3.3 Study of Patterns for G Protein-Selectivity

3.3.1 Mastoparan-X and -S: Secretagogues Selective for Different Gα-subunits

Identification of Distinct Distances of Positive Charges at Mastoparans

In initial studies, the available structures of peptides that showed significant selective activity towards distinct Gα-subtypes were investigated for different structural patterns, such as residue positions, side chain orientations and distances of hydrogen bond acceptors, hydrogen bond donors and charge distances.

Structural information of the tetradecapeptides mastoparan-X (MPX) and mastoparan-S (MPS) were used for these primary considerations. Both were available in a vesicle-bound and a G protein-interacting state.

The conformation of the vesicle-bound MPX peptide, which was solved in the presence of perdeuterated phospholipide vesicles (Wakamatsu *et al.*, 1992), shows an amphipathic helix (Fig. 1-3). Its membrane-associated side is fully non-polar. The other side includes all hydrophilic residues, including three lysine residues (Lys4, Lys11 and Lys12). Structural analysis of MPX interacting with G proteins Gαi/Gαo from entry 1A13 (Kusunoki *et al.*, 1998) from the PDB (Berman *et al.*, 2000) showed conformations similar to those of vesicle-bound structures (Fig. 1-3). Similarly, a separation into a rather hydrophobic and a rather hydrophilic side was observed, which is supported by the predominantly α-helical conformation of the peptide (Sukumar and Higashijima, 1992).

The vesicle-bound conformation of MPS (Sukumar *et al.*, 1997) is similar to the above-mentioned structures of MPX (Fig. 1-3). The structure of vesicle-interacting MPS is also amphipathic, based on its mainly α-helical conformation. But there are a few differences in both sequences. MPS includes an additional hydrophilic residue (Ser8 instead of Ala) but only two basic residues (Lys4 and Arg11). The 3rd basic amino acid of MPX (Lys12) was replaced by a glutamine (Gln12) in MPS. In addition, MPS includes α-amino-isobutyric acid (Aib, U) at position 10 and substitution Leu13→Val. Aib is an amino acid occurring naturally only in peptides of microorganisms. Here, it was integrated into MPS to stabilize the α-helical backbone conformation in its surrounding region (Sukumar *et al.*, 1997; Karle and Balaram, 1990). G protein-interacting structures of MPS (solved by NMR in the presence of Gαs, Gαo and Gαi, respectively) do not show the straight α-helical conformations of all other investigated peptides (Sukumar *et al.*, 1997). An obvious kink at methionine Met9 interrupts

the helical backbone of the peptide. Nonetheless, the amphipathic characteristics of MPS still exist (see our own preparatory work in section 1.2.1 and Fig. 1-3).

3D-structure comparisons of positions as well as distances of hydrogen-bond acceptors, hydrogen-bond donors and charges revealed a characteristic pattern matching the described G protein-selectivity of MPX and MPS: the distances of positive charges (Fig. 3-17). The shown distances of positive charges take into consideration the rotamer flexibility of side chains of the investigated peptides. For Gαi-activating mastoparan-X (MPX), the vesicle-interacting structure showed distances of positive charges in a range of 6 to 12 Å, whereas the charges of G protein-interacting MPX are in the range of 12 ± 3 Å. In contrast, the distances of positive charges for MPS, which is selective for Gαs, are 17 to 23 Å and 18 to 25 Å for the structures in vesicles and G proteins, respectively (Fig. 3-17).

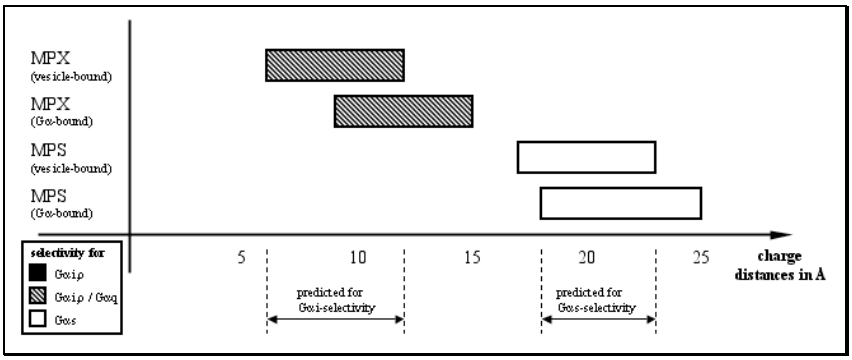


Figure 3-17: Visualization of the distributions of positive charges of the investigated peptide structures. The charge distances and the experimentally shown G protein-selectivity are comparable for MPX and MPS.

Determination of Similar Distances of Complementary Charges at Gα-subunits

Following these initial investigations and applying the information about interactions sites for G proteins (Azpiazu and Gautam, 2001; Gilchrist *et al.*, 2001; Hamm *et al.*, 1988; Kostenis *et al.*, 1998; Lambright *et al.*, 1996; Muradov and Artemyev, 2000; Natochin *et al.*, 2000; Onrust *et al.*, 1997), the available structures of Gα-subunits Gαi, Gαo, and Gαs, and the homology model of Gαq were probed regarding complementary distances of negative charges. Additionally, hydrophilic residues such as asparagines and glutamines were included into these investigations because of their possibility to accept hydrogen bonds by the ligand's positive charges, which may also be important for interaction.

Different groups of distance pairs between negative charges at Gαi, Gαq and Gαs could be identified. They are located in a common area near β6, β5 and the N-terminal helix, predicted to be the interaction site with GPCRs (Fig. 3-18). The detected distances of negative charge are classified in groups of ranges of 6 to 12 Å, 10 to 16 Å and 17 to 24 Å at the surfaces of Gαi, Gαq and Gαs, respectively.

