GIP receptor

GIP receptor: expression in neuroendocrine tumours, internalization, signalling from endosomes and structure-function relationship studies

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Introduction

GIP is well known as a peptide regulating metabolic functions. Here, we first summarize our works on expression of GIPR in neuroendocrine tumours and then present molecular and signalling aspects of this receptor, in particular, the mechanism of GIPR internalization, GIPR signalling from early vesicles of internalization, identification of the GIP binding site, identification of functional elements of the transmembrane domain associated with GIPR activation and its coupling to G proteins.

GIPR expression in neuroendocrine tumours: diagnostic and therapeutic potential

There is a more than 30 years history of gut hormone receptors known to be expressed in specific human cancers. For instance, somatostatin receptors can be massively overexpressed in a large variety of neuroendocrine tumors, and these receptors were found subsequently to be ideal targets for radiolabeled somatostatin analogs, both for tumor diagnosis and therapy. However, not all neuroendocrine tumors express the somatostatin receptors in a sufficient density for therapeutic intervention. Therefore, other peptide receptors came into the focus of recent research and were found to be overexpressed in specific cancers. Specifically, the GIPR have been identified in the past few years in human pathologies, particularly, in tumors. This finding may be of medical interest since these receptors may be specific targets for drug applications, similar to the somatostatin receptors. This paragraph summarizes the currently available data on the expression of the GIP receptor.

GIPR can be overexpressed in a variety of human tumors, as shown with *in vitro* receptor autoradiography techniques [1]. Indeed, impressive GIP receptor expression is found in functional pancreatic neuroendocrine tumors (NET), such as insulinomas and gastrinomas, as well as in nonfunctional pancreatic NET and ileal NET. They are also found in most bronchial NET. The high receptor content in NET contrasts with the low physiological GIP receptor expression in corresponding healthy human tissues, with only a few gastrointestinal tissues showing measurable receptor quantities, primarily the islets of the pancreas, where GIP exerts its main physiological action. A strong proof for the identification of specific GIPR in tumors and physiological target tissues is not only given by the clear pharmacological binding behavior in the autoradiography study, in both tumoral and physiological GIP target tissues, but also by the results at the mRNA level using RT-PCR technology in the same tissues [1].

Quite unexpectedly, the GIPR were also identified in another type of tumor, in human thyroid C-cell neoplasia [2]. Indeed, the great majority, namely 89%, of human medullary thyroid carcinomas massively overexpress GIPR. Since none of the GIPR are found in the normal thyroid, the GIP receptor expression may be directly linked to the neoplastic transformation. Conversely, the epithelial and stromal gastrointestinal tumors, including gastric, colonic, and hepatocellular carcinomas, cholangiocarcinomas, and gastrointestinal stromal tumors, as well as lung adenocarcinomas are usually GIP receptor-negative. However, 26% of pancreatic adenocarcinomas express the GIPR [1].

A significant result of our study [1] that compared GIP receptor expression with the somatostatin receptor expression in NET is that the GIP receptor incidence often compared advantageously with the incidence of somatostatin receptor expression in this particular group of gastroenteropancreatic and bronchial NET [1]. Of particular interest is that somatostatin receptor-negative tumors often retain the GIPR; this may be of clinical significance, as GIP receptor imaging and therapy may be developed that may replace or complement somatostatin receptor imaging and therapy in these tumors. Other interesting cases are the insulinomas: the GIPR are expressed not only in all benign insulinomas, including the somatostatin receptor-negative ones but also in malignant insulinomas, known to often lack another gut peptide receptor, the GLP-1 receptor. As such, GIP receptor imaging may be considered an almost universal marker for NET. For comparison, medullary thyroid carcinomas have a very high GIP receptor expression and incidence, while somatostatin receptor expression and incidence in this type of tumors is very low, making a clinical application of GIP analogs in medullary thyroid carcinomas particularly interesting, as there are presently poor diagnostic and therapeutic options in this type of cancer.

In a further study [3], comparing the GIPR and somatostatin receptors with the MIB-1 proliferative index, a marker for tumor stage, in a selection of high somatostatin receptor and GIP receptor expressing gut and lung NETs, it was found that the GIPR, but not the sst2 somatostatin receptors, correlated with the MIB-1 index. GIP receptor levels gradually increased in a subset of insulinomas and nonfunctioning pancreatic NET, and decreased in ileal and bronchopulmonary NET with increasing MIB-1 rate. MIB-1 levels were identified, above which GIP receptor levels were consistently high or low. These MIB-1 levels were different from those defining tumor grade. In grade 3 NET, GIP receptor levels were always low, while sst2 somatostatin receptor levels were variable and sometimes extremely high [3].

