of ligand docking and refinement as well as site-directed mutagenesis.[31–35]

A recent publication compares a homology model of β_2-AR based on bovine rhodopsin with the new β_2-AR crystal structures.[36] Two models were created, one with the rhodopsin-like second ECL and the other had the second ECL built de novo, and the inverse-agonist carazolol was docked into each of these models. In the former of the two structures the ECL interfered with binding of carazolol, whereas in the later of the two models the ECL did not affect the binding of carazolol. These results are consistent with the experimental differences in the second ECL (Figures 16.2 and 16.3) for rhodopsin and β_2-AR.

De novo structure prediction

The inherent limitations of homology modeling and the unique structural template of the GPCR 7TM region has prompted several groups to develop de novo-based approaches to generating GPCR models that may be applied to receptors in families other than Family A, which have more remote homology with the bovine rhodopsin template.

Two de novo methods have emerged lately that are very similar in their approach to constructing a GPCR model structure, which are the PREDICT[37,38] and MembStruk[39–41] methods. The protocol for predicting GPCR structures by these methods consists of roughly the following steps: (1) predict the TM regions using hydrophobicity analysis and other sequence analysis techniques; (2) construct the individual helices and pack them together; (3) have each putative structure in the previous step undergo coarse grain optimization; and (4) perform full optimizations of the structures.

Validation of the structures predicted by these methods was twofold. First, for the structure of bovine rhodopsin built by these techniques, a direct comparison with the crystal structure was made and found to be in close agreement. Second, for models in which there is no experimental crystal structure, the docking and assessment of ligand-binding energies was performed and found to be consistent with experimental values. Furthermore, some of these docked structures provided useful insights regarding the nature of the ligand binding interactions.[37–41]

DOCKING STUDIES

Manual docking

Molecular docking is perhaps one of the most illuminating and sublime procedures used by computational chemists.[4–7] It has the ability to reveal aspects of ligand binding that are neither obvious nor trivial and cannot be ascertained from the ligands alone. The literature is filled with numerous studies in which the binding of small molecules and peptides was examined.[43–52] This usually involved a homology model of the receptor and the docking

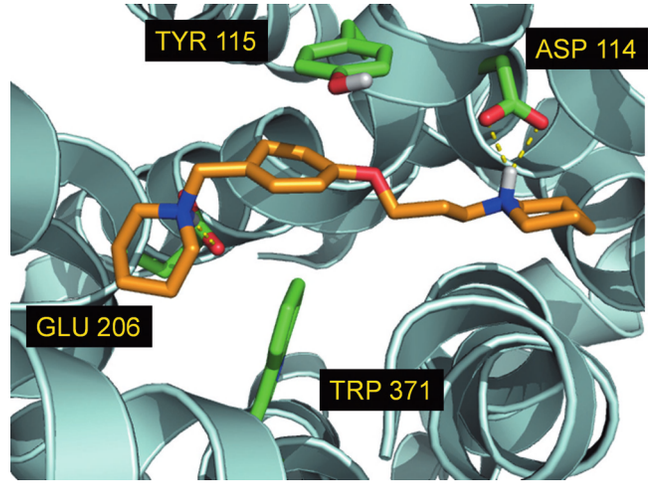

Figure 16.4. Histamine H_3 antagonist docked in the binding site of a homology model followed by 200 ps of molecular dynamics simulation.[36] Image was created with PYMOL 0.99 (http://pymol.sourceforge.net/).

of the ligand was either manual and/or semiautomated. Moreover, site-directed mutagenesis data were often used to determine key residues responsible for endogenous ligand activity as well as antagonist activity too.

For example, homology models have been used to study ligand binding in several types of aminoergic receptors, including dopamine,[44,45] histamine,[33,36] β-andrenergic,[34,43,44] and serotonin.[45] Several docking studies involving the β-andrenergic receptors and antagonists and their natural ligand[34,43,44] usually include the basic amine interaction with the aspartic acid side chain on helix 3 (D3.32), which is consistent with the β_2-AR structure with carazolol bound (Figure 16.3). In addition, the catachol hydroxyl groups make hydrogen bonding interactions with the two serine side chains on helix 5 (S5.42 and S5.46).

Other examples of docking include the histamine receptors.[33,36] Currently, there are four known histamine subtypes (H_1, H_2, H_3 and H_4).[36] Antagonists of H_1 and H_2 comprise some of the better known "blockbuster" drugs on the market today. Like all aminoergic receptors there is a highly conserved aspartic acid on helix 3 (D3.32)[5] that interacts with the basic amine of the natural ligand histamine as well as exogenous antagonists and antagonists (Figure 16.4).

In the H_3 docking model there is an additional basic amine that is based on the model and existing structure/activity relationships are postulated to interact with a glutamic acid (GLU 206) side chain on helix 5.

Fast docking and virtual screening

There is an ever-increasing use of 3D drug targets to rapidly dock compound collections into their active site to discover novel leads for that target especially when no lead compounds are known.[6,7] Several studies involving this type of virtual screening applied to GPCRs has appeared in the literature.[46–52] Most of these studies relied on homology