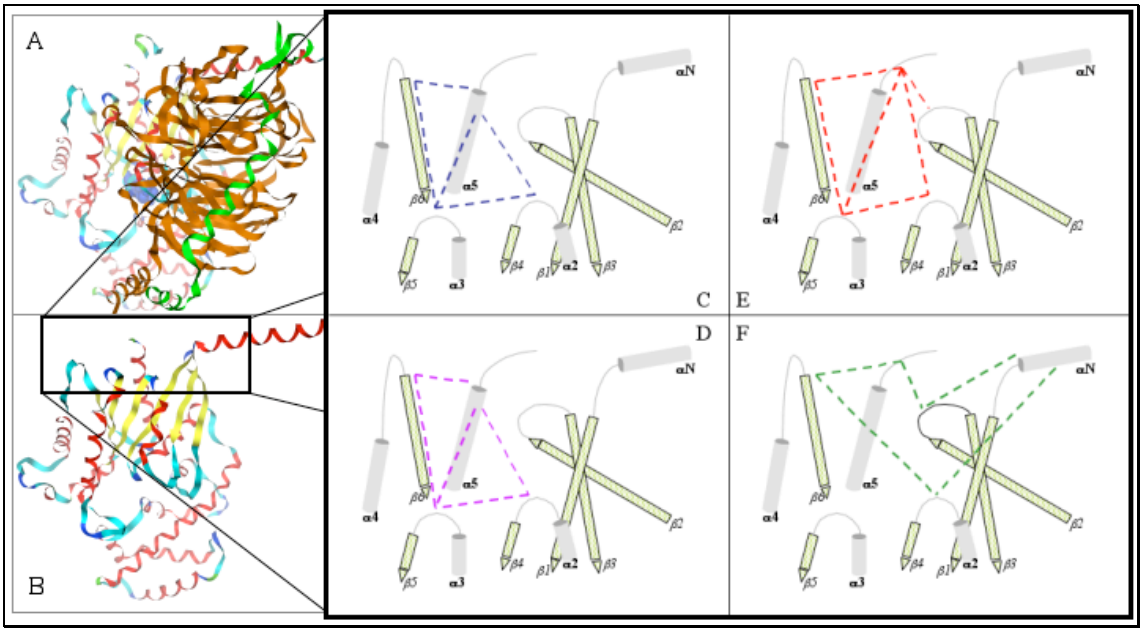


Figure 3-18: **The interaction sites in Gα-subunits show typical distance patterns of negative charges. The complex of Gα, Gβ and Gγ is shown (A) from this point of view the Gα-subunit with removed βγ-complex is shown in the other pictures (B, C, D, E, F). Studies of distances of negative charges in different Gα-subunits clearly revealed distinct distances in the neighborhood of the Gα's C-termini. The negative charges on Gαi (C) and Gαo (D) are only located at loops α2-β4, α3-β5, at sheet β6 and helix α5, leading to small charge distances (6 to 12 Å). At Gαq (E) the charges in helix α5 are shifted to the C-terminus leading to an increase to medium distances (10 to 16 Å). The additionally introduced charge at loop β2-β3 is also in this range. On Gαs (F) the distances of negative charges are medium and large (17 to 24 Å), which is based on removal of charges in loop α3-β5 and additional negatively charged residues in the N-terminal helix αN.**

Possible Interaction Site of Mastoparans Based on Distances of Complementary Charges

To study, whether these distances of negative charges are essential for interaction of ligands with G proteins, manual dockings of MPX on Gαi and MPS on Gαs were performed. The discussed positions are elements of an array, where the complementary charge distances of G proteins and mastoparans match each other (Fig. 3-19).

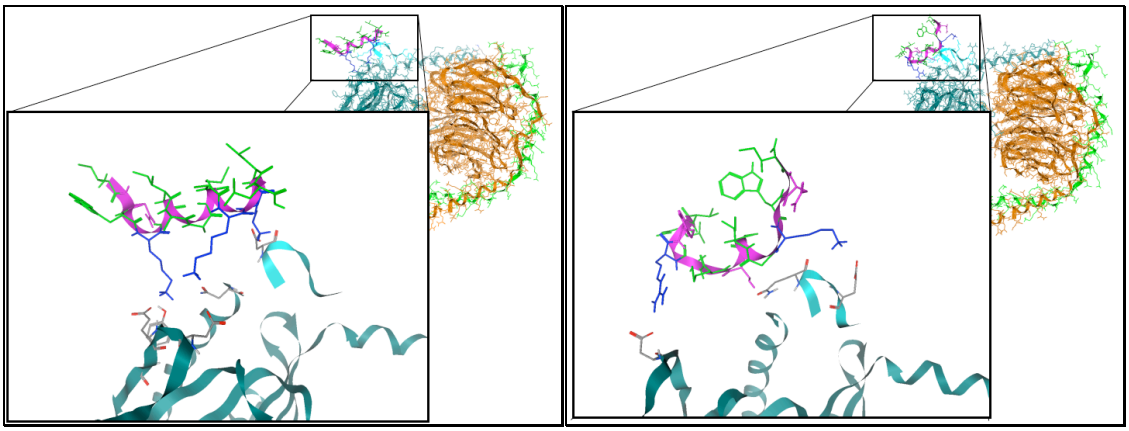


Figure 3-19: **Manually docked structures of mastoparan-X (left side) and mastoparan-S (right side) at interaction sites of Gαi and Gαs, respectively. Using above-mentioned information about complementary charge distances and interaction sites of G protein α-subunits, the G protein-interacting structures of Gαi-selective MPX and Gαs-selective MPS could be properly arranged.**

For Gαo and Gαi, which both include a C-terminal cysteine (*e.g.* Cys351 in SwissProt-entry: GBI1_HUMAN), this hypothesis was already proven experimentally. Bulky ADP-

ribosylation of Cys351 by induction with pertussis toxin (PTX) normally uncouples these G proteins from their receptors. This PTX-induced ribosylation, which is an established test for Gαi-interaction of GPCRs, also interrupts the activation of Gαi by MPX and other secretagogues (Mousli *et al.*, 1990). These findings support the hypothesis, that these peptides are interacting at the same site of Gαi as Gαi-selective GPCRs.