As mentioned above, the high GIP receptor expression in specific NET and low expression in normal tissues represent the molecular basis for an *in vivo* NET targeting for diagnostic and therapeutic purposes. Although this is true for those tumors expressing solely the GIPR, the frequent concomitant expression of the GIPR with the somatostatin receptors and even the GLP-1 receptors in many NET suggest also the possibility of multiple receptor targeting of the respective tumors [4]; one may conceive injections of a cocktail with established radiolabeled somatostatin analogs and GLP-1 analogs, together with putative GIP analogs, that may be an attractive possibility for a potent tumor imaging and targeted radiotherapy of NET. A recent *in vitro* autoradiography study showed that this could become reality since the *in vitro* targeting of gut and lung NETs with a cocktail of ^{125}I-Tyr^{3}-octreotide, ^{125}I-GLP-1(7-36 amide) and ^{125}I-GIP(1-30) permitted the detection of all tested tumors [5], whereas the single radioligands, applied alone, never detected all tumors.

At the present time, there are no clinical investigations ongoing using radiolabeled GIP in NET patients, which would support the presented *in vitro* data in human tumor tissues. However, a recent *in vivo* biodistribution study [6] in nude mice showed that ^{111}In- or ^{68}Ga-DOTA-conjugated

GIP(1-30) derivatives (EG4) could specifically target the GIP receptor-expressing INR 1G9-hGIPr xenografts. This proof-of-principle study in animals indicates the feasibility of imaging the GIPR in tumors, opening new avenues for GIP theranostics in patients with NET. However, in order to exploit the GIPR as a therapeutic target, new GIP receptor ligands with less kidney uptake will have to be developed.

Mechanism of internalization of the GIPR

Endocytosis or internalization of receptors is a physiological process, which can be useful for delivery of drug within cell interior and, which makes tumor treatment by radiolabeled ligands more efficient. In general, activation of membrane G-protein coupled receptors by natural or pharmacological ligands, including therapeutic agents, is followed by biochemical events leading to signal desensitization and receptor internalization. Among these biochemical events, there is rapid receptor phosphorylation on Serine and/or Threonine by kinases (PKA, PKC) activated by a second messenger, and by specialized kinases named GRK (G protein Receptor Kinases) [7-10]. Then, phosphorylated GPCRs recruit scaffolding proteins such as β-arrestins, which initiate internalization of receptors and, in addition, serve as adaptators for activation of signalling proteins, especially those of the MAP kinases pathway. Internalization of GPCRs occurs through invagination of the plasma membrane, most often at clathrin-coated pits with the participation of another scaffolding protein, adaptin 2 (abbreviated AP2). The internalization vesicles are then separated from the membrane by the action of a mechano-protein, dynamine, leading to the formation of early endosomes. Finally, the internalized GPCRs are directed to lysosomes, to be degraded, or to recycling vesicles, to reappear on the cellular surface [7-10]. GPCRs therefore follows a spatio-temporal dynamic regulated by their agonist ligands.

Concerning GIPR, so far, regulation of its presence at the cell surface in tissues where it is expressed remains poorly understood. Thus, although exposure of pancreatic islet cells to GIP has been shown to produce homologous desensitization of the GIPR, the impact of GIPR internalization and trafficking on GIP response has not been investigated in detail yet [11, 12].

These facts, together with the renewed interest for the development of GIP analogues of therapeutic value, led us to study internalization of the GIPR following pharmacological stimulation by GIP, as well as the cellular and molecular underlying mechanisms. These studies were carried out in HEK cells, a reference cell model for receptor internalization analysis, and published in 2015 [11].

In the experiments, GIPR was traced using fluorescent irreversible labeling of the GIPR with AlexaF647-GIP or with the GFP tag, and GIPR internalization was observed by confocal microscopy. We found rapid and abundant internalization of the GIPR immediately after GIP stimulation. Once internalized, GIPR poorly recycles to the cell surface but rather was targeted to late endosomes and lysosomes. Furthermore, converging results obtained with chemical inhibitors (chlorpromazine or fillipin) and GFP tagged proteins (Lca clathrin-eYFP or caveolin1-GFP) of both clathrin-coated pits

and caveolae support that internalization of the GIPR occurs mainly through clathrin-coated pits. GIPR internalization was strongly diminished and delayed by a dominant-negative or a chemical inhibitor of dynamin, indicating the involvement of the GTPase in the fission of GIPR-containing endocytosis vesicles from the cell plasma membrane.

