

Contents

1	Introduction	1
1.1	General Introduction	1
1.2	G-Protein Coupled Receptors	1
1.2.1	Pharmaceutical Relevance and Classification	1
1.2.2	Molecular Structure and Signal Transduction	3
1.2.3	The Structure of Bovine Rhodopsin	4
1.2.4	Agonists, Antagonists and Inverse Agonists	6
1.3	Histamine	6
1.4	The Histamine Receptor Family	8
1.4.1	The Human H ₃ -Receptor	9
1.4.2	The Human H ₄ -Receptor	20
2	Methods	25
2.1	Generation of Homology Models	25
2.1.1	Introduction	25
2.1.2	Sequence Analysis Tools	25
2.1.3	Sequence Structure Alignment	30
2.1.4	Adding Amino Acid Side Chains	30
2.1.5	Prediction of Protonation States	30
2.1.6	Force Field Methods: Energy Minimisation and MD Simulations	32
2.1.7	Model Evaluation	36
2.2	Conformational Analysis of Ligand Molecules	37
2.3	Ligand Superposition Techniques	38

2.4	Analysis of Interaction Fields	39
2.5	Ligand Docking	40
2.6	Pharmacophore Models for Screening Structural Databases	40
I	Molecular Modelling Studies of Bovine Rhodopsin	43
3	Scope	45
4	Results	47
4.1	System Setups for the Simulation of Bovine Rhodopsin	47
4.1.1	Generation of Model Structures of Bovine Rhodopsin	47
4.1.2	Bovine Rhodopsin in a CCl ₄ /Water Environment	49
4.1.3	Bovine Rhodopsin in a DPPC/Water Environment	51
4.1.4	Calculation of pK _a -shifts of Titrable Amino Acid Residues	53
4.2	Truncated versus Entire Protein Models	54
4.3	Consideration of Internal Water Molecules	55
4.4	CCl ₄ /Water versus DPPC/Water Environment	59
4.5	Choice of the Correct State of Protonation	61
4.5.1	Calculation of pK _a -shifts Using the UHBD Program	61
4.5.2	MD Simulations Comparing Different States of Protonation for Residues D83/2.50, E122/3.37, and E181/4.70	63
4.6	Studying the Conformational Adaptations after Introduction of <i>all-trans</i> -Retinal	67
4.7	Simulation Setup for the Analysis of Interhelical Contacts	69
5	Discussion	75
5.1	System Setup	76
5.2	Effects of an <i>N</i> -terminal Truncation	76
5.3	Effects of Internal Water Molecules	77
5.4	Influence of the Simulation Environment	80
5.5	Choice of Protonation States	81
5.6	Conformational Adaptations after Introduction of <i>all-trans</i> -Retinal	82
5.7	Deriving Interhelical Contacts as Potential Constraints in GPCR Simulations	84

II Molecular Modelling Study of the Human Histamine H₃-Receptor	87
6 Scope	89
7 Results	91
7.1 Generation of a Homology Model of the Human Histamine H ₃ -Receptor . . .	91
7.1.1 Sequence Analysis Tools	91
7.1.2 Sequence Structure Alignment	93
7.1.3 Placing Amino Acid Side Chains	95
7.1.4 Simultaneous Side Chain and Ligand Placement	101
7.2 MD Simulations of hH ₃ R Models	103
7.2.1 MD Simulations of Uncomplexed hH ₃ R Models	103
7.2.2 MD Simulation of Inverse Agonist/hH ₃ R Complexes	109
7.3 Model Validation via Screening of a Focused Database	121
7.4 Application of the Generated Binding Pocket Conformation as Filter in HTS	127
7.5 Pharmacophore Based Screening	131
7.6 The hH ₃ R Binding Site, Suggested Structures and Implications for the hH ₄ R	140
7.6.1 The hH ₃ R Binding Site	140
7.6.2 Suggested Structures for Experimental Testing	144
8 Discussion	149
8.1 Generation of a Homology Model of the Human Histamine H ₃ -Receptor and Comparison with other hH ₃ R Models	149
8.2 High Throughput Screening by Docking	156
8.3 Pharmacophore Based Screening	157
8.4 Analysis of the hH ₃ R Binding Pocket	160
8.4.1 Orientation of H ₃ R Ligands in the Binding Pocket	160
8.4.2 Species Differences for the H ₃ R	162
8.4.3 Agonism versus Inverse Agonism	163
9 Summary	167

10 Appendix	169
10.1 Force Field Terms	169
10.1.1 Bonded Interactions	169
10.1.2 Non-Bonded Interactions	171
10.2 Example Parameter Input File for an MD Simulation in GROMACS	172
10.3 One Letter Code for Amino Acids	177
10.4 List of Abbreviations	178