3.3.2 Lipoamines – Small Synthetics Selective for Different Gα-subunits

To test whether the found complementary charge distances also count as selective recognition patterns for other G protein-ligand interactions, peptidomimetics were studied, which have been also known as G protein modulators (Nürnberg *et al.*, 1999). Alkyl-substituted amino acid-derivatives – termed lipoamines – have already been described to interact with G proteins in such a distinct mode of interaction (Leschke *et al.*, 1997; Nürnberg *et al.*, 1999; Breitweg-Lehmann *et al.,* 2002). Based on existing data of G protein-selectivity of many of these compounds, searches for their conformational space were made and analyzed regarding distances of positive charges. Thereby, the charge distances of compound FU132 that was published as G protein-activating peptidomimetic (Breitweg-Lehmann *et al.*, 2002) cluster in ranges of about 10±5 Å.

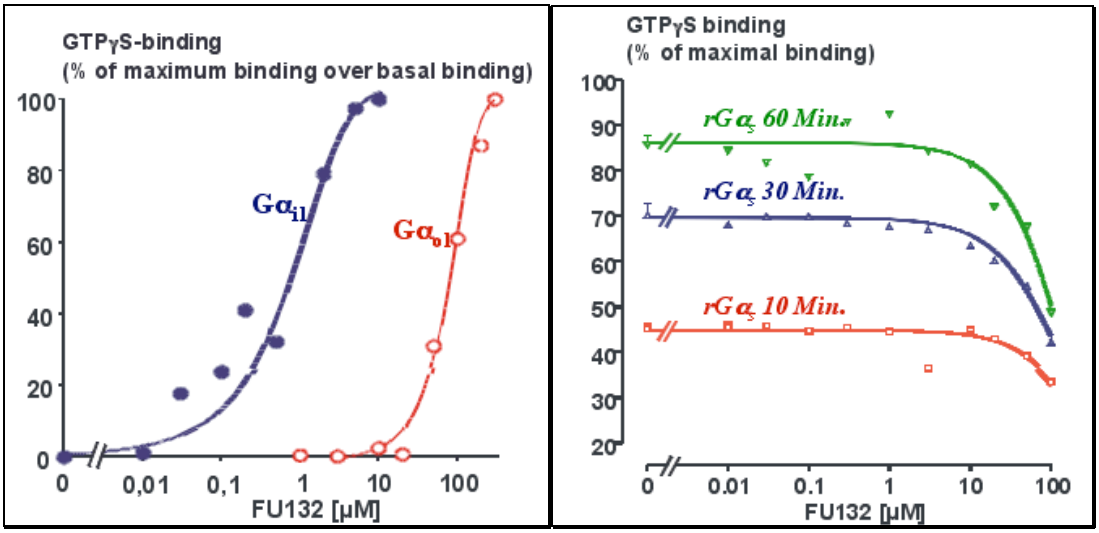


Figure 3-20: Activation of different recombinant Gα-subunits by compound FU132. Left side: Gαi1 and Gαo1 are targets for activation by FU132. Right side: Gαs cannot be activated as is shown by incubation with FU132 for 10, 30 and 60 minutes.

Following the ideas developed on MPX and other secretagogues, FU132 should be selective for Gαi. Pharmacological characterization and testing of FU132 for modulation of Gαi and Gαs confirmed these hypothesises experimentally. FU132 shows clear modulation for Gαi subunits but not for Gαs subunits (Fig. 3-20).

To provide further support for charge distances as recognition patterns in G protein-ligand interactions, FU132 was docked on Gαi as shown before for MPX on Gαi and MPS on Gαs (Fig. 3-21).

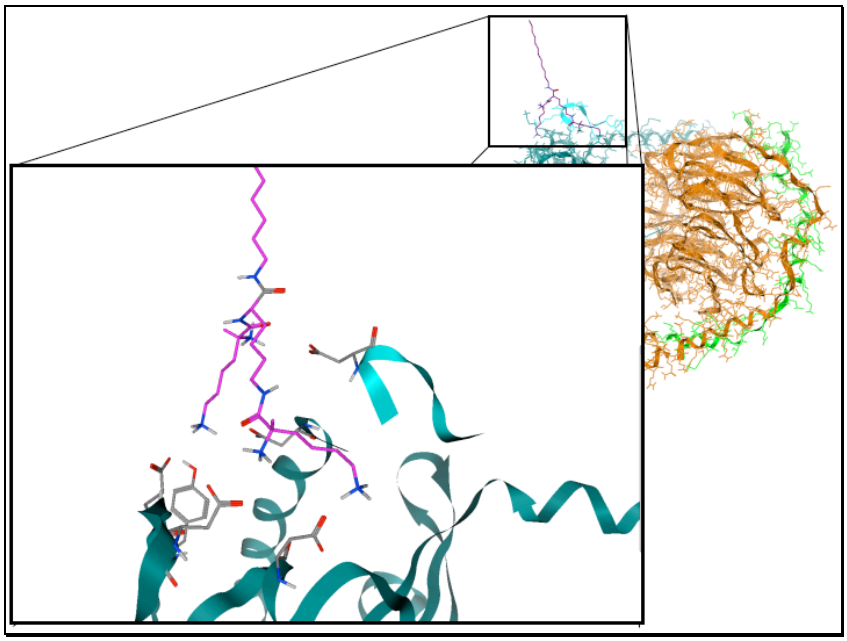


Figure 3-21: **Lipoamine compound FU132 manually docked at the ligand interaction site of Gα-subunit Gαi. The positive charges of the ligand interact with complementary negative charges at Gαi.**

Also in this part of investigation, distances of complementary charges match, revealing an interaction site on Gαi close to its C-terminus. FU132 interacts closely to the cysteine residue, which is PTX-dependent ribosylated. Using this ADP-ribosylation assay, it was demonstrated clearly that FU132 interacts on a position at Gαi, which is also used by Gαi-selective GPCRs (Breitweg-Lehmann *et al.*, 2000).

