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G-PROTEIN COUPLED RECEPTOR AS MULTI-DOMAIN ALLOSTERIC MODULATOR: EMERGING OPPORTUNITIES AND PROSPECTS

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Abstract: G protein-coupled receptors (GPCRs), also known as seven-(pass)-trans membrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptors, and G protein-linked receptors (GPLR), form a large group of evolutionarily related proteins that are cell surface receptors that detect molecules outside the cell and activate cellular responses. Since they are coupled with G proteins, they pass through the cell membrane seven times in form of six loops (three extracellular loops interacting with ligand molecules, three intracellular loops interacting with G proteins, a N-terminal extracellular region and a C-terminal intracellular region) of amino acid residues, which is why they are sometimes referred to as seven-trans membrane receptors. Ligands can bind either to the extracellular N-terminus and loops (e.g. glutamate receptors) or to the binding site within trans membrane helices (rhodopsin-like family). They are all activated by agonists, although a spontaneous auto-activation of an empty receptor has also been observed.

G protein-coupled receptors are found only in eukaryotes, including yeast, choanoflagellates, and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein-coupled receptors are involved in many diseases.

There are two principal signal transduction pathways involving the G protein-coupled receptors:

- The c-AMP signal pathway and
- The phosphatidylinositol signal pathway.

When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR can then activate an associated G protein by exchanging the GDP bound to the G protein for a GTP. The G protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type (Gαs, Gαi/o, Gαq/11, Gα12/13). GPCRs are an important drug target and approximately 34% of all Food and Drug Administration (FDA) approved drugs target 108 members of this family. The global sales volume for these drugs is estimated to be 180 billion US dollars as of 2018. It is estimated that GPCRs are targets for about 50% of drugs currently on the market, mainly due to their involvement in signaling pathways related to many diseases i.e. mental, metabolic including endocrinological disorders, immunological including viral infections, cardiovascular, inflammatory, senses disorders, and cancer. The long ago discovered association between GPCRs and many endogenous and exogenous substances, resulting in e.g. analgesia, is another dynamically developing field of the pharmaceutical research.

Keywords: GPCR, GTP, GEF, GPLR, 7-TM, FZD

Introduction: G-protein coupled receptors (GPCRs) are cell surface receptors that play a critical role in cell signaling. GPCRs are the largest and most diverse protein family in the mammalian genome. They contain seven membrane helices, with an intracellular C-terminus connected by three intracellular and three extracellular loops and an extracellular N-terminus; thus giving rise to their other names 7-TM receptors, heptahelical receptors, and seven transmembrane receptors. They are comprised of about 800-1000 members, making up about 3 – 5 % of the human genome. These were first discovered by **Stephen Foord** and his co-workers in the Receptor Systems and Cell Biology groups at GSK (Stevenage, U.K.). The GPCRs family Is subdivided into 7 main classes including Class A rhodopsin-like, Class B secretin-like, Class C metabotropic glutamate/pheromone, Class F frizzled (FZD), Taste receptors (TAS1R, TAS2R), Vomeronasal receptors (VN1R, VN2R) and 7-TM orphan receptors, in which class A rhodopsin like GPCRs is the largest.[1]

They make up about 48% percent of all GPCRs. The division is based on the type of stimuli that activates the GPCR and sequence similarity. Although most GPCRs have the same seven trans membrane structures, they all have differences in the N-terminus and manner in which their corresponding stimulus binds, thus giving rise to many different functions and sequences. The largest class, class A consists of light receptors and adrenaline receptors with a highly conserved Asp-Arg-Tyr motif at the cytoplasmic side of the third trans membrane domain. Class B consists of hormone and neuropeptide receptors. Class C class receptors are composed with GPCRs with an exceptionally large N-terminus.

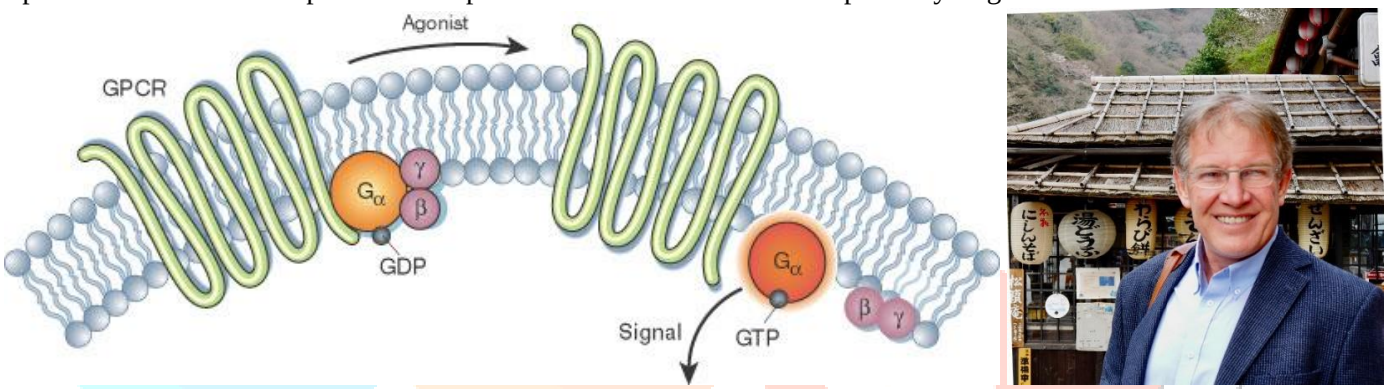


Figure-1: Structure of GPCR & Inventor [Steve Foord Consultant to Pharma and Biotech Stevenage, England, United Kingdom]

As their name implies, GPCRs initiate signaling via G-proteins. The G-proteins coupled to GPCRs are heterotrimeric and consist of alpha (α), beta (β), and gamma (γ) subunits. The GPCR, in essence, is a guanine-nucleotide exchange factor (GEF) for the Gα subunit. GPCR signaling is initiated by the binding of an agonist to the GPCR, causing a conformational change of the GPCR, which results in the activation of GEF activity toward the Gα subunit. This GEF activity causes an increase in the dissociation of GDP from Gα, allowing the rapid exchange of GDP for GTP, which is present in high intracellular concentrations. The activated, GTP-bound Gα subunit then promotes dissociation of the heterotrimeric complex. The GTP-bound Gα subunits and Gβγ dimers then go on to activate a number of second messenger generating pathways including the activation of phospholipase C and the activation/inhibition of adenylate cyclase in addition to a variety of other pathways. The signaling is terminated by the intrinsic GTPase activity of the Gα subunit, which cleaves GTP to form GDP, inactivating Gα and resulting in the re-association of Gα with Gβγ in a heterotrimeric, inactive complex. G protein–coupled receptors are found only in eukaryotes, such as yeast, choanoflagellates and animals. They mediate effects of light, neurotransmitters, lipids, proteins, amino acids, hormones, nucleotides, and chemokines. GPCRs family is predicted to be present throughout the majority of sequenced eukaryotic genomes. Classically GPCRs activate a chemosensory transduction pathway through a change in the associated heterotrimeric G-protein activity. Animal sensory cells from nematodes to vertebrates express hundreds of GPCR genes that play critical roles in both olfaction and gustation through heterotrimeric G-protein activation. Fungi and Amoebozoans, also utilize GPCRs in chemo sensation. In yeast, this receptor class has been shown to play important roles in their nutrient and pheromone sensing pathways.[2]

A pheromone (from Ancient Greek φέρω (phérō) 'to bear', and hormone) is a secreted or excreted chemical factor that triggers a social response in members of the same species. Pheromones are chemicals capable of acting like hormones outside the body of the secreting individual, to affect the behavior of the receiving individuals. There are alarm pheromones, food trail pheromones, sex pheromones, and many others that affect behavior or physiology. Pheromones are used by many organisms, from basic unicellular prokaryotes to complex multicellular eukaryotes. Their use among insects has been particularly well documented. In addition, some vertebrates, plants and ciliates communicate by using pheromones. The ecological functions and evolution of pheromones are a major topic of research in the field of chemical ecology. The pheromone consists of an aromatic compound (2-phenylundecane), cuticular hydrocarbons (pentacosane and heptacosane),

fatty acids (palmitic acid and trans-vaccenic acid), and cholesterol; the pheromone induces long-term aggregation at new nesting and feeding sites.[3]

