A BINDING FREE ENERGY BASED COMPUTATIONAL PATHWAY FROM CHEMICAL TEMPLATES TO LEAD-LIKE MOLECULES: A CASE STUDY OF CYCLOOXYGENASE -2 INHIBITORS

by

N.LATHA
DEPARTMENT OF CHEMISTRY

SUBMITTED

IN FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

to the

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

HAUZ KHAS, NEW DELHI

INDIA

OCTOBER 2004

CERTIFICATE

This is to certify that the thesis entitled "*A Binding Free Energy Based Computational Pathway from Chemical Templates to Lead-like Molecules: A Case Study of Cyclooxygenase-2 Inhibitors*" being submitted by **Ms. N. LATHA** to the Indian Institute of Technology, Delhi for the award of the degree of **Doctor of Philosophy** in Chemistry is a record of bonafide research work carried out by her. Ms. N. Latha has worked under my guidance and supervision, and has fulfilled the requirements for the submission of this thesis which, to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to other University or Institute for the award of any degree or diploma.

(B. JAYARAM)
Professor
Department of Chemistry,
Indian Institute of Technology, Delhi

Date: 6/1/05

Acknowledgements

It is a pleasure to thank the many people who made this thesis possible. Many people have been a part of my journey in education, as friends, teachers, and colleagues.

Life blessed me with the opportunity to pursue my higher studies at IIT Delhi after 10 years of service as a lecturer in the Department of Biochemistry at Sri Venkateswara College, University of Delhi.

Firstly, for giving me an opportunity to do research and keeping his confidence in my capabilities, I am extremely grateful to my supervisor, Prof. B. Jayaram. I could not have imagined having a better advisor and mentor for my Ph.D. This work is a small tribute from a student still anxious to learn from him. I thank him for his guidance and unconditional support that has its own magic. He helped me to ground my research efforts more firmly by giving me a better foundation in this field. His unquenchable curiosity for the subject and total dedication towards work are probably the most valuable lessons that I have learned during this period of study. I am deeply indebted to him for his rejuvenating encouragement, which gave lots of self-confidence and inspired me to pursue the research in the right direction.

I would avail this opportunity here to specially thank Dr. Madhan Mohan, Director, Department of Biotechnology, Government of India for the helpful discussions on emerging trends in bioinformatics and computational biology in India four years ago and also recommending me to Prof. B. Jayaram at IIT Delhi.

I wish to express my sincere gratitude to Sri Venkateswara College for giving me an opportunity to pursue my higher studies and supporting me throughout the period of study. I am thankful to Dr. A. Sankara Reddy, Principal, Sri Venkateswara College for his support and encouragement. My heartfelt thanks to my colleagues- Dr. Hemalatha Reddy, Meenakshi Kuhar and Dr.Nandita Narayansamy at the Department of Biochemistry, Sri Venkateswara College for their help and valuable friendship. My special thanks to Hema, who stood by me at all times and for her patient hearing on all matters – be it scientific or personal. I am very grateful to Dr. V. Krishnamurthy (former-Principal, SVC), Dr. Panda, and Dr. V. C. S. Rao for their sound advice and guidance. I *owe my thanks to the non-teaching staff of the Department of Biochemistry, SVC and administrative staff of SVC, especially Mr. T. S. R. Murthy, Mr. Ramanamurthy and Mr. M.L.N. Murthy for all the administrative help.*

I am extremely grateful to Prof. A. K. Tyagi, Prof. V. K. Chaudhary, Prof. P. C. Ghosh and Prof. Debi Sarkar at Department of Biochemistry, University of Delhi, South Campus for their encouragement and guidance.

I am thankful to the Head, Department of Chemistry, IIT Delhi for providing me full access to the departmental facilities to carry out the research. I thank all the teachers at the Department of Chemistry, IIT Delhi for their support. I am also grateful to all the teachers of IIT Delhi – Prof. Alok Ray, Prof. J. K. Deb, Dr. S. N. Naik and Dr. Satyawati Sharma who guided me during the pre-Ph. D coursework.

I acknowledge the financial support of UGC, INSA and Department of Science and Technology, Government of India for attending the conference at Albany, USA.

This thesis would not have been possible without the support and love showered upon me by the lab members I owe my special thanks to Surojit and Pooja who stood by my side at all times and were always there to cheer me up. I am very thankful to Tarun for all his help in the drug design project. Many thanks go out to Pankaj for his enthusiasm and keen interest in discussing great diversity of topics with all the lab members during the tea/coffee breaks – ranging from scientific discussions on drug design to music to daily affairs. I thank Kumkum for all the discussions and editing suggestions that contributed to the final copy of the thesis. I must thank Saher for all the help and useful scientific discussions on molecular dynamics. I thank Gandhimathi for her assistance in the initial stages of the drug design project. Thanks are also due to Sonal, Arunima, Vidhu, Praveen, Poonam, Samrat, Pradeep and Bipasha for their good company. I am also thankful to my Ph.D seniors – Parul for her assistance during my initial period of struggle with computational studies and Surjit Dixit for his motivating talks and making my stay comfortable during my trip to Albany, USA.