Summarizing, the hypothesis that specific distances of complementary charges code for Gα-subtypes in G protein-ligand recognition could be extended to FU132. For further proof, a new compound with predicted G protein-selectivity should be designed by narrowing-down the distance of positive charges to exclude all other Gα-subunits except Gαi. Results of MPX also indicated that hydrophobic patches in-between the positive charges may additionally favor Gαi-selectivity. The newly designed lipoamine should include a combination of small distances of positive charges and a certain amount of hydrophobicity. Hence, on the base of FU132 different combinations of aliphatic chains, aromatic rings and positively charged groups were investigated regarding their conformational space (Fig. 3-22) and charge distances (Tab. 3-5).

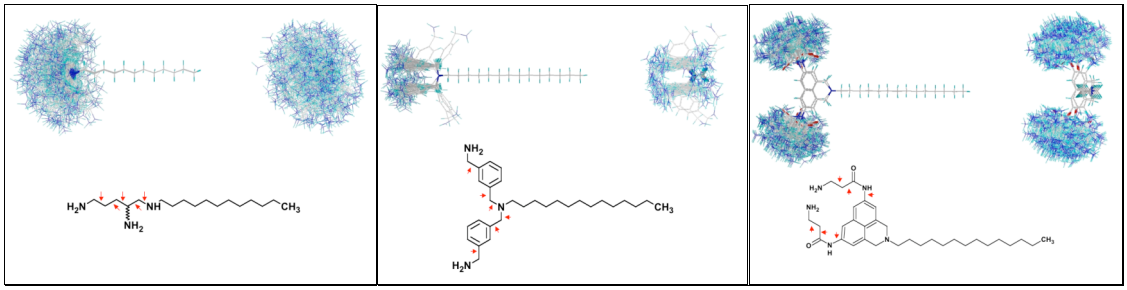


Figure 3-22: Ensembles of molecules from the systematic search within SYBYL6.8. Shown are examples for lipoamines: FUB86 (left), FUB 244 (middle) and FUB 295 (right). FUB86, which unselectively activates Gαi and Gαq, shows positive charges everywhere in its conformational space. In contrast, Gαi-selective FUB244 is highly restricted by aromatic interactions to narrow distances of positive charges. FUB295 is somehow restricted for larger distances and it was experimentally shown not to interact with Gαi (data not shown).

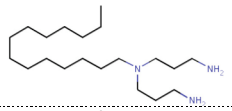
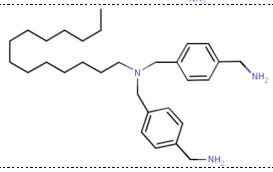
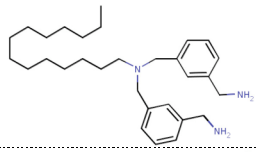
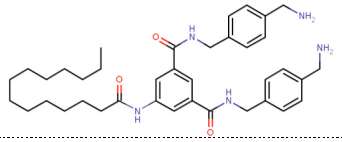
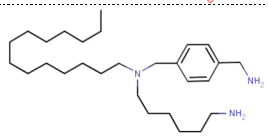
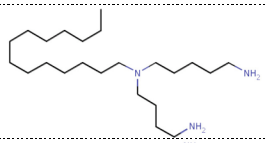
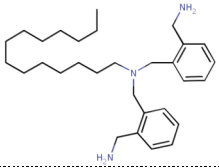
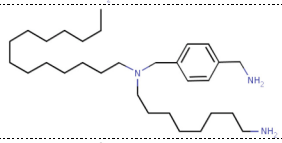
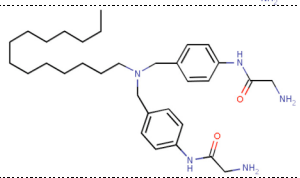
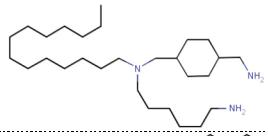
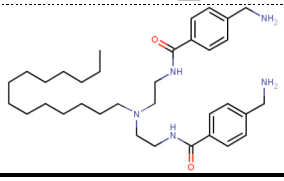
Comparisons of the studies of the conformational space and charge distance distributions with experimental results – ligands were synthesized in the group of Walter Schunack, Freie Universität Berlin and tested by the group of Bernd Nürnberg, Heinrich Heine Universität Düsseldorf – clearly demonstrated that reaching Gα-subtype selective distances is the most prominent but not the only feature in the mechanism of G protein-interaction.

The best ligand from our predictions was FU244, which was predicted to interact with Gαi because of small distances of its positive charges and their very restrictive spatial distribution. The latter may be based on π-stacking interactions of two aromatic rings. The experimental results on FU244 show interaction with G proteins of Gαi-type only. By this, that compound is confirmed as the best Gαi-interacting lipoamine and, thereby, it verifies our hypothesis about small charge patterns for selectivity regarding Gαi.

On the other hand, comparison of the distance ranges of positive charges of compounds FU248 (4 to 20 Å) and FU259 (4 to 17 Å) does not fully explain the experimental results regarding Gαi-interactions (Tab. 3-5). While both compounds show similar distributions of positively charged groups, their interaction modes with Gαi are not comparable. The IC_{50} for interaction with Gαi of FU259 is 0.5 µM and yet FU248 is not able to interact with Gαi.

Hence, the conformational searches have shown additional hints for better characterization of small G protein-activating ligands. For example, applying the charge distance of the lowest energy structures as additional information was a useful tool to weigh up the overall charge distances regarding their Gα-subtype selectivity. In case of above-mentioned example, the lowest energy model of FU248 has a charge distance of 13 Å that is too broad for interactions with Gαi, whereas the charge distance of FU259 of 6 Å is within the typical range of Gαi-selectivity (Tab. 3-5).

Table 3-5: Selection of studied lipoamine compounds. Range of charge distances combined with charge distance of lowest energy structure (both based on conformational space searches) give good indications for Gα-selectivity as demonstrated by experiments regarding Gαi -interaction.