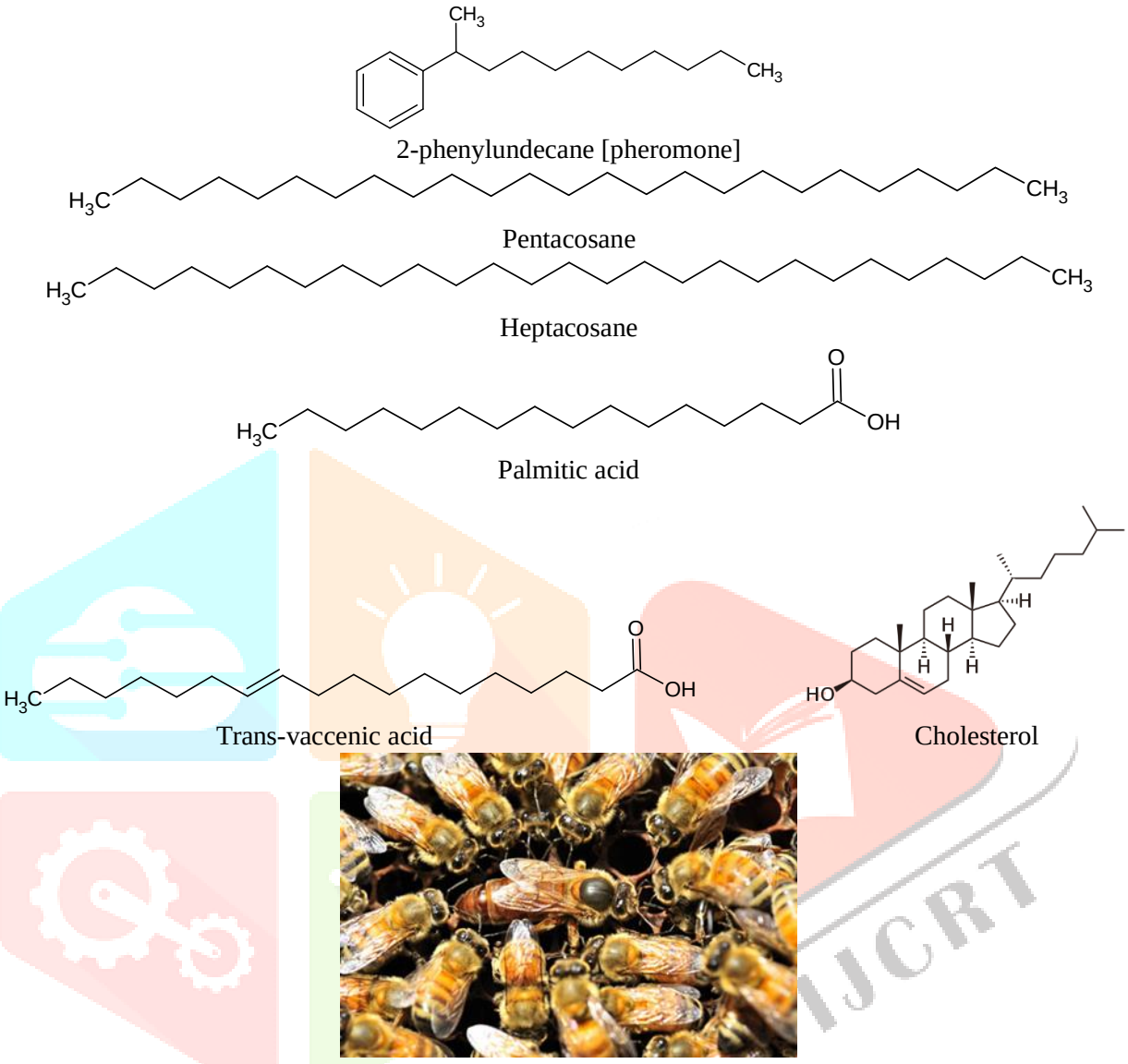
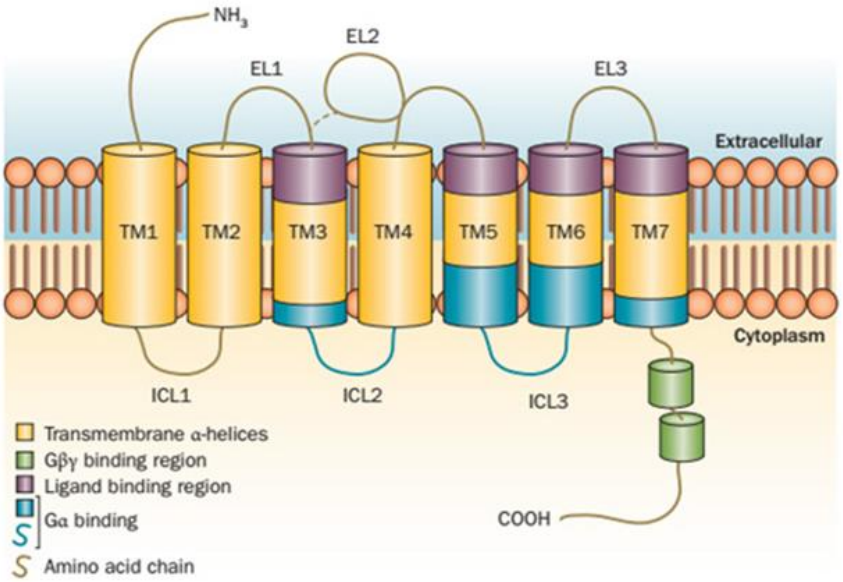


Figure-2: Pheromone constituents

What Do GPCRs Look like?

GPCRs have an extracellular N-terminus, seven trans membranes (7-TM) -helices (TM-1 to TM-7) coupled by three intracellular (IL-1 to IL-3) and three extracellular loops (EL-1 to EL-3), and an intracellular C-terminus. The GPCR forms a barrel-like tertiary structure within the plasma membrane, with the seven trans membrane helices forming a cavity that serves a ligand-binding domain that is frequently covered by EL-2.[4]

However, larger ligands (e.g., proteins or big peptides) may interact with the extracellular loops or, in the case of class C metabotropic glutamate receptors (mGluRs), the N-terminal tail, as shown by the class C metabotropic glutamate receptors (mGluRs). The long N-terminal tail of class C GPCRs, which also comprises a ligand-binding region, distinguishes them from other GPCRs. When glutamate binds to a mGluR, the N-terminal tail undergoes a conformational shift that allows it to connect with the extracellular loop and TM domain residues. All three types of agonist-induced activation result in a change in the relative orientations of the TM helices (similar to a twisting motion), resulting in a broader intracellular surface and "disclosure" of intracellular helices and TM domains critical to signal transduction function (i.e., G-protein coupling). Inverse agonists and antagonists may attach to a variety of locations, but their ultimate function must be to prevent the TM helix from reorienting.[5]