As for molecular events linking GIPR activation to its subsequent targeting to clathrin-coated pits, several lines of experimental evidence support that GIPR internalization does not require β-arrestins. First, both confocal microscopy and BRET studies were unable to show recruitment of β-arrestin 1 or 2, whereas parallel experiments showed this recruitment to the CCK2R used as a reference receptor recruiting β-arrestins [13]. Moreover, elimination of phosphorylatable amino acids by truncation of the C-terminal region of GIPR did not affect internalization of the GIPR. On the other hand, the participation of the AP-2 complex in GIPR internalization was seen. In absence of involvement of β-arrestins in GIPR internalization, additional works are required to identify the component and mechanism by which activated GIPR is targeted to AP-2 complex for its endocytosis.

Therefore, our results on GIPR internalization in HEK cells differ from those reported in transfected 3T3L1 adipocytes [14]. In the last study, it was reported that GIPR constitutively internalizes and recycles to the cell surface and GIP slows GIPR recycling without affecting the kinetics of GIPR internalization. We have no explanation for such different findings excepted that GIPR, like other G-protein coupled receptors, could behave differently according to the cell context [15]. On the other hand, it is interesting to compare the behavior of the GIPR with that of the GLP1R. Indeed, although these two receptors are highly homologous in structure and functions, their insulinotropic responses are differently affected in diabetics. Studies in insulinoma and HEK cells both showed that GLP1 rapidly internalizes in response to its natural agonist but also rapidly recycles to the cell surface [16, 17]. Furthermore, GLP1 was shown to recruit GRK2 and β-arrestin2 upon activation [12]. Thus, with respect to recycling to the cell surface and molecular mechanisms involved in internalization, GIPR seems to differ from GLP-1R. These contrasting features might contribute to the distinct behavior of the two incretin receptors in diabetic humans. It is worthy to note that rapid and abundant internalization of the GIPR found in HEK cells was also found in tumor pancreatic endocrine cells thus opening the possibility of tumor imaging and eradication using radiolabeled GIP [6].

Internalized GIP receptor stimulates adenylyl cyclase on early endosomes

Considering GPCRs subjected to agonist-induced internalization, it was considered, until recently, that only the pool of receptors located at the plasma membrane could trigger G-protein-dependent signalling, and that once internalized, the receptors became unable to produce a signal and therefore became biologically “silent”. This classic concept was challenged and refuted based on experimental arguments initially published in 2009 for the parathyroid hormone receptor (PTHR). Then, a series of elegant works, with different receptors, confirmed this new view whereby

internalized GPCRs can continue to signal. Concomitantly, it has become clear that the cAMP released in different cell compartments does not necessarily produce the same biological effects [18-22]. These data, together with ours showing that GIPR is rapidly internalized following activation, led us to investigate whether internalized GIP stimulates cAMP production in early endosomes.

