models built from the bovine rhodopsin structure or newer de novo methods. However, because of the advent of the β_2-AR structures there are some new virtual screening studies reported in the literature as well.

Three-dimensional virtual screening applications using homology models of the GPCR based on the bovine rhodopsin structure are plentiful.[46–49] In a systematic study of the dopamine (D3), muscarinic (M1), and vasopressin (V1a) receptors by Bissantz et al., three different docking methods with seven scoring functions were used.[46] The binding sites of the GPCR models were preoptimized to be able to accommodate antagonists better. Each model was then used to dock a randomly chosen set of 990 druglike molecules plus ten known antagonists for each receptor. The hit rates from these procedures ranged between 5 and 40%. Another systematic study employing PREDICT built models for five different receptors, including biogenic amine, peptide, and chemokine receptors.[38] One docking method and multiple scoring functions were used to screen ~1.6 million druglike compounds available from >20 vendors worldwide. Hits rates between 12 and 21% were achieved for the five receptors, and in most cases the best hit was a novel and potent (1–100 nM) compound. A recent study of the melanin-concentrating hormone receptor identified six novel chemotypes of 187,084 druglike compounds screened, which amounted to a tenfold improvement over random high-throughput screening.[47] In a final 3D virtual screening study on the histamine H_4 receptor, close to nine million compounds that were available commercially were screened in silico, of which 255 compounds were ordered and 16 were considered active.[48]

Another common technique is to use a hierarchical approach in which a large compound collection is first screened using pharmacophores and the compounds obtained from that filtering process are then docked in the receptor active site.[50,51] This approach takes advantage of the speed and well-established success of pharmacophore searching while potentially eliminating many hits that match the pharmacophore but are poor candidates for the target due to steric clashes with the protein target.

In a recent article, the newly published β_2-AR structure was used to test the ability to dock β_2-AR antagonists in to the binding site.[52] First, a series of seven known β_2-AR antagonists were docked and compared with the experimentally bound carazolol compound. The docked structures of the seven beta-blockers, which included carazolol, were in very good agreement with the binding mode adopted by carazolol in the crystal structure. Similar interactions with the conserved aspartic acidic group on helix 3 and hydrogen bond donor/acceptor groups on helix 5 were achieved. Also, the placement of hydrophobic groups that extended beyond the region of the carbazol macrocycle were reasonable. Next, high-throughput docking with an in-house proprietary (~400,000 compounds) database was performed. In the top 30 compounds, 11 known beta-blockers were found. Finally, a second high-throughput docking experiment involving 4 million compounds was performed. The docking identified compounds that appear to bind in two very different parts of the binding site. One binding region is the traditional site occupied by the known antagonists like carazolol; the second is a region near one of the extracellular loops. Many of the compounds predicted to bind in the second region are unique chemotypes for β_2-AR antagonists. This second binding site is in the loop region of the β_2-AR structure that differs from the bovine rhodopsin structure (Figure 16.2). So it is unlikely that these compounds would be found in a virtual screening of a rhodopsin-based homology model. The experimental results of these new findings are not yet published; however, the ability to make very novel predictions like this is the single most important advantage of the 3D structure-based approach.

MOLECULAR DYNAMICS SIMULATIONS

Molecular dynamics (MD) simulation is an important tool for studying the flexibility, stability, and large-scale motions of molecules often in a condensed-phase environment.[4] These approaches are still very computationally intensive and are still not used routinely in industry for drug discovery. However, these methods are often needed to predict properties of a system that an individual structure cannot provide such as average properties like structure and thermodynamic properties like binding energies.[4] Two ways of carrying out these calculations[4] are (1) to surround the protein system with explicit solvent and impose a periodic boundary condition and (2) to use continuum methods like the generalized Born model. MD has also become a very important technique used to construct and anneal model structures.[4]

Explicit bilayer and solvent

Simulation of protein/ligand interactions are often greatly influenced by the environment in which a protein system resides. This is particularly true for GPCRs that straddle two very different physical regions that have different hydrophilic/hydrophobic properties, namely water and lipid.

In a recent article[53] a 40 ns simulation on the bovine rhodopsin structure examined the average structure of the protein, the average structure of the retinal binding site, the large-scale motions, and the lipid/water interactions. During the simulation the structure of the protein was well maintained, especially the helices. There were larger deviations in the loop and N- and C-terminal regions. A change was observed in the hydrogen bonding near the retinal chromophore that leads to some shifts in the tilts of several helices. This was hypothesized to be important for the reaction cycle of the receptor.

Simulations of β_2-AR with epinephrine and butoxamine bound were run using a de novo model.[54] Butox amine