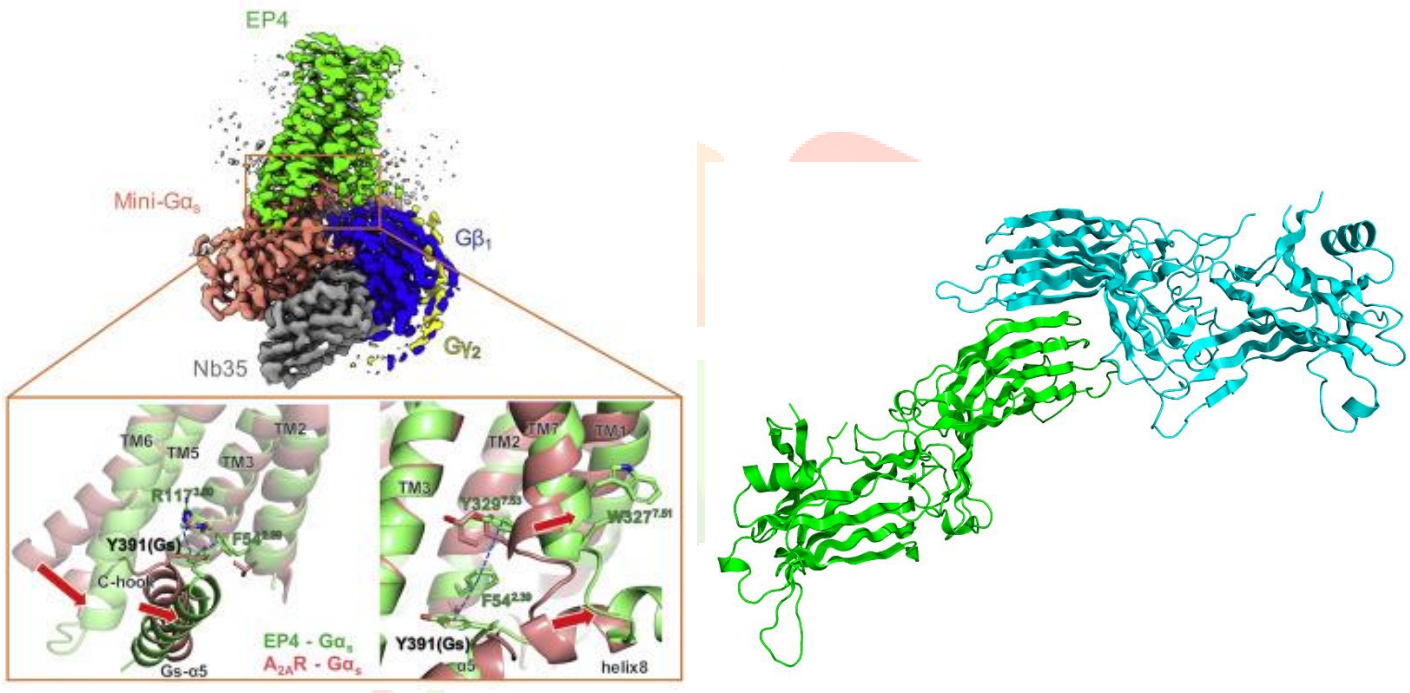


Figure-3: Receptor bed of GCPR & Arrestins

The structure of GPCRs' N- and C-terminal tails could have crucial functions other than ligand binding. M3 muscarinic receptors, for example, have an adequate C-terminus, but the six-amino-acid polybasic (KKKRRK) domain in the C-terminus is required for pre assembly with Gq proteins. The C-terminus, in particular, frequently contains serine (Ser) or threonine (Thr) residues, which, when phosphorylated, increase the intracellular surface's affinity for the binding of scaffolding proteins known as -arrestins (-arr). Once attached, -arrestins sterically impede G-protein coupling and may recruit other proteins, resulting in the formation of signaling complexes that are implicated in the activation of the extracellular signal-regulated kinase (ERK) pathway or receptor endocytosis (internalization). Because these Ser and Thr residues are frequently phosphorylated as a result of GPCR activation, -arr-mediated G-protein dissociation and GPCR internalization are essential processes of desensitization. Furthermore, internalised "mega-complexes" containing a single GPCR, -arr(in the tail conformation), and heterotrimeric G protein exist and may be responsible for endosome protein signaling.[6]

Palmitoylation of one or more locations of the C-terminal tail or intracellular loops is another common structural feature among GPCRs. Palmitoylation is the addition of hydrophobic acyl groups to cysteine (Cys) residues, which has the effect of directing the receptor to cholesterol- and sphingolipid-rich microdomains of the plasma membrane known as lipid rafts. Because many GPCR downstream transducer and effector molecules (including those implicated in negative feedback pathways) are similarly localized to lipid rafts, fast receptor signaling is facilitated. GPCRs respond to extracellular signals

mediated by a wide range of agonists, from proteins to biogenic amines to protons, but they all use a G-protein coupling mechanism to transmit the signal. A guanine-nucleotide exchange factor (GEF) domain, which is predominantly produced by a mixture of IL-2 and IL-3, as well as neighboring residues of the related TM helices, enables this.

G Protein-Coupled Receptors Examples: Beta-adrenergic receptors that bind epinephrine; prostaglandin E2 receptors that bind inflammatory compounds called prostaglandins; and rhodopsin, which contains a photoreactive molecule called retinal that responds to light signals received by rod cells in the eye, are all examples of GPCRs.

Mechanism: An external signal, such as a ligand or another signal mediator, activates the G protein-coupled receptor. This causes a conformational shift in the receptor, resulting in G protein activation. The type of G protein has an additional effect. G proteins are then inactivated by RGS proteins, which are GTPase activating proteins.[7]

G Protein-Coupled Receptors Steps:

Ligand Binding: GPCRs have one or more receptors for the following ligands: sensory signal mediators (e.g., light and olfactory stimulatory molecules); adenosine, bombesin, bradykinin, endothelin, γ-aminobutyric acid (GABA), hepatocyte growth factor (HGF), melanocortins, neuropeptide Y, opioid peptides, opsins, somatostatin, GH, tachykinins, members of the vasoactive intestinal peptide family, and vasopressin; biogenic amines (e.g., dopamine, epinephrine, norepinephrine, histamine, serotonin, and melatonin); glutamate (metabotropic effect); glucagon; acetylcholine (muscarinic effect); chemokines; lipid mediators of inflammation (e.g., prostaglandins, prostanoids, platelet-activating factor, and leukotrienes); peptide hormones (e.g., calcitonin, C5a anaphylatoxin, follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), neurokinin, thyrotropin-releasing hormone (TRH), and oxytocin); and endocannabinoids. Orphan receptors are GPCRs that serve as receptors for stimuli that have yet to be discovered. GPCR ligands, on the other hand, often bind within the transmembrane domain, unlike other types of receptors that have been investigated and where ligands attach outside to the membrane. Protease-activated receptors, on the other hand, are triggered by the cleavage of a portion of their extracellular domain.

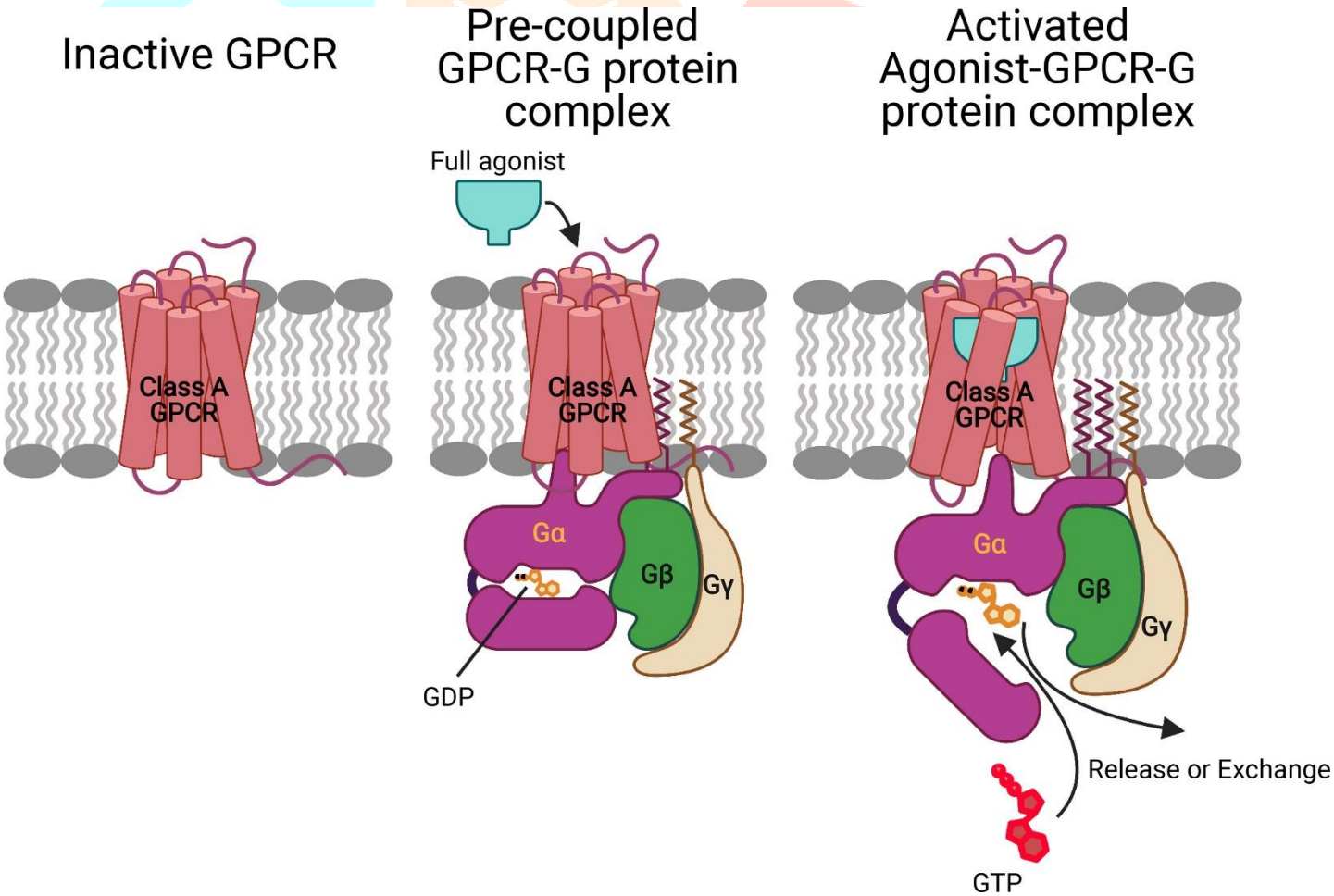


Figure-4: Mechanism of GPCR

Conformational Change: The receptor's signal transduction through the membrane is not totally understood. The GPCR is known to be linked to a heterotrimeric G protein complex in its inactive state. When an agonist binds to a GPCR, the receptor undergoes a conformational shift that is conveyed to the bound G subunit of the heterotrimeric G protein through protein domain dynamics. The activated G subunit exchanges GTP for GDP, resulting in the G subunit's separation from the G dimer and from the receptor. The fragmented G and G subunits engage with other intracellular proteins to continue

the signal transduction cascade, while the released GPCR can rebind to another heterotrimeric G protein to form a new complex ready to start a new round of signal transduction. A receptor molecule is thought to be in a conformational balance between active and inactive biophysical states. Ligand binding to the receptor may cause the equilibrium to shift toward active receptor states. There are three categories of ligands: Agonists are ligands that cause the equilibrium to shift in favour of active states, inverse agonists are ligands that cause the equilibrium to move in favour of inactive states, and neutral antagonists are ligands that have no effect on the equilibrium. It's still unclear how the active and inactive states differ from one another.[8]

