

**TABLE OF CONTENTS**

	<b>TABLE OF CONTENTS</b>	<b>1</b>
	<b>COOPERATIONS</b>	<b>6</b>
	<b>ABBREVIATIONS</b>	<b>7</b>
<b>1</b>	<b>INTRODUCTION</b>	<b>9</b>
<b>1.1</b>	<b><i>The Thyroid Stimulating Hormone Receptor</i></b>	<b>9</b>
<b>1.2</b>	<b><i>Structural architecture of the TSHR and previously identified inter-/intramolecular functionalities of extracellular receptor components</i></b>	<b>11</b>
1.2.1	<i>Extracellular structure of the TSHR</i>	12
1.2.1.1	<i>N-terminal structure</i>	12
1.2.1.1.1	<i>Cysteine-box 1</i>	12
1.2.1.1.2	<i>Leucine-rich repeat domain</i>	12
1.2.1.1.3	<i>Hinge region</i>	14
1.2.1.1.3.1	<i>Cysteine-box 2</i>	14
1.2.1.1.3.2	<i>Cysteine-box 2/3 linker</i>	16
1.2.1.1.3.3	<i>Cysteine-box 3</i>	18
1.2.1.2	<i>Extracellular loops</i>	18
1.2.1.2.1	<i>Extracellular loop 1</i>	18
1.2.1.2.2	<i>Extracellular loop 2</i>	19
1.2.1.2.3	<i>Extracellular loop 3</i>	19
1.2.1.3	<i>TSH - TSHR interaction</i>	20
1.2.1.4	<i>Molecular Models of the GPHRs</i>	21
1.2.1.4.1	<i>LRR domain</i>	21
1.2.1.4.2	<i>Receptor models including extracellular loops and the hinge region</i>	22
<b>1.3</b>	<b><i>Activation and Inactivation of the TSHR</i></b>	<b>22</b>
1.3.1	<i>Activity states of the TSHR</i>	22
1.3.2	<i>Phenotypes of Mutations</i>	24
1.3.2.1	<i>Constitutively activating mutations</i>	24
1.3.2.2	<i>Inactivating mutations</i>	25

1.3.2.3	<i>Databases of mutation phenotypes</i>	25
1.3.2.4	<i>Modulation of TSHR activity by low molecular weight ligands</i>	26
<b>1.4</b>	<b><i>Aims of the studies</i></b>	<b>26</b>
<b>1.5</b>	<b><i>Strategy</i></b>	<b>27</b>
<b>2</b>	<b>MATERIAL AND METHODS</b>	<b>30</b>
<b>2.1</b>	<b><i>Bioinformatics, Molecular modelling and Docking procedures</i></b>	<b>30</b>
2.1.1	<i>Amino acid sequence alignment of the GPHRs</i>	30
2.1.2	<i>Molecular modelling of TSHR and LHCGR; LMW ligand docking procedures</i>	30
2.1.2.1	<i>The serpentine domain of TSHR and LHCGR</i>	30
2.1.2.2	<i>Cysteine-box 1 and the leucine-rich repeat hormone binding domain</i>	31
2.1.2.3	<i>Cysteine-box 2 and cysteine-box 3</i>	32
2.1.2.4	<i>S281 at cysteine-box 2 and the extracellular loop 1</i>	32
2.1.2.5	<i>Extracellular loop 2 and 3</i>	33
2.1.2.6	<i>Docking complexes of the TSHR and the LHCGR with a LMW ligand</i>	33
2.1.3	<i>Sequence-Structure-Function resource</i>	34
2.1.3.1	<i>Data Set</i>	34
2.1.3.2	<i>Alignment</i>	34
2.1.3.3	<i>Numbering</i>	35
2.1.3.4	<i>3D models and structural templates for GPHR models</i>	35
2.1.3.5	<i>Database Technology</i>	36
2.1.3.6	<i>Search functions and Output options</i>	36
<b>2.2</b>	<b><i>Characterization of mutant phenotypes</i></b>	<b>37</b>
2.2.1	<i>Site-directed mutagenesis</i>	37
2.2.2	<i>Cell culture and transient expression of mutated TSHRs</i>	38
2.2.3	<i>FACS analyses</i>	38
2.2.4	<i>cAMP accumulation assay</i>	38
2.2.5	<i>Stimulation of inositol phosphate formation</i>	39

