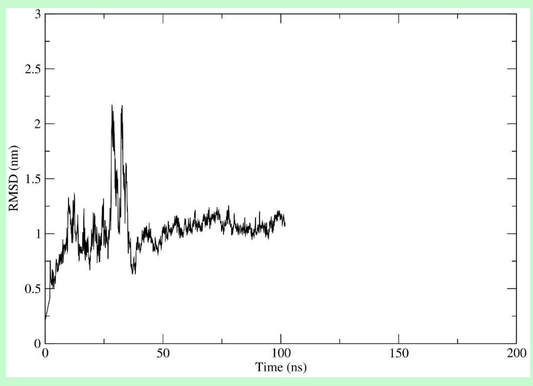

Figure.5

Plots of root-mean-square deviations of (figure.4) protein main chain atoms and (figure.5) ligand heavy atoms along the MD simulation time.

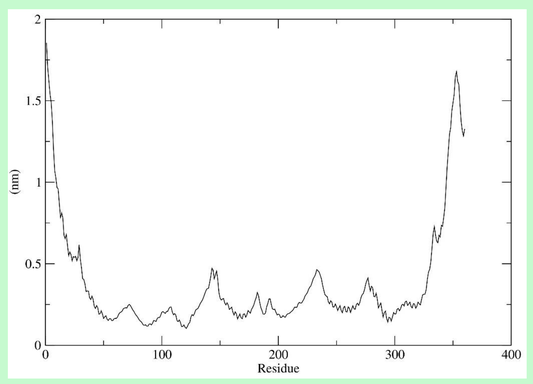

Figure6. RMS fluctuation

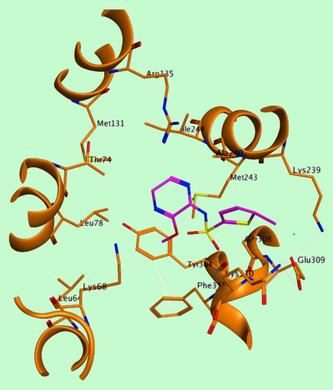

Figure7. Docked pose of compound 1 (magenta, sticks) the allosteric pocket of CCR4 (orange, cartoon).

Conclusions

In this work, the potential presence of an IABS in CCR4 was explored. Overlay of the structure of CCR4 on the crystallized coordinates of CCR2 showed high sequence identity and similarity in the IABS of 80% and 100%, respectively and an RMSD of 1.983 Å. A 100-ns MD simulation of the inactive state of CCR4 bound to a pyrazinyl sulfonamide compound, a previously reported CCR4 inhibitor, showed high stability, supporting its potential binding to the IABS of CCR4. Our results support the presence of an IABS in CCR4 similar to that of CCR2, highlighting key residues crucial for allosteric modulation and thus provide structural insights for future design of ligands binding in this pocket.

Future Prospects

- Calculate the free binding energy of compound 1 in CCR4 receptor.
- *In vitro* CCR4 binding assay to confirm allosteric binding of compound 1.
- Structural modifications of compound 1 to improve its binding affinity.
- Conducting virtual screening studies to find other novel CCR4 allosteric modulators.

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