G-protein Activation/Deactivation Cycle: The GEF domain may be attached to an inactive -subunit of a heterotrimeric G-protein when the receptor is inactive. These "G-proteins" are a trimer of subunits (known as G, G, and G, respectively) that are rendered inactive when reversibly attached to Guanosine diphosphate (GDP) (or, alternatively, no guanine nucleotide) but active when bound to guanosine triphosphate (GTP). The GEF domain allosterically activates the G-protein by enabling the exchange of a molecule of GDP for GTP at the G—subunit protein's upon receptor activation. The cell maintains a 10:1 cytosolic GTP: GDP ratio to ensure GTP exchange. The G-protein subunits detach from the receptor and from each other at this stage, resulting in a G-GTP monomer and a tightly coupled G dimer that can now control the activity of other intracellular proteins. The palmitoylation of G and the presence of an isoprenoid moiety that has been covalently attached to the C-termini of G, however, limit the amount to which they can spread.

Because G can also hydrolyze GTP to GDP, the inactive form of the -subunit (G-GDP) is eventually regenerated, allowing reassociation with a G dimer to create the "resting" G-protein, which can bind to a GPCR and await activation. The effects of another family of allosteric modifying proteins known as Regulators of G-protein Signaling, or RGS proteins, which are a form of GTPase-Activating Protein, or GAP, often speed up the rate of GTP hydrolysis. Many of the key effector proteins (e.g., adenylate cyclases) that are activated/inactivated by G-GTP also exhibit GAP activity. GPCR-initiated signalling has the ability to self-terminate even at this early stage in the process.[9]

Signaling: The signaling pathways activated by GPCRs are constrained by the GPCR's primary sequence and tertiary structure but are ultimately controlled by the conformation maintained by a particular ligand and the availability of transducer molecules. GPCRs are thought to use two sorts of transducers at the moment: G-proteins and -arrestins. The majority of signaling is ultimately dependent on G-protein activation because -arr's have a high affinity only for the phosphorylated version of most GPCRs. The possibility of contact, on the other hand, allows for G-protein-independent signaling.

G-protein-dependent signaling: There are three main G-protein-mediated signaling pathways, each mediated by four sub-classes of G-proteins (Gαs, Gαi/o, Gαq/11, and Gα12/13), which are characterized by sequence homology. Each sub-class of G-protein consists of several proteins, each of which is the result of multiple genes or splice variants, which can result in modest to significant variances in signaling capabilities, although they appear to be categorized into four classes in general. These classes are defined by the isoform of their -subunit because the signal-transducing properties of the different potential combinations do not appear to differ much. While most GPCRs are capable of activating multiple G-subtypes, they have a preference for one over the other. Functional selectivity occurs when the subtype activated is dependent on the ligand bound to the GPCR (also known as agonist-directed trafficking, or conformation-specific agonism). However, because any single agonist may be capable of maintaining more than one conformation of the GPCR's GEF domain during the course of single contact, it may also trigger activation of numerous different G-proteins. In addition, if the chosen isoform of Gα is not available, a conformation that activates one isoform of Gα may activate another. In addition, feedback mechanisms may cause receptor changes (such as phosphorylation) that modify G-protein preference. Regardless of these intricacies, the GPCR's preferred coupling partner is usually determined by the G-protein that is most clearly activated by the endogenous ligand in most physiological or experimental situations.

G-protein-independent signaling: GPCRs may communicate through G-protein-independent methods, and heterotrimeric G-proteins may perform functional roles independent of GPCRs, despite the fact that they are traditionally assumed to only work together. Many proteins already listed for their functions in G-protein-dependent signalling, such as -arrs, GRKs, and Srcs, may signal independently through GPCRs. For example, -arrestin signalling mediated by the chemokine receptor CXCR3 was found to be required for full effectiveness chemotaxis of activated T cells. Further scaffolding proteins involved in GPCR subcellular localization (e.g., PDZ-domain-containing proteins) may also operate as silencing proteins.

c-AMP and PIP2 Pathways: The cAMP signal pathway and the phosphatidylinositol signal pathway are the two main signal transduction pathways involving G protein-linked receptors.[10]

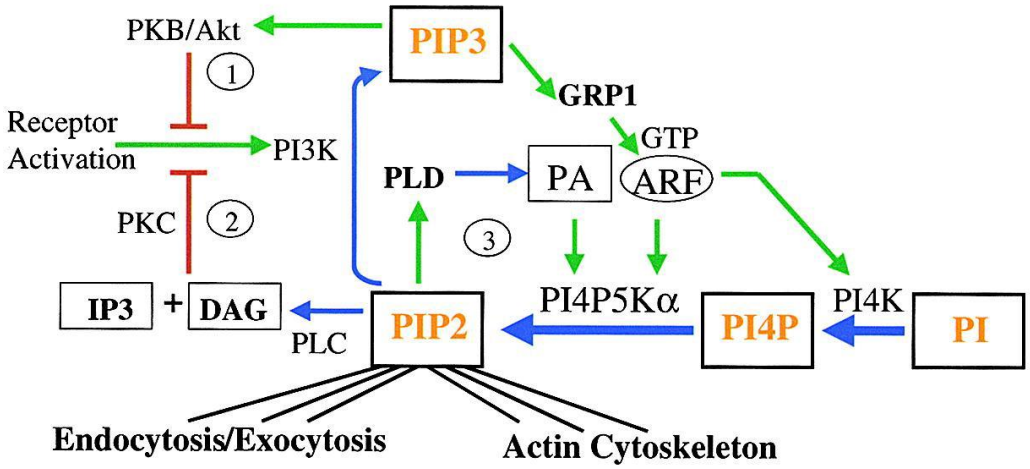


Figure- 5: PIP2 & PIP3 Pathways

cAMP Signal Pathway: Simulative hormone receptor (Rs) or inhibitory hormone receptor (Ri); simulative regulative G-protein (Gs) or inhibitory regulative G-protein (Gi); adenylyl cyclase; protein kinase A (PKA); and cAMP phosphodiesterase are the five key components of the cAMP signaling pathway. The simulative hormone receptor (Rs) can bind with simulative signal molecules, whereas the inhibitory hormone receptor (Ri) can interact with inhibitory signal molecules. Simulative regulative G-protein is a G-protein that is related to the stimulative hormone receptor (Rs), and its component can stimulate the activity of an enzyme or other intracellular processes when activated. Inhibitory regulative G-protein, on the other hand, is coupled to an inhibitory hormone receptor, and its subunit could impede the action of an enzyme or other intracellular processes when activated.