The study, published in 2016 [23] required a battery of tools and methods used to act on the internalization of the GIPR or to measure the production of cAMP and the activation of the PKA in different cell compartments. These are mentioned in Figure 1, where internalization process of GIPR and its intracellular fate are also indicated: - (1) the fluorescent analogue of the GIP, DY647-GIP. DY647-GIP, by binding to the GIPR, allowed to visualize by confocal microscopy, the GIPR on living cells. The study of the kinetics of co-location between DY647-GIP and GIPR (marked by a fluorescent fusion protein) revealed that the ligand, DY647-GIP, and GIPR remain associated for at least two hours after the start of stimulation. The DY647-GIP thus enabled the localization of GIPR on the surface and inside the cells, in the internalization vesicles; - (2) the Bret probe, Rluc-Epac1-citrine or the Fret probe, EYFP-Epac1-ECFP, were used to measure the total production of cAMP; - (3) the plasmids coding for DsRed fluorescent proteins Rab5 or GFP-EEA1 allowed to label early endosomes. - (4) The pharmacological inhibitor of dynamin blocked internalization of GIPR and - (5) the plasmid coding for Dsred-DN-Rab7, the dominant-negative of Rab7, blocked intracellular traffic of internalized receptors at the early endosomal stage [24]. - (6) The activity of GIPR on early endosomes was detected, on the one hand, using a nanobody fused with GFP and recognizing the active form of the Gs alpha subunit [25], and, on the other hand, - (7) by means of a cAMP sensor specifically located on early endosomes (abbreviated: FYVE-EPAC FRET). This cAMP probe is composed of EPAC1 protein, known to bind cAMP, with the N- and C-terminal ends fused with EYFP and ECFP fluorescent proteins. Illumination of cells expressing this chimeric protein, EYFP-EPAC1-ECFP, produces a FRET signal having an intensity which depends on cAMP concentration. This cAMP sensor is usually located in the cytosol. In order to detect cAMP on early endosomes, a "FYVE" sequence derived from EAA1, a protein specifically present on the surface of early endosomes, was introduced at the C-terminal end of the ECFP. -(8) PKA activity was measured using the FRET probe, AKAR3 FRET [26]. This probe is, in fact, a chimerical protein comprising at its ends the fluorescent proteins ECFP and EYFP and in its central part, a peptide substrate of the PKA and a peptide containing a binding domain of phosphorylated amino acids (FHA). Following the phosphorylation of this chimeric protein by the PKA, a change of conformation occurs, resulting in increasing the FRET signal.

Results obtained with these different tools indicated that once internalized and located in the membrane of early endosomes, GIPR continues to be activated and induces cAMP production. First, the kinetics of cAMP production and PKA activation are dependent on the internalization of GIPR and its intracellular traffic. Indeed, inhibition of internalization by a chemical inhibitor (dyngo-4a) or a negative dominant of the dynamin suppresses the persistent phase of cAMP production kinetics. In a

different way, we provided indirect but novel evidence of GIP-stimulated endosomal production of cAMP by over-expressing of a dominant-negative Rab-7 mutant which blocked intracellular traffic of internalized GIPR at the early endosomal stage. Indeed, the progression of intracellular traffic after internalization involves an exchange between Rab5, a GTPase present on early endosomes, and Rab-7, the GTPase of late endosomes. Thus, over-expression of a dominant-negative Rab-7 mutant arrests vesicular traffic at the early endosome stage [24]. In HEK cells over-expressing the dominant negative of Rab-7 we observed, by confocal microscopy, an accumulation of the internalized receptor at the early endosomes and a kinetic of cAMP production characterized by a continuous increase over time of cAMP level. In addition, we provide two sets of direct evidences for a biological activity of internalized GIPR. First, using a nanobody, we detected the active form of Gαs on the surface of early endosomes marked by the agonist probe DY647-GIP. Second, we measured a FRET signal accounting for a higher cAMP concentration on the surface of endosomes containing DY647-GIP compared to endosomes not labelled with DY647-GIP, the existence of which was thus independent of GIPR internalisation.

In conclusion of this work, it appears that GIPR belongs to the GPCR group for which G-protein coupling-dependent signalling persists when these receptors are internalized and located in early endosomes. Our data call further studies aimed at understanding the physiological function(s) of the cAMP pool produced by internalized GIPR.

Structure-function relationship studies of the GIPR

Identification of the binding site of GIP in the GIPR

The cloning of GPCRs cDNAs in the 90' made possible to conduct structure-function relationship studies, which help to understand a role of abnormal receptors (for example mutated receptors) in pathology, the activity of certain drugs and to facilitate the design of new drugs. We have dedicated a part of our research to the identification of the binding site of GIP in its receptor [27]. This was achieved by using site-directed mutagenesis and molecular modelling in synergy. At this time, it was already known that the N-terminus of GIP is crucial for the biological activity of the peptide. Therefore, we focused our attention on the identification of amino acids involved in functional interaction with the N-terminus of GIP. We built a homology model of the transmembrane core of GIPR and used the 3-D model of the complex formed between GIP and the N-terminal extracellular domain of GIPR available from crystal structure [28]. The later complex was docked to the transmembrane domains of GIPR allowing *in silico* identification of putative residues of the binding site, which were then subjected to site-directed mutagenesis. All mutants were expressed in HEK 293 cells for binding studies and a functional assay based on the production of cAMP. Mutation of residues Arg183, Arg190, Arg300 and Phe357 caused dramatic shifts in the potency of the GIPR to induce cAMP formation. Further characterization of these mutants, including tests with alanine substituted GIP analogues, led us to propose that Glu3 of GIP interacts with Arg183 of the GIPR.