Compound	Structural Formula	Range of Charge Distances [Å]	Charge Distance of Lowest Energy Structure [Å]	IC_{50} [µM]
FU220		4 – 15	6	50
FU244		3 – 7	6	0.14
FU245		3 – 13	7	0.24
FU248		4 – 20	13	NO EFFECT
FU250		5 – 16	6	1.2
FU257		4 – 13	7	5.0
FU258		4 – 10	7	0.3
FU259		4 – 17	6	0.5
FU260		4 – 7	4	0.6
FU261		5 – 15	11	13
FU274		4 – 7	4	0.2

After all, restricting the possibilities of charge patterns by elements of rigidity, such as intramolecular π-stacking interactions of aromatic rings (see FU244 in Tab. 3-5, Fig. 3-22)

and supporting hydrogen bonds by other nearby functional groups, additionally increases the selectivity for a certain Gα-subtype, such as Gαi.

Taken together, also small non-peptidic lipoamine compounds show selectivity for Gα-subunits based on different Gα-dependent distances of positive charges. Additionally, comparisons of more aromatic compounds (*e.g.* FU250) with similar compounds lacking aromaticity (*e.g.* FU261) demonstrated that aromatic rings and/or highly hydrophobic moieties are increasing the selectivity for Gαi (Tab. 3-5).

As a conclusion, distances of complementary charges on G proteins and their ligands are a recognition signal, whereas additional ligand features based on hydrophobic and/or aromatic interactions may increase the affinity and, thereby, the effects on signalling.

As a result, lipoamines also revealed specific distances of positive charges as patterns of G protein-selectivity: for Gαi distances below 12 Å with an optimum of about 6 Å; for Gαs large distances above 17 Å and for Gαq distances in between 12 and 17 Å.

3.3.3 Gα-Subtype Specific Differences at Endothelin-Receptor Subtypes A and B

To support the hypothesis of complementary charge distances as selective recognition patterns for G protein-subtypes, we wanted to test, whether this can also be assigned to GPCR subtypes with different affinities for Gα proteins. The endothelin-receptor subtypes are known to interact with different Gα-subtypes. ETA favors interaction with Gαq and Gαs, whereas ETB prefers interaction with Gαi and Gαq. It has to be noted that the Gαs-interaction of ETA is not completely accepted, since it has only be found in CHO cells. Because of this, ETA and ETB were investigated for selectivity to Gαq and Gαi, respectively.

Already, first sequence comparisons of both receptor subtypes revealed different distributions of positively charged residues within their intracellular loops ICL1, ICL2, ICL3 and their C-termini (Fig. 3-23).

Intracellular loops ICL1 and ICL2, which share the same length, differ in distribution of positive charges remarkably. So, ETB comprises three positively charged residues (Lys128 in ICL1 plus Lys210 and Lys216 in ICL2) those positions are uncharged in ETA (Gln107 in ICL1 plus Gln194 and Leu200 in ICL2). At ICL3 exchange of Asn284 in ETA to Arg302 in ETB is somehow buffered by simultaneously modification of Arg291 in ETA to Gln308 in ETB.

The receptor models, which are C-terminally truncated to Cys386 (ETA) and Cys403 (ETB), include the cytoplasmic C-terminal helix 8. This helix is attached to the membrane by palmitoylation of cysteine residues. It includes two additional positive charges per receptor,

which could be possible interaction partners for G proteins. Nevertheless, the positions of these charges are unaltered (Fig. 3-24).

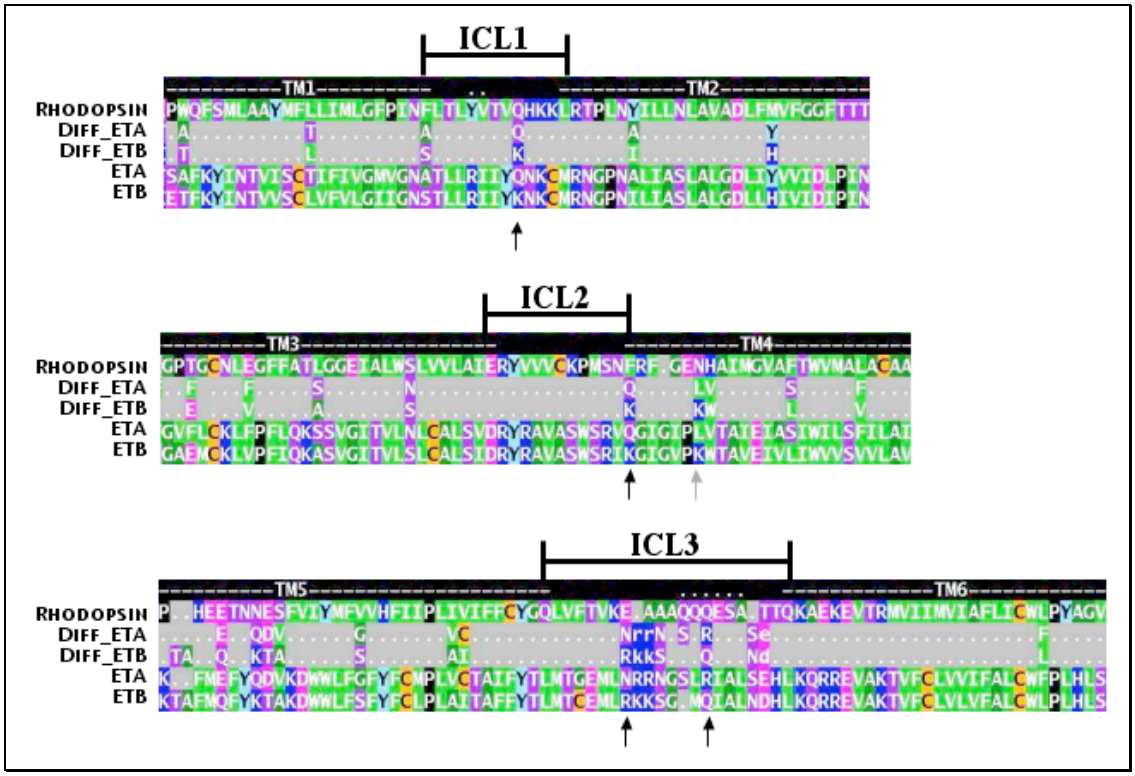


Figure 3-23: **Sequence alignments focusing on intracellular loops ICL1, ICL2 and ICL3 of human ETA and human ETB sequence against rhodopsin. Four residue exchanges modify the distribution of positive charges within the intracellular loops (black arrows) and an additional exchange appears in nearby intracellular portion of transmembrane helix TMH4 (grey arrow). The sequences shown are (top down): Rhodopsin – bovine rhodopsin, Diff_ETA – residues of ETA differing from ETB, Diff_ETB – residues of ETB differing from ETA, ETA – human ETA, ETB – human ETB**

