Chapter 1. Introduction

1.1. Overview

What medically oriented researcher doesn't want to find the cure for cancer, HIV, Parkinson's disease, depression, Alzheimer's disease, or heart failure? Or, even better, maybe there's a magic molecule that can fix all of the above. Unfortunately, humanity can no longer depend on magically finding the proper mix of herbs or potions. Rather, medicines evolve from a precise understanding of the biochemical interactions within the body and the rational design of compounds to modify these interactions in a precise, controlled manner. Thus, rational drug design is an increasingly popular field in academia and the multibillion-dollar pharmaceutical industry. This thesis presents research into the interaction between specific G protein-coupled receptors (GPCRs) and their ligands, in an attempt to validate our method of GPCR structure prediction and to understand subtype specificity within this family of lipid receptors.

Many techniques exist for chemically modifying human physiology, but one of the most common pharmacological mechanisms used for drugs today is alteration of intercellular signaling. GPCRs are the targets of many of the blockbuster drugs on the market, including loratadine (Claritin®), fluoxetine (Prozac®), and zolpidem (Ambien®). GPCRs are proteins that span the cellular membrane, communicating exterior signals into secondary messengers inside the cell. Since these secondary messengers affect every aspect of cellular function, understanding the behavior and chemistry of GPCRs is fundamental to this rational drug design procedure.

Unfortunately, designing molecules to target specific GPCRs is not a simple task. The most effective method to design a drug requires knowledge of the structure of the protein target. Membrane proteins are particularly difficult to crystallize, which means that determining their structure experimentally is a prohibitive challenge. Fortunately, recent advances in computational chemistry afford new insights into GPCR structure and interactions through the complex structure modeling and dynamics simulations that were impossible even a decade ago.

This thesis takes on the challenge of computationally predicting the three-dimensional structure of a family of GPCRs that are activated by lysophospholipids and/or sphingolipids. Prior work outside of the Goddard group has created models of these proteins based on the one well-known, high-resolution crystal structure of a GPCR: bovine rhodopsin (BR). This technique, called homology modeling, is especially effective at quickly building and studying proteins that have similar sequences to the original, template protein. However, homology modeling is less effective when the proteins of interest have low sequence homology to the template.[1-4] The human lipid receptors studied here have amino acid sequences that are approximately 15% identical to the sequence of bovine rhodopsin. Chapter 2 presents further details on the biology of GPCRs, lysophospholipids, and their receptors. Note that there is information provided about LPA_1 and LPA_3 for completeness, but those proteins are the subject of future publications.

The presented research builds on the groundwork undertaken by Drs. Spencer Hall and Rene Trabanino. Dr. Trabanino developed and tested the method we use for predicting the segments of the protein sequence that span the cellular membrane.[5,6] Dr.

Hall developed the first, and some subsequent revisions, of the program used to turn those amino acid sequences into three-dimensional proteins.[7,8] The majority of the structure prediction, though, arose through methods newly developed in our research group. This thesis introduces a new method for finding the optimum helical rotation and for an optimization of the docking methods employed for large ligands. Chapter 3 summarizes these new methods for generating the predicted GPCR structures and isolating binding sites within these proteins. Chapters 4 and 5 provide details about the protein structures of LPA_2 and $S1P_1$ and their binding sites, as well as a discussion on the validation of these findings.

Understanding the simple molecular interactions that initiate activation of a complex signaling process allows for a logical and rational approach for altering that pathway, whether it is to prevent cancer cells from multiplying or to force a cell to produce more hormones. Computational protein structure prediction and drug design has many advantages over current experimental methods, especially when working with membrane bound proteins. This research will further the understanding of GPCR structure and ligand binding, which will enable detailed exploration of potential drug targets for a multitude of diseases.

1.2. References

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