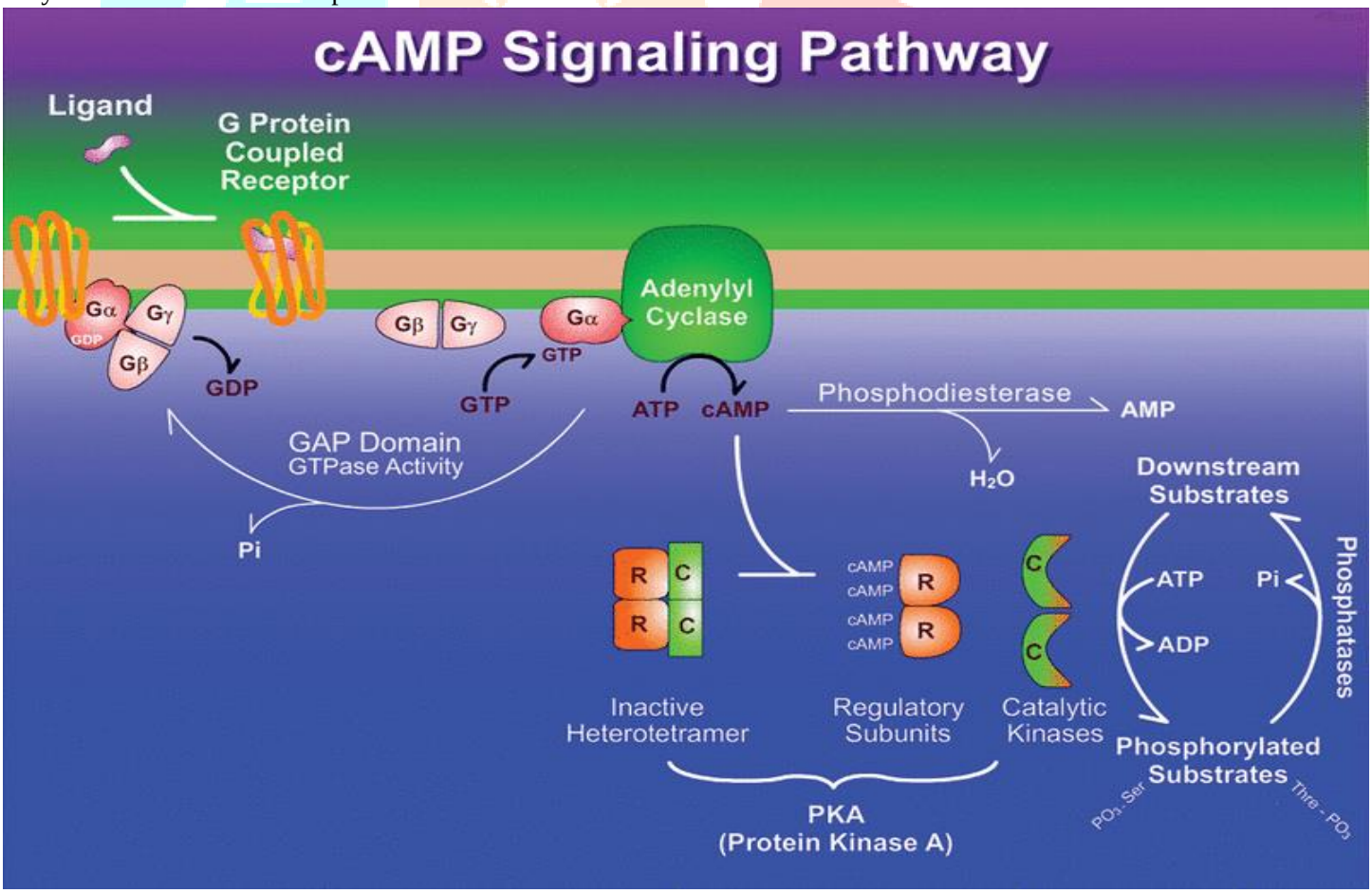


Figure- 6: c-AMP signaling pathway

Adenylyl cyclase is a 12-transmembrane glycoprotein that uses the cofactor Mg^{2+} or Mn^{2+} to catalyze the conversion of ATP to cAMP. The cAMP generated is a second messenger in cellular metabolism and a protein kinase A allosteric activator. Protein kinase A regulates cell metabolism by phosphorylating particular committed enzymes in the metabolic pathway, making it a key enzyme in cell metabolism. It can also control the expression of particular genes, cellular secretion, and membrane permeability. Two catalytic and two regulatory subunits make up the protein enzyme. The complex is

inactive when there is no cAMP present. When cAMP binds to regulatory subunits, their conformation changes, causing the regulatory subunits to dissociate, activating protein kinase A and allowing for additional biological consequences. cAMP phosphodiesterase, an enzyme that converts cAMP to 5′-AMP and inactivates protein kinase A, can then terminate these impulses.[11]

Phosphatidylinositol Signal Pathway: The extracellular signal molecule interacts with the G-protein receptor (Gq) on the cell surface and activates phospholipase C, which is found on the plasma membrane, in the phosphatidylinositol signal pathway. Phosphatidylinositol 4,5-bisphosphate (PIP2) is hydrolyzed by lipase into two-second messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 interacts to the IP3 receptor in the smooth endoplasmic reticulum and mitochondrial membranes, which opens Ca^{2+} channels. DAG aids in the activation of protein kinase C (PKC), which phosphorylates a variety of other proteins, altering their catalytic activity and causing cellular reactions.

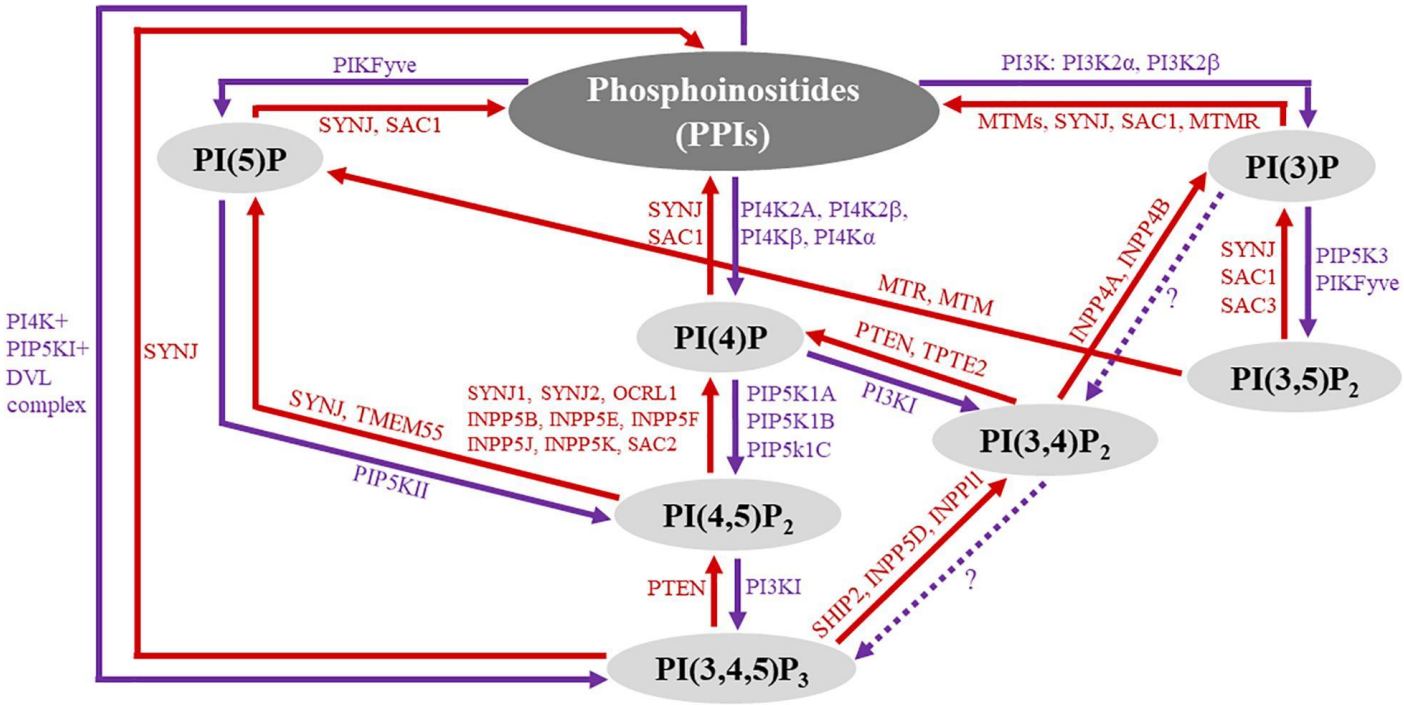


Figure-7: Phosphatidylinositol Signal Pathway

Ca^{2+} has remarkable effects: it cooperates with DAG in activating PKC and can activate the CaM kinase pathway, in which the calcium-modulated protein calmodulin (CaM) binds Ca^{2+}, undergoes a conformational change, and activates CaM kinase II, which has the unique ability to increase its binding affinity to CaM by autophosphorylation, rendering CaM unavailable for the activation of other enzymes. The kinase then phosphorylates and regulates the activity of target enzymes. Ca^{2+}-CaM, which is also a regulatory subunit of adenylyl cyclase and phosphodiesterase in the cAMP signal pathway, connects the two signal pathways.

