

STRUCTURE AND FUNCTION STUDIES OF THE HUMAN  
DOPAMINE RECEPTORS

Thesis by

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In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in

Biochemistry and Molecular Biophysics (Chemistry)



California Institute of Technology

Pasadena, California

2004

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This thesis is dedicated to my biological and scientific parents:

*Mohammad Kalani, LL.M., and Afrouz Mehrazarin, LL.M.*

*William A. Goddard, III, Ph.D., Kendall N. Houk, Ph.D., and Vaidehi Nagarajan, Ph.D.*

## Acknowledgments

I hereby would like to thank all those who have played a part in my development as a member of the society and the scientific community.

First and foremost, I would like to thank my parents and family, whose support and guidance have helped me sail through difficult chapters of my life. My father and mother have been ultra-supportive, and this thesis is dedicated to their efforts and sacrifices. I would also like to thank my grandmothers (here is your first “doctorate grandson”).

I would also like to acknowledge the efforts, guidance, and support of my scientific parents Bill Goddard, Ken Houk, and Vaidehi Nagarajan. All these individuals have had a direct impact on my personal and professional life. Thank you for your support; thank you for teaching me how to do science and how to deal with the politics of science.

Furthermore, I would like to thank my brother, friends, and scientific colleagues who have supported me throughout my academic years. Thank you (*in alphabetical order*) Peter “Helix-Envy” Freddolino (A.K.A. Adrenaline), Spencer E. Hall (A.K.A. Grandpa), Maziyar A. Kalani, Emile C. Pinera, Caius Radu, Nancy L. Tran, Professor Owen Witte, and Li Yang. Also, thank you members of the MSC who have at one point or another been helpful to me.

I would also like to take this time to thank Mrs. Hallie Fraser (formerly Miss Hallie Lecker), my Taekwondo instructor, mentor, and friend for all of her help and training. Mrs. Fraser was my inspiration and role model during my teenage years and I thank her for the direction and motivation she provided for me.

The next chapter of my life will take me to medical school at Johns Hopkins University School of Medicine where I will pursue my studies towards a Doctor of Medicine degree. Thank you to all who supported me as I prepared for medical school. Most notably, I would like to thank Drs. G. Jennifer Wilson, Robert N. Schwartz, Dominic Thomas, David Cohen (RIP), Steve Hardinger, and Steven G. Clarke.

This thesis is appropriately dedicated to the individuals whose support, love, and dedication have prepared me for a career as a medical scientist: Mom, Dad, Bill, Ken, and Vaidehi.

## Abstract

Dopamine neurotransmitter and its receptors play a critical role in cell signaling process responsible for information transfer in neurons functioning in the nervous system. Development of improved therapeutics for such disorders as Parkinson's and schizophrenia would be significantly enhanced with the availability of the three-dimensional (3-D) structure for the dopamine receptors and of the binding site for dopamine and other agonists and antagonists. In this thesis, I report the 3-D structures of the 5 subtypes of the human dopamine receptors, predicted from primary sequence using first principles theoretical and computational techniques. I use the term "first principles" to mean that we do not use the high resolution crystal structure of rhodopsin as a template, nor do we use homology modeling or threading of any kind to determine the structure. Predicting the binding sites, and the relative binding affinities of endogenous ligands and various pharmaceuticals to the 5 receptors validates the predicted structures. These structures correctly predict the critical residues for binding dopamine and several antagonists, identified by mutation studies and give relative binding affinities that correlate well with experiment. The predicted binding site for dopamine and agonists is located between transmembrane helices (TM) 3, 4, 5, and 6, while the best antagonists bind to a site involving TM helices 2, 3, 4, 6, and 7 with minimal contacts to TM 5. We identify characteristic differences between the binding sites of agonists and antagonists, as well as factors that cause differential binding to the 5 subtypes of the human dopamine receptors.

This thesis consists of five chapters that have, or will shortly result in publications. The first chapter is a brief introduction to the field, the motivation for the project, my scientific contributions, and contribution of others on the team. Chapter two introduces the methods and their successes at reproducing experimentally known results for the human D<sub>2</sub> dopamine receptor; it discusses, in great detail, the active site of pharmaceutical agonists and antagonists to the human D<sub>2</sub> dopamine receptor, and highlights the strengths and shortcomings of homology modeling for membrane bound proteins; this chapter will be submitted for publication to the *Journal of Molecular*

*Biology*. Chapter three reports the results of a blind study performed in collaboration with Aventis Pharmaceuticals. For this study, we were provided with the two-dimensional structure of 9 antagonists and were asked to predict their binding sites, binding affinities, and to explain the differential binding of the ligands to the human D<sub>2</sub> and D<sub>3</sub> dopamine receptors and the human  $\alpha$ 1A adrenergic receptor. The results of this study are in preparation for submission to the *Journal of Medicinal Chemistry*. Chapters four and five of the thesis give preliminary results of comparative studies of the agonist and antagonist binding sites of the five subtypes of the human dopamine receptors. Chapter 6 contains results of another blind study on the G2A receptor with Professor Owen Witte.

In addition to the six main chapters, this thesis contains 6 independent appendices that report results of similar studies in other systems. The first 2 appendices are work that has already been published. The remaining 4 appendices will shortly result in publications, but at this time, they are not publication worthy; these appendices represent data that has been analyzed but has not been written in paper format.

In addition, I would like to make note of the studies that I have conducted on the 9 subtypes of the human adrenergic receptors with Mr. Peter Freddolino, the 4 human histamine receptors that were conducted with Mr. Freddolino and Mr. Maziyar Kalani, and the 4 G2A-like lipid receptors conducted with Mr. Rene Trabanino, Dr. Radu, Dr. Yang, and Professor Owen Witte of the Howard Hughes Medical Institute at the David Geffen School of Medicine at the University of California, Los Angeles.

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M. Yashar S. Kalani, Nagarajan Vaidehi, William A. Goddard, III, The Structure and Function of the Human D<sub>2</sub> Dopamine Receptor and the Agonist and Antagonist Binding Sites, *Proceedings of the National Academy of Sciences, U.S.A.* 101, 3815-3820, 2004.

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