INTRODUCTION

Computational modeling of G-Protein Coupled Receptors (GPCRs) has recently become an interesting area of research, as GPCRs are targets for 50% of all existing medications (1). GPCR’s are thought to be in equilibrium between an active and inactive state and are affected by the binding of ligands. Most ligands are classified as agonists which shift the receptor into an active state, inverse agonists which shift the receptor into an inactive state, or antagonists which maintain the equilibrium and compete for binding. The binding of a ligand will alter the conformation of the receptor enough to cause a change in the heterotrimeric G-protein coupled to it. A GTP will replace a GDP attached to the alpha subunit of this G-protein, resulting in a change in a nearby effector molecule, which in turn sends out second messengers such as cAMP throughout the cell. This signal pathway is amplified through each step as many G-proteins can activate many effectors which in turn activate a large number of second messengers (2, 3). There are five general families of receptors including the rhodopsin-like GPCRs, the secretin-like GPCRs, the cAMP receptors, the fungal mating pheromone receptors, and the metabotropic glutamate receptors (4). All of these families are characterized by the presence of seven transmembrane helices composed of hydrophobic sequences; however, the actual sequences vary greatly. The G-Protein Coupled Receptors have a “mean pairwise amino acid identity” of only 17%, making it challenging to both classify and create homology models (5).
1. **One specific aim of this project will be to model the melatonin receptors (MT1 and MT2)** using bovine rhodopsin through structural motif comparison, specifically an aromatic cluster in transmembrane 6 and the “NPXXY” motif in transmembrane 7.

2. **The second aim of this project will be to dock various ligands into the receptor using both a Monte Carlo Simulated Annealing Algorithm and a Lamarckian Genetic Algorithm.**

Homology modeling is performed on proteins that commonly have more structural rather than sequential similarities. The “target protein” is aligned with the “template,” allowing conserved and variable sequences to be determined. From this alignment, the structures for the conserved sequences can be generated using the template protein. The variable sequences often constitute loops at the surface of the protein and can often be modeled from structures in other proteins. Using different scoring functions and conformational searches, the full structure of the target protein can be produced. Target structures can be checked through programs such as 3D-Profiler and ProCheck. The criteria to be used in the analysis includes torsion angles, hydrogen bonding, side-chain conformation, bond angles, and bond lengths (6).

The melatonin receptors are closely related to bovine Rhodopsin, the first protein crystal structure deposited in the Protein Data Bank. Two specific motifs in these receptors differ enough to raise questions as to whether or not function is conserved despite slight amino acid changes. The “aromatic cluster” motif has been recognized to bind to the ligand and cause changes in G-Protein interactions, and the “N(P)XXY” motif is involved in stabilization of the inactive receptor. Our hypothesis is that changes in specific amino acids of these motifs should change the 3-D conformation of the receptor and ultimately its function and stability.
STRUCTURAL MOTIFS

The aromatic cluster motif is assumed to interact with a ligand through aromatic interactions, resulting in a reduction in the TM 6 kink and a loss of interaction between residues E6.30 and R3.50 (the “arginine cage”). The consequent altering of TM 3 produces a change in G-Protein interactions. In melatonin receptors, residue 6.52 is thought to interact specifically with the ligand. In many GPCRs, this position is occupied by phenylalanine; however, an asparagine residue replaces this phenylalanine in melatonin receptors. Since asparagine is nonaromatic, it is probable that the melatonin receptors are at least somewhat constitutively active, as N6.52 is unable to hold W6.48 perpendicular to the plasma membrane. Rather it will move and displace TM 6, causing activation of the receptor, even without the binding of a ligand (7).

The “N(P)XXY” motif in TM 7 provides stabilization of the inactive GPCR. In most receptors Y7.53 is thought to interact with Y7.60. Residue 7.60 is located in Helix 8, which lies parallel to the lipid bilayer and is an extension of TM7. Melatonin receptors contain a phenylalanine at residue 7.60, conserving aromaticity; however, the interaction is not as strong. Stabilization of the inactive state also occurs through an interaction between TM2 and TM7. Most GPCR receptors contain an asparagine at residue 7.49 followed by a proline forming a highly pronounced proline kink (8), allowing N7.49 to interact with D2.50. In the melatonin receptors an alanine replaces the proline residue, leaving a question as to whether or not this transmembrane interaction can occur (7).
METHODS

Modeling of the melatonin receptors will be done using MOE and BioMedCache. Ligands will be docked into the receptors using a FastDock engine, available in Cache. FastDock uses a Lamarckian Genetic Algorithm and scores using a potential of mean force (PMF). PMF uses 34 ligand atom types and 16 protein atom types to create 544 unique pair potentials. Docking in Cache does not allow for covalent bond formation and adds non-bonded terms to the PMF so that atoms stay at non-bonded distances (9).

The genetic algorithm is based on the biological principles of evolution and natural selection. Data is stored in chromosomes and segregated into genes that represent the orientation or phenotype of the protein/ligand. Using multiple operators, including crossover (recombination), mutation, and maturation operators, “parent” chromosomes will generate offspring that will gradually lead to an optimized “solution” (10). The Lamarckian aspect of this algorithm pertains to the local searches performed on the offspring to minimize the phenotype (structure), and then in essence turn the phenotype into the genotype of the new offspring. This algorithm is named after Jean Batsite de Lamarck who believed that phenotypic characteristics one obtained during a lifetime could become heritable and incorporated in one’s genotype. Most Lamarckian algorithms employ an inverse mapping function to map a minimized phenotype back into the genotype, however docking algorithms continuously map genotypes to phenotypes until an optimized phenotype is found during these local searches (11).
The results of this research project will provide verification of the homology modeling done on the melatonin receptors using MOE. Docking will hopefully provide a better insight into the structural motifs of the melatonin receptors and how certain agonist and antagonist bind. The docking programs in BioMedCache and MOE are very different. Instead of a genetic algorithm MOE can employ either a Monte Carlo Simulated Annealing Algorithm or a Tabu Search (12).

A Monte Carlo Algorithm creates small changes in the conformation, orientation, or displacement of the ligand and then scores the new structure based on empirical force field equations. If the move lowers the energy of the system, the new conformation is accepted, otherwise the Boltzmann factor \( p = e^{-\frac{E}{kT}} \) is computed. The new conformation is then accepted only if the probability is greater than a random number between zero and one. The environment of the ligand/receptor begins at a very high temperature allowing for large global searches of conformational space. As time progresses the temperature is lowered so that local searches can be made to optimize the final structure. The Tabu Search, a stochastic approach like Monte Carlo, is a less common approach to the Docking problem. It blocks moves that result in a structure too similar to the previous one by calculating the root mean squared (RMS) deviation (12). The purpose of this approach is to avoid local minima and search as much of the “solution space” as possible. This is accomplished by avoiding the retracing of steps by keeping a series of moves in what is called a “tabu list” (13).

RESULTS
The primary goal of this research project is to provide structural verification of the melatonin (MT1 and MT2) receptors which have already been modeled using MOE. The docking methods utilized in the research project contain various algorithms that should complement one another in an attempt to obtain the structure of the docked receptor. By the end of this research project, the function of the structural motifs in the melatonin receptors will hopefully be clarified, and the results will demonstrate whether or not they follow the same pattern as other GPCRs of the same Rhodopsin family.

REFERENCES

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