# Table of Contents

1 Introduction 1

1.1 Olfaction 1

1.2 Olfactory Receptors 1

1.3 Odorant Receptors and Their Signalling 2

1.4 Structure and Function of Odorant Receptors 3

1.5 Neuroanatomy of Olfaction 4

1.6 Evolution of Odorant Receptors 4

1.7 Functional Expression of Odorant Receptors in Heterologous Cell Systems and OR Based Sensors 7

1.8 Intracellular Traffic of Membrane Proteins 9

1.9 Odorant receptors and Metals 12

1.10 Odour Chemistry 12

1.10.1 Odorant Structure Does Not Predict Odour Quality 12

1.10.2 Choice of odorants 13

1.10.3 Metabolic Conversion of Odorants in the Nasal Mucus 15

1.11 Smell 15

1.12 Aims of this work 17

2 Materials and Methods 18

2.1 Cell Culture Methods 18

2.1.1 Cultivation of Cells 18

2.1.2 Transfection of Cells in 100 mm Plate Format 19

2.2 Ca\textsuperscript{2+} Assays 19

2.3 Luminescence Assays 20

2.4 Surface ELISA 20

2.5 Immunocytochemistry 20

2.6 Preparation of Plasma Membranes 21

2.7 Immunoblot Analysis 22

2.8 Bioinformatics 22

2.9 Analysis of Published Cognate Odorant-Receptor Assignments 23

2.10 Calculation of Concentration Response Curves 23

2.11 Preparation of Agonist Dilution Series 24

2.12 Calculation of the Z'-factor 24

2.13 Calculation of Trafficking-Motif Biodiversity 26

2.14 Calculation of the possible trafficking motif combinations 27

2.15 Molecular Biological Methods 27

2.15.1 Molecular Cloning 27

2.15.2 Polymerase Chain Reaction 28

2.15.3 Reverse Transcriptase-Polymerase Chain Reaction 28

2.15.4 Agarose Gel Electrophoresis 28

2.15.5 Site-Directed Mutagenesis 29

2.15.6 Kit-Based Isolation, Preparation and Processing of Nucleic Acids 29

2.15.7 Restriction Digest of DNA 30
Results

3.1 Analysis of Deorphaned OR and Their Cognate Odorants
3.1.1 Physico-Chemical Properties of Cognate Ligands for Phylogenetically Related Odorant Receptors
3.2 Identification of Odorant Receptors for Thiol Key Food Odorants and Establishment of a Ca^{2+}-Imaging Based Bioassay
3.3 Cell Systems Enabling the Detection of Second Messenger cAMP
3.4 Identification of Odorant Receptors for Thiolic Key Food Odorants with the Luminescence Based Assay Platform
3.5 Identification and Characterization of Odorant Receptors for the Enantiomeric Key Food Odorants (+)/(−)-Carvone
3.6 Optimisation of Cell System Performance by Co-Expressing Gy13
3.7 Identification and Statistical Evaluation of Trafficking Motifs in OR
3.8 Functional Impact of C-terminal Trafficking Motifs on GPCR
3.9 Functional Impact of Trafficking Motifs on Odorant Receptor Expression
3.10 Do the Transport Motifs act on the Intracellular Translocation of GPCR?
3.11 Functional Impact of Putative ICL3 Trafficking Motifs
3.12 Trafficking Motifs in Other Chemosensory GPCR
3.13 A Spaced Dibasic Motif in RTP1S is Required for the Functional Expression of Odorant Receptors
3.14 Approaches to Optimize Functional Expression of OR in Heterologous Cell Systems

Discussion

4.1 Ligands for Odorant Receptors – Does the Nature of Published Ligands Imply a Special Role for Key Food Odorants?
4.2 Class-i Odorant Receptors Have More Hydrophilic Ligands Compared to Class-II OR
4.3 Library Screen Versus Thiolic Odorants and Putative Constituents of the Odorant Binding Pocket of Cognate Odorant Receptors
4.4 Deorphanisation of Odorant Receptors for (+)/(−)-Carvone and Cell-Dependent Characteristics
4.5 The Odorant Binding Pocket and Determinants for Enantioselectivity of Olfr43 Other Than OR1A1
4.6 Co-Expression of Gy13
4.7 Odorant Receptors are Massively Loaded with Combinations of Highly Conserved Functional Short Amino Acid Motifs
4.7.1 A Proximal C-Terminal Dibasic Motif is Required for Functional OR1A1 Expression in HEK293 Cells But Not in NxG Cells
4.7.2 Do C-terminal Trafficking Motifs in OR Regulate the Membrane Expression of OR in Heterologous Cell Systems?
4.7.3 Do ICL3-Trafficking Motifs in OR Regulate the Membrane Expression of OR in Heterologous Cell Systems?
4.7.4 Hypothesis on the Physiological Relevance of Trafficking Motifs in OR: Regulation of Axon Targeting of OSN
4.7.5 Naturally Occurring SNPs in C-Terminal Trafficking Motifs May Affect Olfactory Perception and Add to Personalized Noses

4.7.6 Trafficking Motif Equipment of Orphan Versus Deorphaned Odorant Receptors

4.7.7 Comparable Occurrence of Trafficking Motifs in Chemosensory Receptors of Different Classes and Groups

4.7.8 Genetic Fingerprints of Trafficking Motif Equipment in Recent Human Evolution

4.8 In RTP1 Also a Spaced Dibasic Motif May be Involved in the Interaction with Odorant Receptors

4.9 Similar Screening Approaches Provide Differential Results

5 Outlook

6 References