Furthermore, these studies strongly supported a binding mode of GIP to GIPR in which the N-terminus of GIP was sited within transmembrane domains (TMD) 2, 3, 5, and 6 with biologically crucial Tyr1 interacting with Gln224 (TMD3), Arg300 (TMD5) and Phe357 (TMD6).

Identification of functional elements of the GIPR associated to receptor activation and G protein coupling

We further pursued structure-activity relationship studies on GIPR by characterizing functional elements of the transmembrane domain (TMD) associated to receptor activation and G protein coupling. For this purpose, residues putatively involved in key interactions we mutated in the GIPR. The study was published in 2015 [29].

As the framework to interpret our pharmacological experiments, we built a homology model of the inactive GIPR based on the inactive glucagon receptor (GR) structure [30], one of the two structures of secretin-like or class B1 receptors available at that time. GR has 53% sequence identity with GIPR on the TMD. The other structure available was the inactive corticotropin-releasing factor receptor 1 (CRF1R, with only 27% sequence identify with the GIPR) in complex with a negative allosteric modulator [31]. Since no active structure of a class B1 receptor was available, we also created an active model of the GIPR by incorporating active features from the structure of the (class A) β2-adrenergic receptor in complex with Gs [32].

Today the structural framework has remarkably improved, with 19 class B1 structures deposited in the Protein Data Bank (https://www.rcsb.org/) covering 8 out of the 16 class B1 receptors, allowing to revise the proposed mechanism of activation. The TMD of GIPR has not been yet resolved experimentally, despite the appearance of new GPCR structures in recent years. GIPR is a part of the glucacon subfamily of receptors together with GR, GLP1R and GLP2R. They all have sequence identities with GIPR in the TMD of about 50%. The structures of the GLP1R are of remarkable interest since they cover both inactive [33, 34] and active states [35, 36], thus helping to understand the molecular determinants of each state. For GR there are only inactive structures available, whereas no structures for the GLP2R has yet been solved. Structures for receptors of other B1 subfamilies are available for CRFR1 (inactive), calcitonin and calcitonin like receptors (CALR and CALRL; active), parathyroïd hormone receptor 1 (PTH1R; inactive and active) (see https://gpcrdb.org/structure/). Collectively, these structures provide a global of the mechanisms of ligand binding [37], receptor activation and G protein binding at the residue level.

Figure 2 shows a computational model of the active GIPR in complex with GIP and with the cognate Gs protein. The overall complex and receptor fold are very similar to other B1 receptors that have been determined. The comparison between inactive and active states indicate that the main changes between them are: i) the opening of the extracellular half of transmembrane (TM) helices 6 and 7 to open a cavity to accommodate the N-terminus of the endogenous peptide within the TMD and ii) the formation of a large kink in TM6 that facilitates the binding of the protein (see Figure XB).

Some of the residues that we mutated in our study published in 2015 are responsible for those changes. The residues form networks of interactions organized in layers including a "central polar network", an "intracellular polar network" and the residue of the "G protein binding site" [37] (see Figure 2A).

We revisited our proposed mechanisms by considering the structures available today. In general, our predictions were reasonably correct with only small differences associated with the unpredicted highly kinked TM6 with the unwinding of its central portion and the associated changes in the binding of the C-terminus of Gα. All these features are conserved in class B1 receptors. TM6 in class A receptors contains a smaller kink without unwinding of the helix. However, the location of residues in the cytoplasmic half of TM6 is quite different from the location in the class A homology model.

As part of the "central polar network", we studied an intracellular link between residues at TMs 2, 6 and 7 that is specific of the glucacon subfamily of the receptors. We proposed a triangle of interaction involving Arg183$^{2.60b}$, Ser381$^{7.46b}$ and Glu354$^{6.53b}$ (numbers in superscript indicate the general numbering system for the class B [38]) in the inactive state that would break upon agonist binding. Arg183$^{2.60b}$ is precisely one of the most important residues recognizing the N-terminus of GIP via interaction with Glu3 [27]. Our proposal is consistent with the Glu354$^{6.53bAla}$ being constitutively active on cAMP production and more potent in response to GIP. These interactions are a part of the machinery that permits the concerted opening of TMs 6 and 7 away from TM2 to accommodate the N-terminus of the endogenous peptides.

