5 SUMMARY

The glycoprotein-hormone receptors (GPHRs), thyroid stimulating hormone (TSH, thyrotropin) receptor (TSHR), lutropin (LH)/ choriogonadotropin (CG) receptor (LHCGR) and follitropin (FSH) receptor (FSHR) are related members of the rhodopsin/adrenergic receptor family within the GPCR superfamily. TSH and the TSHR are pivotal proteins in the control of thyroid function.
The TSHR is activated by TSH, however it can also be activated by constitutively activating mutations (CAMs), antibodies, small synthetic ligands, tryptic cleavage and deletions of epitopes in the extracellular domain (ECD) or the serpentine domain (SD). Dysfunction of the TSHR causes several diseases including hyper- and hypothyroidism.

Therefore, the delineation of the molecular activation mechanism by investigation of sequence-structure-function relationships in the TSHR is of high importance to understand the molecular causes of frequently occurring receptor dysfunctions. Moreover, knowledge about structural localization and biochemical/biophysical properties of signalling sensitive amino acids or specific epitopes regarding inactivation or activation of the TSHR will allow better understanding of the complex activation mechanism and reveal new ideas of sites for pharmacological interventions. This might open the door for new therapeutic perspectives for treatment of TSHR-mediated diseases with the TSHR as a direct target.

The aim of this study was to provide conceptual progress in the understanding of how glycoprotein hormone receptors convey the signal from the externally bound hormone towards the transmembrane domain in order to achieve the transition between the basal and active conformations. The detailed aims were: i) structural and functional characterization of determinants that are prerequisites for the signal transduction process, ii) molecular description of localization and binding-modes of small molecules, iii.) generation of a sequence-structure-function analysis resource for phenotypes of GPHRs.
Using an iterative cycle of hypothesis and experimental proof of sequence-structure-function relationships by combining molecular homology modeling and site directed mutagenesis, the border between physiological and structural aspects is crossed by confirming predictions of molecular and structural determinants for TSHR activation.
In these studies structural features of the leucine-rich repeat hormone binding domain (LRRD) were described and for the first time provided deep and important insights in the

structural architecture of this special receptor domain that is responsible for the hormone and antibody binding process. It was shown, that the LRRD is comprised of eleven leucine-rich repeats instead of the formerly predicted nine repeats. Homology models predicted a structure and shape of the LRR domain that is completed by the flanking cysteine boxes as stabilizing portions of the LRRD. This shape and structure was later on confirmed by the published crystal structure of the FSHR LRRD. This information shed new light on the extracellular inter- and intermolecular interactions of GPHRs.

Furthermore, assembled comparative models led to the identification of new epitopes at the structurally unknown hinge region that are essential for the activation process. In detail, the hypothesis was developed that portions of the intramolecular components at cysteine-boxes C-b2 and C-b3 in the N- and C-terminal extracellular hinge regions interact in tight spatial cooperation and are localized at the interface between the ecto- and serpentine domains. As one major result, modeling driven mutagenesis studies showed that these structural features are of high significance in the primary and/or secondary activation steps of TSHR. Point mutations led on the one hand to the identification of 5 new positions in the TSHR for constitutive receptor activation by mutations (C-b2: K291A; C-b3: D403A, E404K, N406A, P407D) that stabilise the basal receptor state. On the other hand inactivating mutations for TSH mediated signaling (P400A, P407A, E409A) were identified and provided new hints for components involved in the formation of the activated receptor state. Considering the spatial proximity of these portions, they very likely form components of an intramolecular structural switch (internal transmitter) which is important for the stabilization of the basal, partially active state of TSHR as well as the signal transduction from the ectodomain towards the serpentine domain.

Additionally, several mutations in the ECL2 (K565A), and the ECL3 (P652A-V656A), led to inactivation of the TSHR (also selective for different G-protein subtypes), suggesting that these extracellularly located amino acids are important for the transduction of the signal from the ectodomain to the transmembrane domain by initiating the coupling of the hormone to the ECD.

The second major result is the identification of a molecular hydrophobic contact site and interface between the ECL2 and the TMH6 of the TSHR. This functional interface is most probably involved in triggering different activity states and the subsequently conformational adjustment of TMH6 by ECL2. In detail, the isoleucines 640 in TMH6 and 568 in ECL2 were identified and characterized as complementary knob and hole counterparts and key players in the TSHR signaling process. Slight side chain alterations at both positions by site-directed

mutagenesis lead to a decreased or increased basal activity, which confirmed the predicted mutual shift. This result confirmed the fundamental rule of TMH6 movement for different activity states of a GPCR. Moreover, the rule is augmented by identifying a regulating counterpart at ECL2 in the case of the TSHR.
Taken together, this study identifies several intramolecular signaling determinants of the extracellular region of TSHR that can be functionally and structurally described as part of an internal signaling transmitter. It is demonstrated, that signaling sensitive interfaces and molecular contacts between structural receptor components (e.g. between ECL2 and TMH6) are important for the modulation of activity states of the TSHR.

Moreover, based on the refined molecular models, docking studies are also conducted for a small molecular weight ligand (LMW) that activates the TSHR and the homologous LHCGR. This study describes allosteric binding modes of a LMW ligand to the TSHR and LHCGR in detail. Molecular localization, direct interactions between the LMW ligand and TSHR are suggested and confirmed. This information is a prerequisite for the rational development and refinement of highly potent LMW ligands (antagonistic and agonistic).

Finally, a web accessible resource system for Sequence-Structure-Function Analysis (www.fmp-berlin.de/ssfa) was developed for GPHRs using functional data from about 900 mutations. Complementary to known databases, the data set and developed tools allow the linking of functional and biochemical properties of wild type and mutation phenotypes of GPHRs with spatial features to reveal structure-function relationships. Moreover, a semi-quantitative analysis allows a discrimination of molecular and structural determinants that are responsible for different functionalities such as selective G-protein-mediated activation of GPHRs.