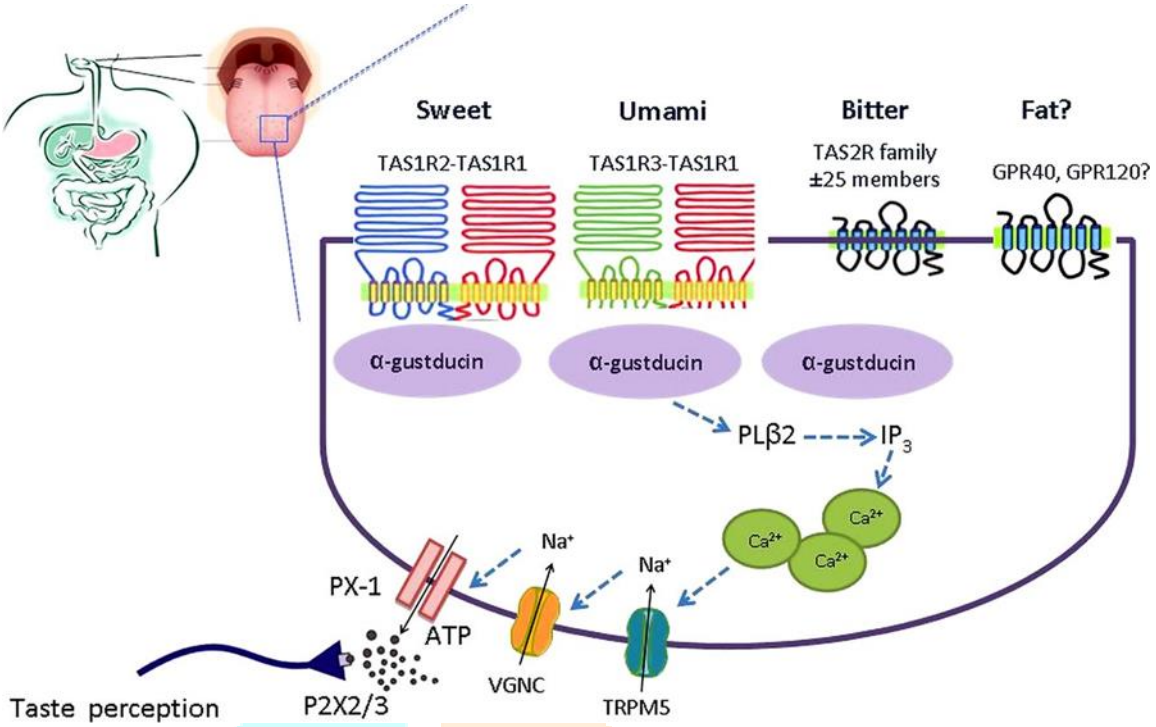


Figure-8: Effect of GCPR in taste bud

G Protein Receptor Physiological Roles: GPCRs have a role in a number of physiological processes. The following are some examples of their physiological roles:

The Visual Sense: Opsins convert electromagnetic radiation into cellular messages through a photo isomerization event. Rhodopsin, for example, accomplishes this by converting 11-cis-retinal to all-trans-retinal. Opsins convert electromagnetic radiation into cellular messages through a photo isomerization event. Rhodopsin, for example, accomplishes this by converting 11-cis-retinal to all-trans-retinal.[12]

The Gustatory Sense (Taste): Gustducin is released by GPCRs in taste cells in reaction to bitter, umami, and sweet-tasting stimuli.

The Sense of Smell: The olfactory epithelium has receptors that bind odorants (olfactory receptors) and pheromones (vomeronasal receptors).

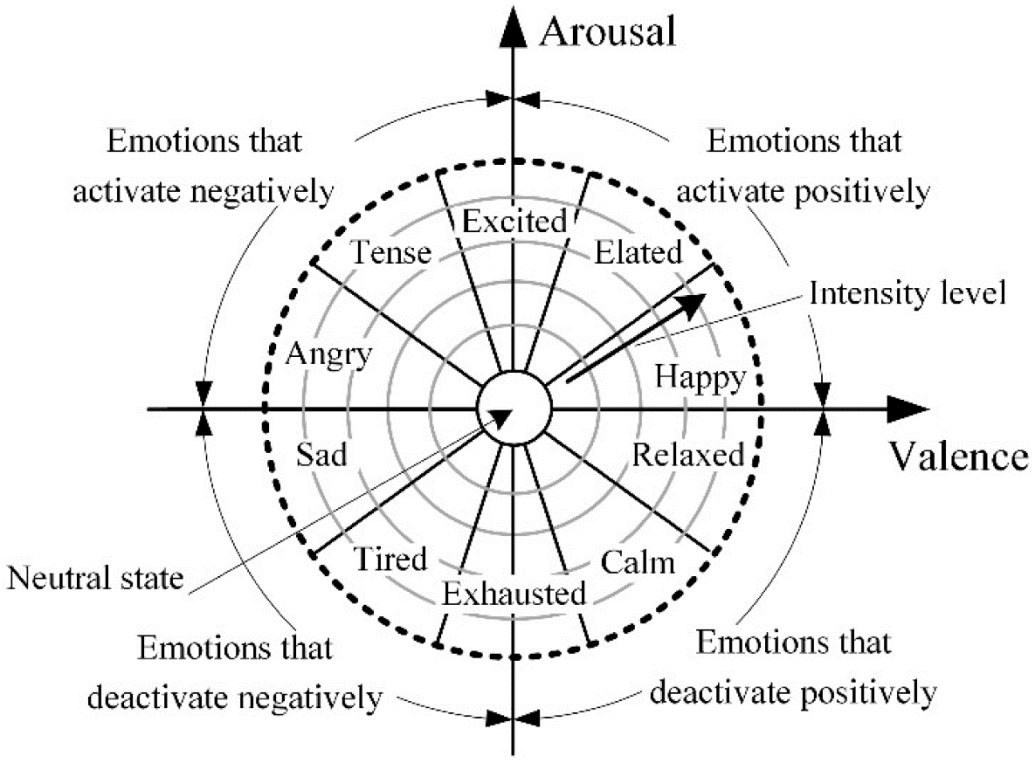


Figure-9: GCPR activates mood changes

Behavioral and Mood Regulation: Serotonin, dopamine, histamine, GABA, and glutamate are among the neurotransmitters bound by receptors in the mammalian brain.

Regulation of Immune System Activity and Inflammation: Histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response; chemokine receptors bind ligands that promote intercellular communication between immune system cells. GPCRs play a role in immunological modulation as well, controlling interleukin induction and inhibiting TLR-induced immune responses in T cells, for example.

GPCR as drug target: Class A: Class A GPCRs, the so called "rhodopsin-like family" consisting of 719 members, are divided into several subgroups: aminergic, peptide, protein, lipid, melatonin, nucleotide, steroid, alicarboxylic acid, sensory, and orphan. They have a conventional transmembrane domain (TMD) that forms ligand-binding pocket and additional eight helices with a palmitoylated cysteine at the C terminal. Given the wide range of their physiological functions, this class of receptors is the most targeted therapeutically among all other classes. By manually curating Drugs@FDA original New Drug Application (NDA) and Biologic License Application (BLA) database (data extracted from August 2017 to June 2020) and cross-referencing with Drug bank, 20 IUPHAR and ChemBL databases, we were able to find the approved drugs associated with this class. Over 500 GPCR drugs target class A and many of them act at >1 receptor: 75% are made against aminergic receptors and 10% for peptidic ligand receptors with indications ranging from analgesics, allergies, cardiovascular diseases, hypertension, pulmonary diseases, depression, migraine, glaucoma, Parkinson's disease to schizophrenia, cancer-related fatigue, etc. Approximately 500 novel drug candidates are in clinical trials. Of them, 134 are for peptide-activated GPCRs, while small molecules still occupy the majority. It is noted that 6% of class A members are sensory and alicarboxylic acid receptors that have broad untapped therapeutic potentials. Chemokine, prostanoid and melanocortin receptors constitute >8% clinical trial targets in this class.[13]

Class A: In the past 3 years, about 20 NDAs were approved targeting mostly peptide and aminergic receptors. Siponimod and ozanimod provide alternatives to fingolimod (approved in 2010) for treating relapsing forms of multiple sclerosis by modulating sphingosine-1-phosphate receptor. Two radiolabeled ligands, gallium 68 dotatoc and lutetium 177 dotatate, have been approved for neuroendocrine tumor and pancreatic gastrointestinal cancer diagnosis, respectively. Pitolisant, a selective inverse agonist of histamine receptor, is used to treat narcolepsy-related daytime sleepiness, while lemborexant, an orexin receptor antagonist, is used for insomnia management.