We also studied residue Asn310$^{5.50b}$ in the junction between the "central polar network" and the "intracellular polar network" because it is highly conserved in the class B1. Although we proposed that this residue could bind the backbone of TM3 (as in the structure of the CRF1R), this interaction has not been consistently observed in other receptors. By contrast, active states show that this residue forms an interaction with the backbone of TM6 that stabilizes the unwind conformation of the central portion of the helix (see Figure 2B). This interaction could also explain that the mutant Asn310$^{5.50bAla}$ showed a decrease in potency.

The "intracellular polar network" is a highly conserved network of residues in the B1 class that is the analog of the "ionic lock" of class A GPCRs [REF: 11375997]. Within this network, Thr343$^{6.42b}$ is crucial to keep TM6 attached to TMs 3 and 7 interacting with both Leu241$^{3.54b}$ Tyr392$^{7.57b}$ in the inactive state. Upon activation, both interactions are lost, unlocking TM6 and enabling the necessary opening to accommodate the G protein. The new active structures revealed that the interaction His173$^{2.50b}$-Glu237$^{3.50b}$ is required for proper functioning but remains the same in inactive and active states. This is consistent with the lack of cAMP signalling of both His173$^{2.50bAla}$ and Glu237$^{3.50bAla}$ mutants.

The "G protein binding site" consists of three basic residues and a group of hydrophobic residues that interact with Leu residues at the C-terminus of Gα. Arg338$^{6.37b}$ interacts with the C-terminal carboxylate group of Leui (with i being the last residue in the Gα), His173$^{2.50b}$ interacts with

Tyri-3 and Arg169$^{2.46b}$ interacts with Glni-4. The Arg338$^{6.37bAla}$/Arg169$^{2.46bAla}$ double mutant was unable to produce cAMP. Leu245$^{2.54Se}$r, Ile317$^{5.61Ser}$, Ile320$^{5.64Ser}$ and Leu339$^{6.33Ser}$ mutants all showed decreased potencies in cAMP production in response to GIP. This was consistent with the interaction with C-terminal Gα residues Li, Li-1 and Li-6 in our β2-adrenergic receptor-based active model. However actual active structures show that as a result of the largely kinked TM6 Leu339$^{6.33}$ does not interact with Gα.

Conclusion

The works reported in this article show that aside its interest in metabolic diseases, GIPR is a diagnosis and therapeutic target in neuroendocrine tumours. The discovery of an efficient radioligand to target GIPR represents the next challenge. This G-protein coupled receptor share a series of molecular features with other members of this superfamily of receptors, but also displays specific behaviours which are difficult to confirm in the physiological or pathological context in humans. Data on its rapid and abundant internalization, its signalling from early vesicles of internalization and on structure-activity relationship, including its binding site, functional elements responsible for its activation and coupling to G-proteins can be useful in the prospect of design of drugs targeting this receptor.

Legends to figures:

Figure 1: Schematic representation of GIP receptor internalization and post-endocytic trafficking and tools used to identify endosomal production of cAMP and PKA activity

GIP-bound-GIPR was identified using DY647-GIP (1). Total cAMP production was measured using cytosolic Bret sensor *R*Luc-Epac1-citrine or Fret sensor EYFP-Epac1-ECFP (2). Early endosomes were labeled with DsRed-Rab5 or GFP-EEA1 (3). Dyngo4-a (4), an inhibitor of dynamin, served to inhibit GIPR internalization whereas DsRed-DN-Rab7 (5) caused accumulation of early endosomes. Activity of GIPR in early endosomes was detected using a nanobody specific of active form of Gαs subunit (6) and an endosomal FRET sensor genetically targeted to early endosomes thanks to FYVE sequence recognizing PI(3)P (7). Activity of PKA was detected using AKAR3 FRET sensor (8).

Figure 2: (A) Computational model of the active GIPR in complex with GIP and Gs constructed based on GLP1R structure with PDB ID 5VAI. Helices are shown as cylinders and loop traces have been smoothed for clarity. GIP and the G protein subunits are shown with a transparent surface. **(B)**

Changes in TMs 6 and 7 that are necessary to open a cavity to accommodate GIP in its binding pocket and another cavity to accommodate the G protein. The inactive structure is based on GR structure 5EE7 and the active structure is as in (A).

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