2.2.6	<i>Linear regression analysis of constitutive activity as a function of TSHR expression (slopes)</i>	39
2.2.7	<i>Confocal laser scanning microscopy (CLSM)</i>	39
2.2.8	<i>Statistics</i>	40
<b>3</b>	<b>RESULTS</b>	<b>41</b>
<b>3.1</b>	<b><i>Molecular analysis of receptor components</i></b>	<b>41</b>
3.1.1	<i>Cysteine-box 1 and the leucine-rich repeat hormone binding domain</i>	41
3.1.1.1	<i>Molecular Model</i>	41
3.1.2	<i>Structural model and functionalities of cysteine-boxes 2 and 3</i>	44
3.1.2.1	<i>Molecular models of C-b2 and C-b3 complexed with the LRRD</i>	44
3.1.2.2	<i>Functional characterization of amino acids</i>	48
3.1.2.2.1	<i>Mutations of residues in cysteine-box 2</i>	48
3.1.2.2.2	<i>Mutations of residues in cysteine-box 3</i>	49
3.1.3	<i>Extracellular loop 1 and the interaction with cysteine-box 2</i>	53
3.1.3.1	<i>Molecular Model</i>	53
3.1.3.2	<i>Functional characterization of amino acids</i>	54
3.1.3.2.1	<i>Mutations of S281 in cysteine-box 2</i>	54
3.1.3.2.2	<i>Functional assessment of the hTSHR mutants at Y279 (C-b2), Y476, H478, Y481, Y482, and H484 (ECL1)</i>	56
3.1.4	<i>Extracellular loop 2</i>	58
3.1.4.1	<i>Molecular Model</i>	58
3.1.4.2	<i>Functional characterization of amino acids in the ECL2</i>	60
3.1.4.3	<i>Identification of amino acids that are involved in the constitutive activation caused by pathogenic mutation I568V</i>	62
3.1.5	<i>Extracellular loop 3</i>	65
3.1.5.1	<i>Molecular Model</i>	65
3.1.5.2	<i>Functional characterization of amino acids in the ECL3</i>	67
<b>3.2</b>	<b><i>Modes of binding of a small agonistic molecule</i></b>	<b>69</b>
3.2.1	<i>Molecular models of the TSHR and the LHCGR in complex with a LMW agonist</i>	69

<b>3.3</b>	<b><i>A semi-quantitative data set for Sequence-Structure-Function Analysis at GPHRs</i></b>	<b>74</b>
3.3.1	<i>Data Set</i>	74
3.3.2	<i>SSFA Tools</i>	75
3.3.3	<i>Pathogenic mutations</i>	79
3.3.4	<i>Constitutively activating mutations</i>	79
<b>4</b>	<b>DISCUSSION</b>	<b>80</b>
<b>4.1</b>	<b><i>A tightly packed signalling interface between the extracellular- and the serpentine domain</i></b>	<b>80</b>
4.1.1	<i>Structural-functional features of the N-terminal cysteine-box 1 and the LRR hormone binding domain</i>	80
4.1.2	<i>The LRRD is oriented in spatial proximity to the serpentine domain and linked via cysteine-box 2</i>	82
4.1.3	<i>Epitopes Y279-K291 of cysteine-box 2 and P400-D410 of cysteine-box 3 are localized at the interface between the ecto- and serpentine domain</i>	82
4.1.4	<i>Intramolecular switches for signalling activity in the TSHR ectodomain</i>	83
4.1.4.1	<i>Signalling sensitive amino acids in cysteine-box 3</i>	83
4.1.4.2	<i>Constitutively activating mutations in cysteine-box 2</i>	85
4.1.5	<i>Modulation of signalling activity by mutations of amino acids in cysteine-box 2 and cysteine-box 3</i>	86
4.1.6	<i>Section summary</i>	89
<b>4.2</b>	<b><i>A fundamental role of the extracellular loop 2 in the signal transduction process</i></b>	<b>90</b>
4.2.1	<i>Lysine 565 in the ECL2 is a key player in the intramolecular signalling processes of the TSHR</i>	90
4.2.2	<i>Mutations with both decreased basal Gas- and decreased hormone-induced Gαq activity</i>	90
4.2.3	<i>The interface between the ECL2 and the TMH6</i>	91
4.2.4	<i>TMH6 may glide along the ECL2 according to different receptor activity states</i>	93

4.2.5	<i>Section summary</i>	95
<b>4.3</b>	<b><i>The extracellular loop 3 is of high functional importance</i></b>	<b>95</b>
4.3.1	<i>A hydrophobic cluster in the center of the third extracellular loop is important for TSH receptor signalling</i>	96
4.3.2	<i>Section summary</i>	97
<b>4.4</b>	<b><i>TSHR activation by a low molecular weight ligand</i></b>	<b>97</b>
4.4.1	<i>Modes of binding of a small agonistic molecule</i>	98
4.4.3	<i>Section summary</i>	99
<b>4.5</b>	<b><i>A Sequence-Structure-Function-Analysis resource</i></b>	<b>99</b>
4.5.1	<i>SSFA concept and modules</i>	99
4.5.2	<i>SSFA Tools</i>	101
4.5.3	<i>Signalling specificities and interrelated determinants of GPHRs revealed by SSFAnalysis</i>	103
4.5.3.1	<i>Amino acids of high importance for the conformation of hormone induced receptor activation</i>	103
4.5.4	<i>Section summary</i>	104
<b>4.6</b>	<b><i>General importance of presented studies</i></b>	<b>105</b>
<b>5</b>	<b>SUMMARY</b>	<b>106</b>
	<b>ZUSAMMENFASSUNG</b>	<b>109</b>
<b>6</b>	<b>REFERENCES</b>	<b>112</b>
	<b>PUBLICATIONS</b>	<b>125</b>
	<b>PUBLISHED ABSTRACTS OF POSTERS AND ORAL PRESENTATIONS</b>	<b>126</b>
	<b>ERKLÄRUNGEN</b>	<b>128</b>