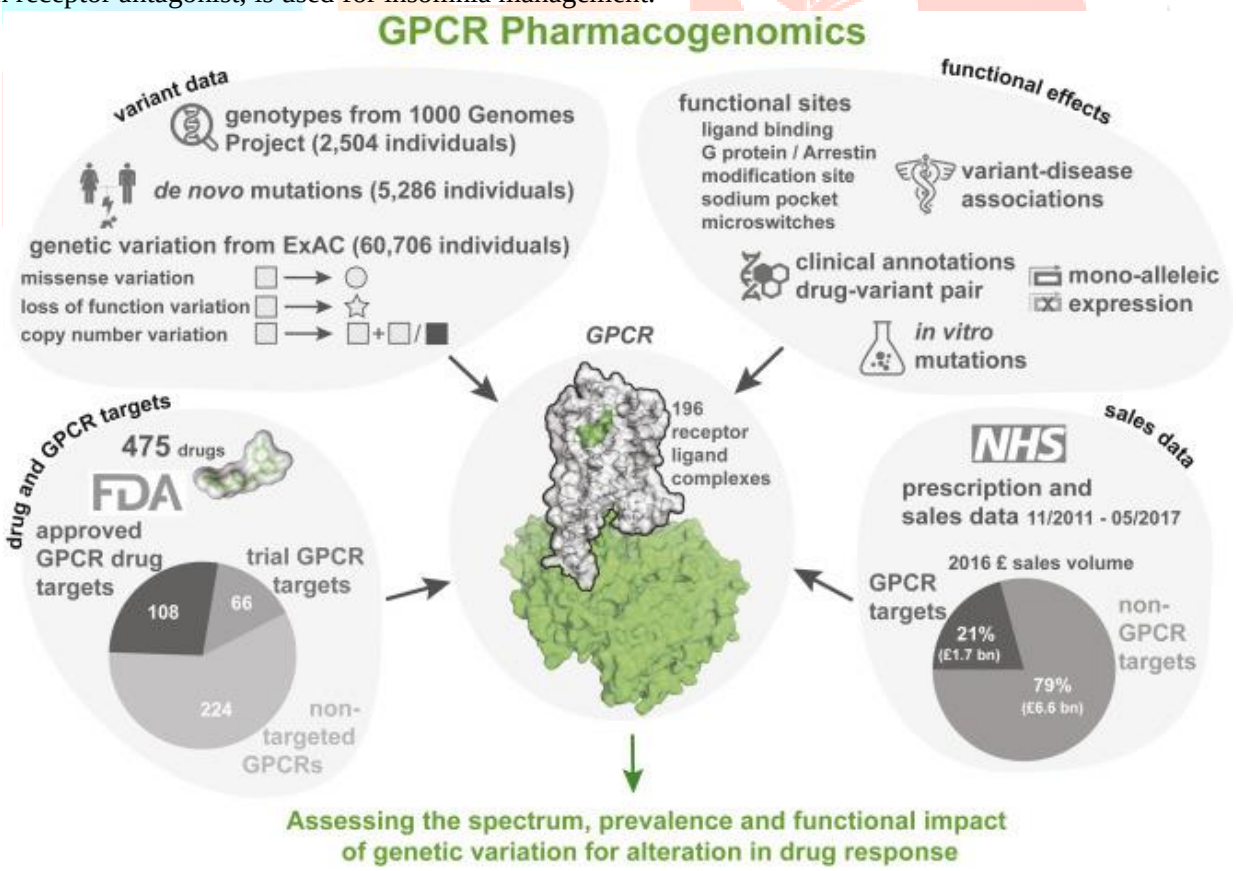


Figure- 10: Types of GPCR on genomics

Gilteritinib (ASP2215) is a small molecule inhibitor of tyrosine kinase. However, it also antagonizes serotonin receptors without any reported pharmacological consequences. Revefenacin is a long-acting antagonist of muscarinic acetylcholine receptors (mAChRs) indicated for chronic obstructive pulmonary disease. Amisulpride, trialed for antiemetic and schizophrenia, was finally approved for antiemetic in 2020. This molecule is acting as an antagonist against dopamine and serotonin receptors. Fosnetupitant, a prodrug of netupitant, was approved for chemotherapy-induced nausea and vomiting. Cysteamine treats radiation sickness via modifying action of neuropeptide Y receptor. Cannabidiol is one the active constituents of the Cannabis plant and was trialed for schizophrenia, graft versus host disease, and anticonvulsant. It was eventually approved in 2018 for the treatment of severe forms of epilepsy—Lennox–Gastaut syndrome and Dravet syndrome. Meanwhile, fostamatinib, indicated for chronic immune thrombocytopenia, targets >300 receptors and enzymes, including adenosine receptor A3.

