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

Thesis by

Shantanu Sharma

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2005

(Defended June 2, 2005)

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.

Acknowledgments

I didn't think Caltech accepted people that hate sci-fi as much as I do, but here I am! This is indeed an odd little place with endearing quirks, from the cult of barefoot undergrads to the cliques of geeks who aim to ostracize seemingly lesser geeks. Nonetheless, my academic experience here has been stimulating and I am very thankful for the training I received. Prof. Bill Goddard gave me the opportunity to learn about computational biology and quantum chemistry, while giving me the freedom and resources to grow independently as a scientist. Bill, I have yet to meet a human being as enthusiastic, energetic, and knowledgeable about science as you are, especially at 4am on a Sunday. If I can walk away from here with some finite element of your stellar memory and keen sense of entrepreneurship, I think I'll be set for a long time.

None of the work presented here would have been completed without the meticulous oversight and nurturing mentorship of Dr. Vaidehi Nagarajan. Vaidehi has many roles in the BioGroup: scientific advisor, older sister, psychiatrist, and head of quality control. She is simply the most compassionate person I have ever met in my life, and the whole scientific enterprise is richer for having her. Vaidehi, I know the real-world is not as caring, so thank you for seeing me through the transition from PDEs to PDAs.

I still don't know how I was able to pull off a "powerhouse" thesis committee consisting of Profs. Harry Gray, Doug Rees, and Bob Grubbs – the most accomplished group of scientists I have had the pleasure of knowing. Harry was my initial research advisor at Caltech, and somehow I managed to learn more about molecular biology and ligand field theory than I had intended to. In his usual manner, he has kept me upbeat, optimistic, and focused on what needs to be accomplished. Thanks, Harry, for letting me

TA one of the most challenging courses at Caltech! Doug is the human Protein Data Bank, and tapping into his deep reservoir of scientific insight has always been just a quick email away. Doug, your personal support over the years has made all the difference. Bob is acknowledged for serving on my committee and for very nicely pointing out the shortcomings in my catalysis research proposal!

The Goddard Group is a bit like the Universe: continually expanding and strikingly diverse. Membrane protein structure prediction is an extremely challenging problem, and accordingly we have assembled a top group of mathematicians, biologists, organic chemists, physical chemists, and…me! I am extremely fortunate to have collaborated with and learned from some of the best in the field: Dr. Wely Floriano, Dr. Rene Trabanino (’04), Peter Freddolino, Dr. Spencer Hall (’05), Jiyoung Heo, Huazhang Shen, Peter Kekenes-Huskey, Victor Kam, Dr. Joyce Peng (’05), Caglar Tanrikulu, Frank Ducheneaux, John Wendel, and Adam Griffith. Outside of the BioGroup, John Keith, Dr. Jonas Oxgaard, and Santiago Solares have kept my spirits up during long hours of frustration.

The work in this thesis was done in collaboration with scientists at Berlex (Schering AG), and it has been a sincere pleasure working/dining/musing with Drs. Richard Horuk, Sabine Schlyer, Sunil Koovakkat, and Monica Kochanny. Berlex was kind enough to fund this work, and the Division of Chemistry supported me through the Mower, Dow, and Rosen Fellowships. The support staff here also deserves special recognition: without Dian Buchness, I’d still be trying to get through my candidacy paperwork, while Natalie Gilmore in the Graduate Office extended a few deadlines so that I could still graduate this year.

Back when I was an unassuming undergraduate, Prof. Ahmed Zewail convinced me that I would learn more in his lab at Caltech than I could working in the Herschbach group at Harvard. In retrospect, I suppose I have him to thank for a few years of sunshine, good surf, interesting science, and awesome company. Prof. David Nesbitt taught me a lot about molecular spectroscopy, lasers, electronics, and living the good life in Colorado. I hope to return there some day. My undergraduate advisors, Profs. Fratiello, Gutierrez, Nickolaisen, and Tikkanen saw me through my tumultuous teenage years and for that alone deserve a doctorate in psychology.

I would be remiss if I did not thank the following people for many years of putting up with me:

Dr. Victor Rucker (Dervan '03) – you've been an ongoing source of support, encouragement, and worldly travel advice. Thanks for letting us crash at your swanky penthouse in SF, and for not pushing me off the ski lift in Tahoe.

Dr. Jeremy Weaver (Gray '05) befriended me during my stint in the Gray Group, and has never forgotten to "surprise" me with post-candidacy and prop-exam inebriants. You're a great friend and a genuinely sharp guy…just watch out for that dude who can see into the future with his glasses. I here he is going to Illinois for grad school.

Dr. Ramez Elgammal (Weitekamp '05) was my first real friend at Caltech, and I'll miss our almost daily conversations about anything that happened to pop into our heads at the time. Thanks for seeing me through candidacy and props.

Pete Huskey – Fix that GUI so people like me can use it! Sorry we never worked through those stock market and poker ideas, but thanks anyway for putting academic life in perspective over Chimay and during our long drives through the desert.

David Robichaud – one of my greatest feats in life was finally convincing you that spending money is a good thing. I am happy to report that your company has been incrementally more enjoyable during our many excursions to Nevada and El Pollo Loco. Stop saving those tortillas, and let me know when you move up to 100/200!

Gabriel Richman for being the coolest roommate I've ever had. Thanks for (literally) showing me why it's not a good idea to eat food made by Korean cult members. Did we even end up getting rated for all that play? Remember I still have pics from AMP!

John Bowen – a Caltech engineer armed with a Harvard MBA coupled with a passion for surfing: you rock. I'm glad to see that scientists can survive in the real world.

Prof. Matthias Selke – where do I begin? From discussions about political economy and fake journals to Red and Adorno…

Dr. Tai Mao – we've known each other since before I was old enough to drive. I'm finally a "doctor" too…. so NOW can I please drive your new Lexus?

Charlene Acdan for working hard to support my ambitious (read: crazy) pursuits in life. It's been a remarkable journey…from near poverty to chartered jets and everything in between. Thanks for showing me what really matters in life.

Finally, I'd like to thank my family for not making fun of me for staying in school this long. To anyone I have forgotten: please accept my deepest apologies and feel free to write in your name here _____.

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.

Table of Contents

List of Tables and Figures

Tables

Figures

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)