The loop structures of ETA and ETB structure models – especially their lengths – are almost identical (Fig. 3-24). But Gαq-selective ETA presents less positive charges in its intracellular regions than Gαi-selective ETB. As a consequence, some distances between positive charges on ETA have to be larger than those of ETB (Fig. 3-24). This is consistent with the hypothesis of charge distance-dependent patterns for Gα-selectivity based on investigations of secretagogues and lipoamines.

To proof this recognition pattern concept of Gα-subtype selectivity also for GPCRs, several positive charged residues were chosen for site-directed mutagenesis. Regions that were already described as sensitive for Gα-selectivity at endothelin-receptor subtypes were taken into account for charge distance calculation but not for mutational studies. The described regions include sequence Met132-Arg133-Asn134 in ICL1 of ETB in interaction with Gα13 (Liu and Wu, 2003), sequence Leu311-Lys312-Gln313-Arg314-Arg315-Glu316-Val317-Ala318 at the intracellular side of TMH5 at ETB interacting with Gαq/1 and Gα12/13 (Liu and Wu, 2003), and the C-terminal helix 8 of ETB (Aquilla *et al.*, 1996).

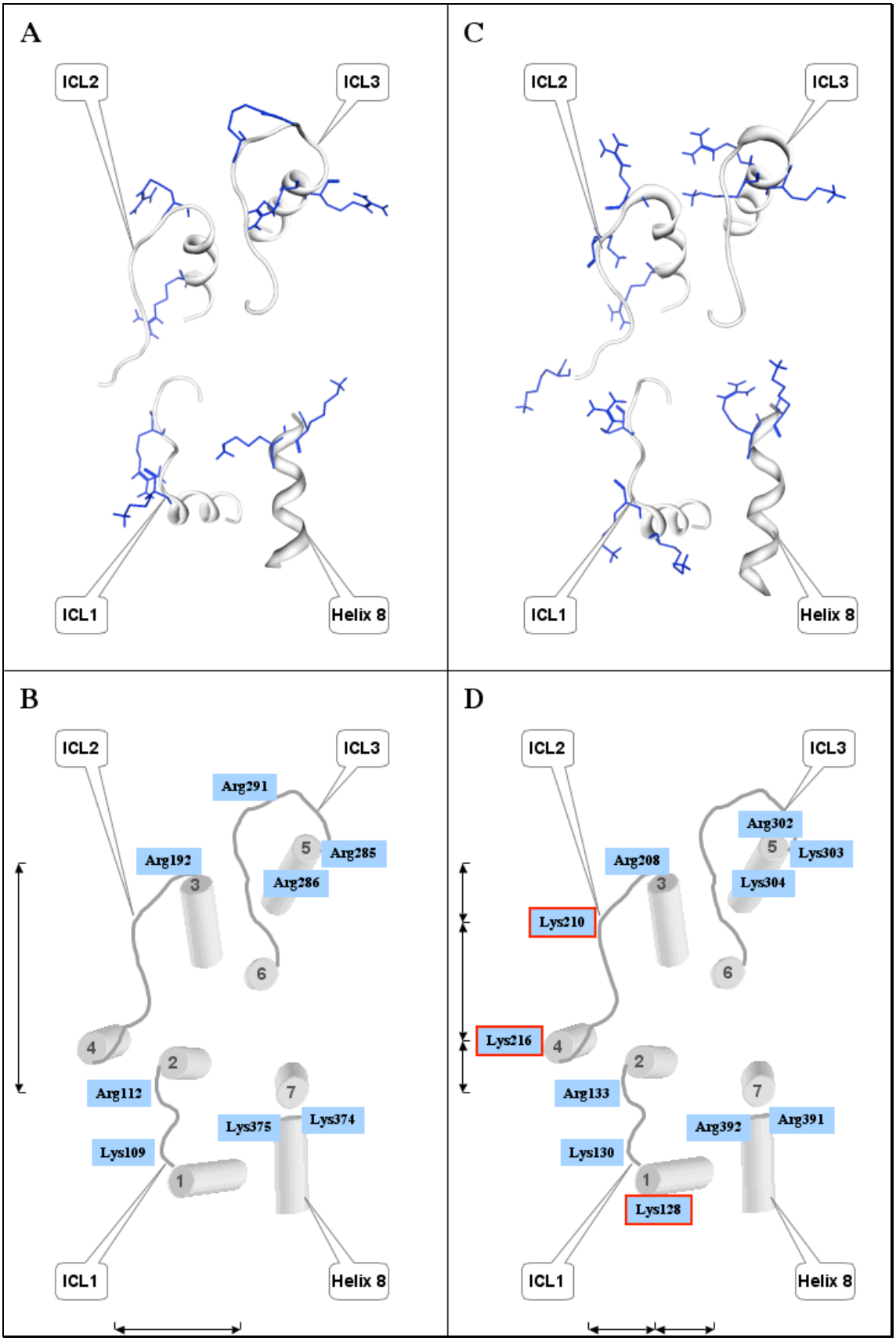


Figure 3-24: **Bottom view of intracellular loops ICL1-3 and C-terminus of ETA and ETB (A and C). For improved clarity both receptor subtypes are also shown as cartoons (B and D). Positively charged residues in ETA and ETB are highlighted in A and C and annotated in blue boxes in B and D, respectively. Marked boxes are basic residues only available in ETB (D). Resulting differences in charge distribution are highlighted by arrows at the left and at the bottom of cartoon images B and D.**

Additionally, chimera studies using loop exchanges clearly demonstrated ICL2 and ICL3 as a major determinant in selective coupling of endothelin receptors with Gαs and Gαi (Takagi *et al.*, 1995).

