

# Prediction of Structure and Antagonist Binding Site in Human and Rodent Chemokine Receptor 1

Thesis by

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*Over the years, I have benefited from the support of many people in both my academic and personal life. It is my hope that this work will stand as a testament to their venerable faith in my ability to succeed as a scientist and become a better human being.*

*Thank you all for your time, generosity, and unconditional love.*

*This thesis is also dedicated to the memory of my loving grandparents,*

*Satyadev and Sudarshan Sharma*

*I wish you were here.*

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## Abstract

Chemoattractant cytokines (chemokines) are small proteins that are known to play a key role in the development of numerous autoimmune and inflammatory diseases. The signal transduction cascade responsible for this pathology is initiated by chemokine binding to a G-protein coupled receptor (GPCR). Since therapeutic intervention would involve inhibition of ligand binding, it follows that detailed understanding of the structures and binding sites of these receptors would lead to the rational design of such drugs. However, GPCRs are a class of integral membrane proteins whose structures are extremely difficult to determine via the conventional method of X-ray crystallography. Additionally, homology models based on the crystal structure of bovine rhodopsin (BR) have offered little structural insight into the remotely homologous chemokine receptors. In light of this information, our laboratory has developed a novel computational approach to predicting the structures and ligand binding sites of GPCRs with no information from the atomic coordinates of the crystal structure of BR.

In this thesis we describe the use of the MembStruk procedure to predict the structure of human, mouse, and rat chemokine receptor 1 (CCR1). Interhelical interactions that stabilize the conformation of each receptor are discussed in detail, and where appropriate comparisons are made to information gleaned from the crystal structure of BR. The side chain placements of conserved residues are found to be different across the human and rodent species, accounting for binding differentials not previously explained by homology models. To improve the binding of a low affinity small molecule antagonist, point mutation candidates in human CCR1 are predicted.

Validation of the human CCR1 structure is achieved through prediction of the antagonist binding site, to which a series of known antagonists are docked and scored for comparison to experimental structure-activity data. The ligand binding energies are in excellent agreement with the experimentally known trend in binding affinities, and results from a virtual ligand screening calculation (Dr. Sabine Schlyer, Berlex/Schering AG) also support the validity of the structural model. This work in this thesis provides the basis for the design of receptor-specific antagonists to human and rodent CCR1, thus accelerating the drug discovery process.

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## Abbreviations

(h/m/r)CCR1	(human/mouse/rat) chemokine receptor 1
GPCR	G-protein coupled receptor
AVGB	Analytical Volume Generalized Born
RBMD	rigid body molecular dynamics
MSA	multiple sequence alignment
ESP	electrostatic potential
CMM	Cell Multipole Method
QEq	charge equilibration
vdW	van der Waals
BR	bovine rhodopsin
BE	binding energy
FF	force field
TM	transmembrane
MD	molecular dynamics
HF	Hartree-Fock
PB	Poisson-Boltzmann
$K_i$	binding affinity (experimental)
nM	nanomolar (experimental)