Finally, I would like to thank my family whose presence helped make the completion of my work possible. I must thank my husband Narayanan, who put up with my varied swings between excited optimism and pessimism during the research work. I thank him for his abundant patience and his encouragement to get me into higher studies.

I must thank Shriya, my only daughter for waiting for me patiently in the evening and missing me with tears in her eyes during my trips to conferences. I am grateful to my parents for raising me as they did and for instilling in me a sense of curiosity and dedication towards work. Many thanks are due to my mother-in-law for all her care and support. I thank my brothers- Ram and Shyam for their encouraging talks over phone, my sister-in-laws Uma, Sudha and Vandana for their love and the chatting that were sometimes required.

Lastly I totally owe to that supreme power- The 'almighty' of whose constant favour, kindness and grace made me wade through all the way to reach the finish line. I dedicate this thesis to Lord Sri Venkateswara.

****

N. Latha

The dawn of the 21st century heralds a new era in the drug discovery process. Access to the complete human genome sequence as well as to the complete sequences of pathogenic organisms (http://**www.ncbi.nlm.nih.gov/**Genbank/) provides us with information that can result in an avalanche of therapeutic targets. As more protein target structures become available with advances in crystallography, nuclear magnetic resonance and bioinformatics, the need grows for a set of computational tools that can identify and analyze active sites and suggest compounds that can bind to these sites specifically. Automation of lead compound design *in silico* given the structure of the protein target and a definition of its active site vies for the top of the wish list in any drug discovery programme. Several conceptual and methodological challenges remain before this goal could be accomplished. Some of the concerns are the novelty of the candidates generated, their geometries, the partial atomic charges, a proper account of the flexibility of the candidate molecule and the target, a consideration of solvent and salt effects in binding, a reliable methodology for developing quantitative estimates of binding affinities and finally the druggability of the candidates generated. This thesis is aimed at charting a computational pathway for lead design at the current state of the art tackling some of the above issues. The pathway developed is tested on a series of known drugs for cyclooxygenase-2 as the target enzyme and this forms the focus of the thesis.

The thesis is divided into seven chapters. The motivation behind this study and a background to computer-aided drug design, the role of biomolecular drug targets and some recent computational methods forms the main focus of **Chapter-1**. A comprehensive

computational protocol of starting from chemical templates to the design of lead-like molecule is presented in **Chapter 2**. This chapter discusses the strategy adopted for the design of template library and candidate molecules there from, development of their geometries and force field parameters, docking the candidates in the active site of the target protein and preparing the protein-ligand complexes for subsequent energy based processing. Binding free energy estimates as applied to protein-ligand complexes obtained from docking and energy minimization can at best be qualitative from a theoretical perspective. These are critically assessed in **Chapter 3**. The success of the method in sorting drugs from non-drugs for a given target is demonstrated using cyclooxygenase as a proof of concept. A case study of COX-2 inhibitors to test the methodology proposed is presented in **Chapter 4**. Flexibility of the target and that of ligand, roles of solvent and salt effects are of serious concern in any viable computational procedure for ranking candidates. Additionally, Boltzmann averaging tends to reduce the magnitudes of the binding free energies obtained from energy-minimized structures (single point calculations). These concerns are addressed by separate molecular dynamics simulations on the systems corresponding to initial and final states of the binding process. **Chapter 5** illustrates the application of molecular dynamics simulations in conjunction with the theory presented for a semi-quantitative estimate of binding free energies. An important question relating to generality of the method and transferability of the protocol is undertaken by testing binding of flurbiprofen, an NSAID specific to COX-1. A detailed structural and energetic analysis is undertaken in **Chapter 6** to account for specificity of flurbiprofen to cyclooxygenase-1. This is presented in this chapter together with some computational issues related to specificity. Finally a critical assessment of the steps in the proposed computational pathway and further improvements envisioned are summarized in **Chapter 7**.

CONTENTS

Chapter 1: Introduction

References

Chapter 2: A computational pathway from chemical templates to generation of structures of target -candidate complexes

References

Appendix

: An ab-initio approach to the estimation of ree energy of protein-ligand complex formation

References

Chapter 4: Sorting drugs from non-drugs based on binding affinities – A case study of COX-2 inhibitors

References
Appendix

Chapter 5: Molecular dynamics simulations on COX-2 inhibitor complexes and post-facto analyses of binding energetics

References

Chapter 6: The issue of specificity: A computational study of the binding of flurbiprofen to COX-1 and COX-2

References

Chapter 7: Summary and Perspectives

Biodata of the candidate