The charge distances between all intracellular basic residues were collected and compared between both receptor subtypes. Different patterns were observed in intracellular loops ICL1 and ICL2. As a result, several amino acids were selected for site-directed mutagenesis (Tab. 3-6) and validated experimentally (by cooperation with Alexander Oksche, FU Berlin; Jens Furkert, FMP).

On ETA, Gln107 (ICL1) and Leu200 (ICL2) were chosen for mutation to the corresponding residues of ETB to shift the Gα-subtype selectivity to Gαi. Similarly, mutation of Lys128 (ICL1), Lys210 (ICL2) and Lys216 (ICL2) in ETB should shift its Gα-selectivity to Gαq. Additional alanine mutations of Arg192 (ICL2) in ETA and Lys130 (ICL1) in ETB were predicted to abolish the selectivity for Gαq and Gαi, respectively (see Table 3-6).

Table 3-6: **Predicted Mutations for Modification of Gα-selectivity at ETA and ETB.**

			Mutant	Expected Effect
ETA	*ICL1*		Gln107→Lys	Increase of GαI (Lys128 in ETB)
		ICL2	Gln107→Lys Leu200→Lys	Increase of GαI (Lys128 and Lys216 in ETB)
			Leu200→Lys	Increase of Gαi (Lys216 in ETB)
			Arg192→Ala	Decrease of Gαq (Removal of positive charge)
ETB	*ICL1*		Lys128→Gln	Decrease of GαI (Gln107 in ETA)
			Lys130→Ala	Decrease of GαI (Removal of positive charge)
		ICL2	Lys128→Gln Lys130→Ala Lys216→Leu	Decrease of GαI (Gln107, Leu200 in ETA and removal of positive charge)
			Lys210→Gln	Decrease of GαI (Gln194 in ETA)
			Lys216→Leu	Decrease of GαI (Leu200 in ETA)

Site-Directed Mutagenesis Studies on Gα-Subtype Selectivity of Endothelin Receptor Subtypes ETA and ETB (Preliminary Results by Work of A. Oksche, J. Furkert)

Mutants of the endothelin receptor subtypes that include predicted residue exchanges (Tab. 3-6) were cloned and transiently expressed in COS-M6 cells by Alexander Oksche (Institut für Pharmakologie Charité - Campus Benjamin Franklin, 14195 Berlin, Germany) and Jens Furkert (Cellular Signalling, FMP Berlin, 13125 Berlin-Buch). G protein-selectivity was studied by assays on [^{3}H]-Inositol-1,3,4-triphosphate (IP3) formation.

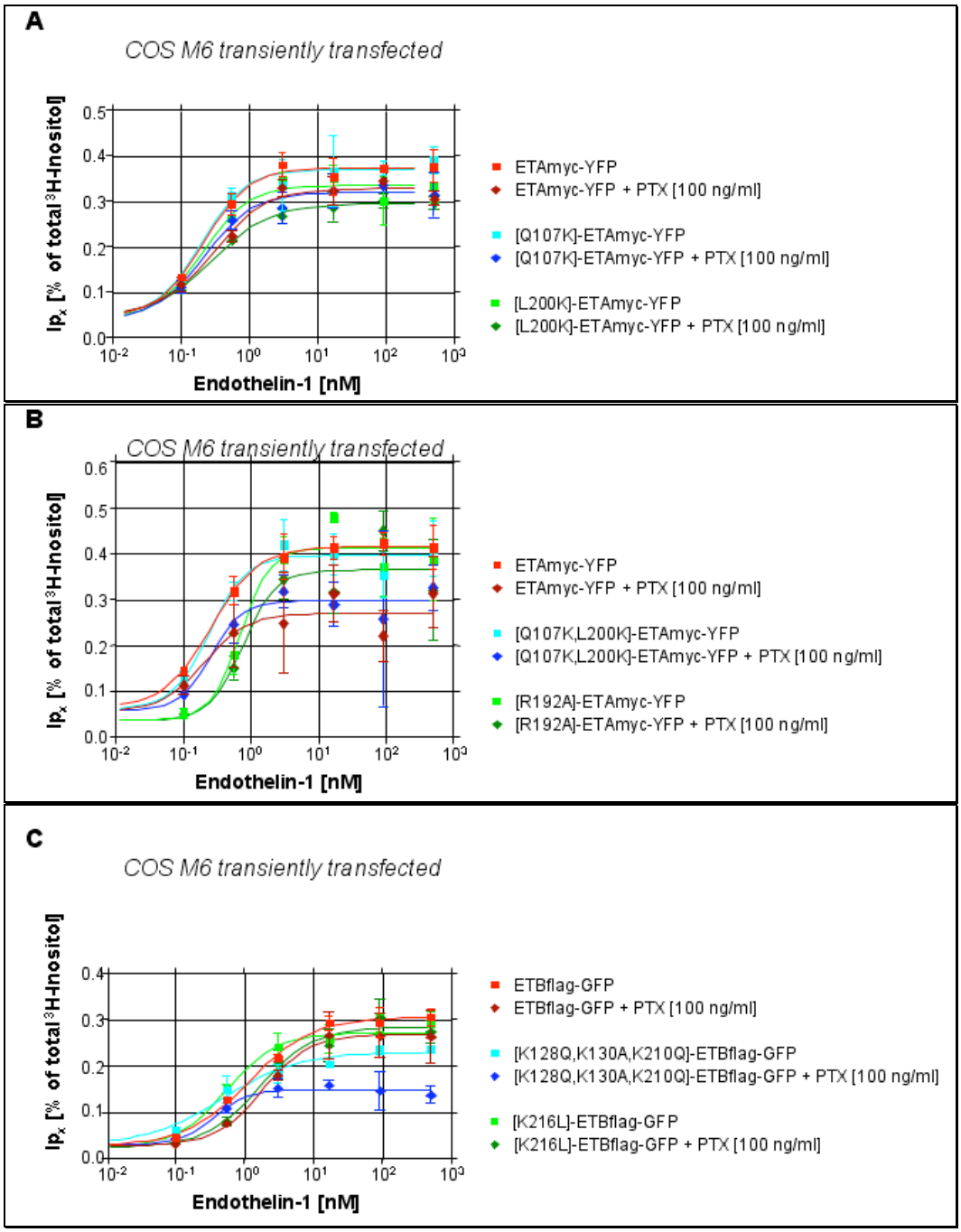


Figure 3-25: Preliminary experimental results of Gα-selectivity of predicted endothelin receptor mutants. Wild-type receptors and mutants were tested both with and without Pertussis-Toxin to separate the amount of total ^{3}H-Inositol formation induced by Gαi.

In ETA, the myc-YFP-tagged wild-type receptor was compared with myc-YFP-tagged mutants Gln107→Lys, Leu200→Lys (Fig. 3-25a) as well as Arg192→Ala and the double mutant Gln107→Lys / Leu200→Lys (Fig. 3-25b). The flag-GFP-tagged wild type of ETB was compared with Lys216→Leu and the triple mutant Lys128→Gln / Lys130→Ala / Lys210→Gln (Fig. 3-25c). All receptors showed normal expression and localization at the cell membrane. As already described in investigations on secretagogues and lipoamines, Gαi-

sensitivity of PTX was used to abolish the effects of Gαi to estimate the amount of IP3 formation induced by this Gα-subtype.

Both, ETA and ETB show sensitivity on the PTX-induced abolishment of Gαi-based IP3 formation. But ETA mutants Gln107→Lys, Leu200→Lys and Gln107→Lys / Leu200→Lys do not. The graphs of ETA mutant Arg192→Ala are shifted to the right and indicate importance for interaction with Gαi.

Both investigated mutants of ETB act differently towards the wild-type receptor. The triple mutant Lys128→Gln / Lys130→Ala / Lys210→Gln demonstrated more influence by Gαi on formation of IP3 than wild-type ETB, whereas Lys216→Leu seems to abolish the interaction with Gαi.

A comparison of the experimental results of predicted mutations is shown in Table 3-7.

Table 3-7: Comparison of predicted and experimental results of predicted endothelin receptor subtype mutants.

Receptor Mutant	Predicted Effect	Maximal activity IP_x [% of total ^{3}H-inositol] without PTX	Maximal activity IP_x [% of total ^{3}H-inositol] with PTX	Result
ETAmyc-YFP		0,415 [CI_{95} 0,395 – 0,435]	0,269 [CI_{95} 0,219 – 0,319]	
[Q107K] ETAmyc-YFP	Increase of Gαi	0,370 [CI_{95} 0,345 – 0,395]	0,3195 [CI_{95} 0,300 – 0,339]	No difference in Gαi-sensitivity
[L200K] ETAmyc-YFP	Increase of Gαi	0,334 [CI_{95} 0,314 – 0,355]	0,295 [CI_{95} 0,283 – 0,307]	No difference in Gαi-sensitivity
[Q107K,L200K] ETAmyc-YFP	Increase of Gαi	0,396 [CI_{95} 0,368 – 0,424]	0,298 [CI_{95} 0,250 – 0,345]	No difference in Gαi-sensitivity
[R192A] ETAmyc-YFP	Decrease of Gαq	0,412 [CI_{95} 0,372 – 0,452]	0,364 [CI_{95} 0,317 – 0,412]	Decrease of Gαi
ETBflag-GFP		0,305 [CI_{95} 0,291 – 0,319]	0,269 [CI_{95} 0,242 – 0,295]	
[K128Q,K130A,K210Q] ETBflag-GFP	Decrease of Gαi	0,230 [CI_{95} 0,215 – 0,246]	0,150 [CI_{95} 0,138 – 0,162]	Increase of Gαi
[K216L] ETBflag-GFP	Decrease of Gαi	0,271 [CI_{95} 0,252 – 0,291]	0,284 [CI_{95} 0,261 – 0,308]	Gαi not existing

These experimental results clearly demonstrate that both endothelin receptor subtypes ETA and ETB interact with Gαi and Gαq. Additionally, three residues were detected, which have remarkable influence on selectivity to Gα-subtypes. Within ETA, the removal of the positive charge at position Arg192 by mutation to alanine led to a decrease of the receptors selectivity for Gαi. In ETB, the triple mutant Lys128→Gln, Lys130→Ala, Lys210→Gln resulted in increased Gαi-selectivity, whereas the removal of Lys216 by mutation to leucine abolished interaction with Gαi.

The additional mutant ETBflag Arg208→Ala (comparable to ETAmyc Arg192→Ala) has still to be generated and tested.

Conclusions about Gα-subtype selectivity at endothelin receptor subtypes

By identification of several residues critically involved in an interaction with Gαi, we could demonstrate that our observations of GPCR-G protein interaction induced by complementary charge patterns generally exist. The positive charge distances within GPCRs are recognized by patterns of negative charges at G proteins.

First results of the still on-going experimental investigations clearly demonstrate an involvement of intracellular loop ICL2 in interaction of endothelin receptors with Gαi. In ETA, for the mutant Arg192→Ala the interaction with Gαi was decreased, while the ETB mutant Lys216→Leu was not able to interact with this Gα protein-subtype anymore.

The latter mutant is interesting because the *vice versa* mutant of ETA (Leu200→Lys) did not show any significant alteration in G protein-interaction. Because of this, one can assume that both endothelin receptor subtypes – although homologous receptors with similar length of their intracellular G protein-interacting domains – use interfaces for Gα protein-selectivity that are not based on the same intracellular domains. Another interesting mutant for this is the triple mutant of ETB including Lys128→Gln, Lys130→Ala, Lys210→Gln. Two of these residues (Lys128, Lys210) were exchanged by the complementary amino acids of the Gαi-unselective receptor subtype ETA, the resulting ETB mutant showed increased Gαi-selectivity. This clearly indicates different patterns of G protein-selectivity at the homologous receptor subtypes ETA and ETB.