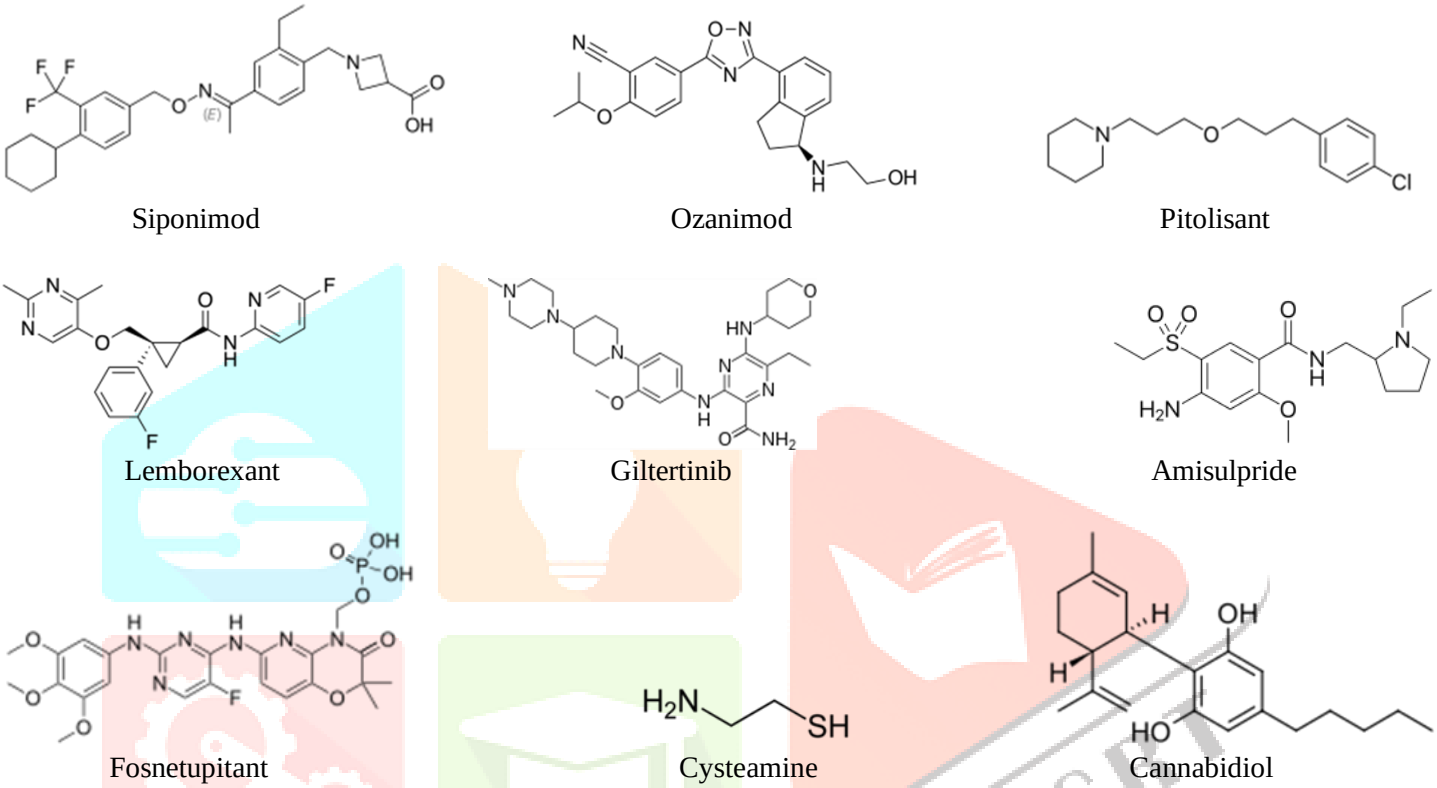
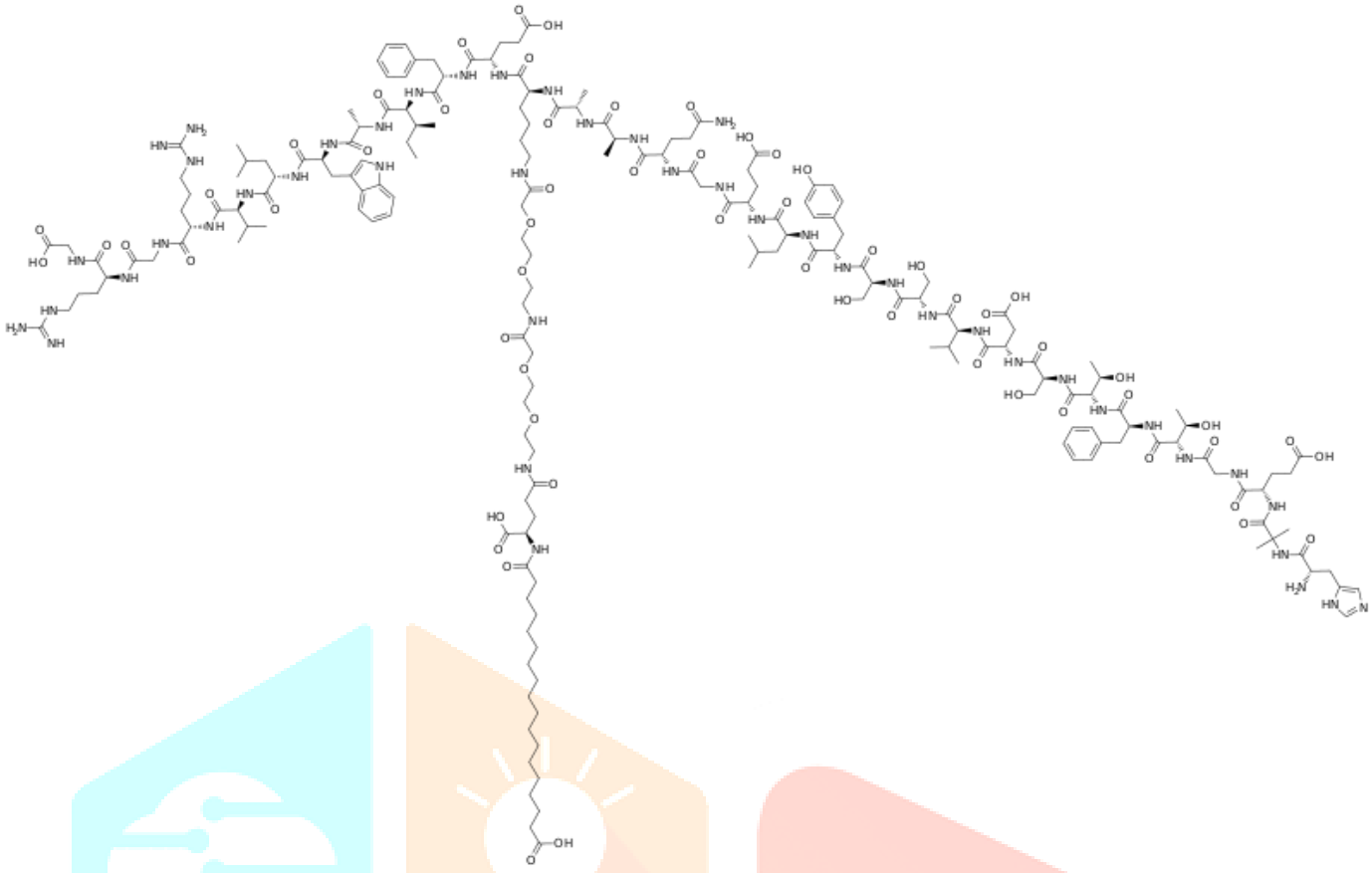
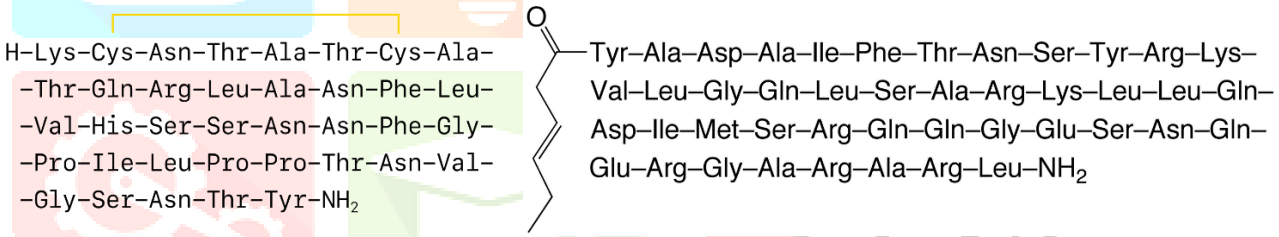


Figure-11: Class A Drugs: Siponimod, Ozanimod, Pitolisant, Lemborexant, Giltertinib, Amisulpride, Fosnetupitant, Cysteamine, Cannabidiol

Class B: This class of GPCRs is divided into two subfamilies: secretin (B1) and adhesion (B2), containing 15 and 33 members, respectively. Secretin subfamily members are characteristic of large extracellular domains (ECDs) and bind to vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), corticotropin-releasing factor (CRF), parathyroid peptide hormone (PTH), growth hormone-releasing hormone (GHRH), calcitonin gene-related peptide (CGRP), glucagon, and glucagon-like peptides (GLPs), respectively. Adhesion subfamily has nine subgroups, possessing unique N-terminal motifs, such as epidermal growth factor, cadherin, and immunoglobulin domains. They are distinguished from other GPCRs due to their roles in cell adhesion and migration. Apart from the long N-terminal domain, other unique features of the B2 subfamily are the GPCR autoproteolysis-inducing domain and the proteolysis site that are responsible for signaling activation through a Stachel sequence (a tethered agonist) and producing N-terminal fragment (NTF) and C-terminal fragment.[14]

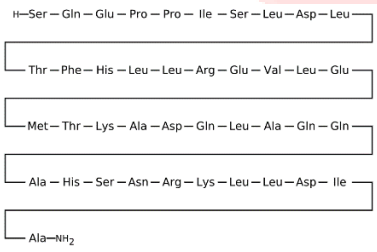


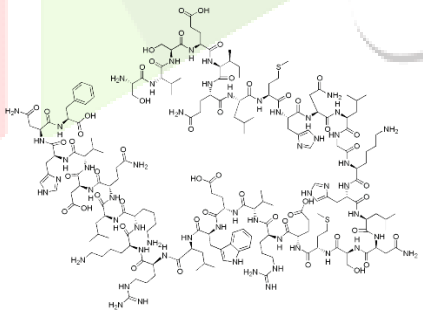
Semaglutide

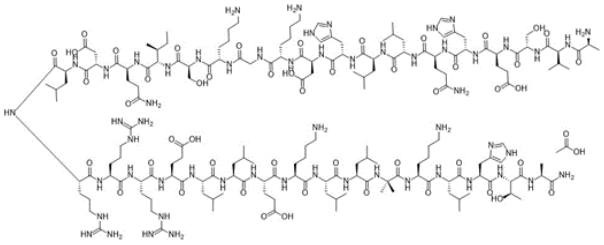


Pramlintide

Tesamorelin







Corticorelin Sermorelin Teriparatide

Figure-12: Class-B Drugs: Semaglutide, Pramlintide,Tesamoreli, Corticorelin, Sermorelin & Teriparatide

The hallmarks of the B2 GPCR subfamily are a two-step activation model, the ligand–NTF interaction and the Stachel signaling/basal activity. Adhesion receptors can also signal independently of fragment dissociation and this has complicated pharmacological consequences. In this class, receptors of glucagon family peptides, followed by CGRP, PTH, GHRH, CRF, VIP, and PACAP, constitute major targets for therapeutic intervention of various diseases, including obesity, T2DM, osteoporosis, migraine, depression, and anxiety. To date, multiple GLP-1 receptor (GLP-1R) agonists have been developed by a combination of selective amino acid substitutions, enzymatic cleavage blockade, and conjugation to entities that increase binding to plasma proteins. These methods not only slow down fast renal clearance of the peptides but also extend their half-lives. Dose-dependent side effects such as nausea and gastrointestinal adverse events are the main drawbacks that are becoming more of a compliant with dose scaling. For instance, one newly approved GLP-1R agonist, semaglutide, has a noticeable half-life of 168 h thereby allowing weekly subcutaneous administration, while oral semaglutide (approved in

2019) formulated using absorption enhancer shows a similar half-life but is dosed daily with reported side effects. CGRP family has a considerable clinical relevance. For instance, pramlintide that targets amylin receptor is utilized to treat both type 1 and type 2 diabetes. Salmon calcitonin has been explored as a treatment for Paget's disease and metabolic disorders. Furthermore, the association of migraine and CGRP elevation led to FDA-approved monoclonal antibodies (mAbs) against its receptor, e.g., erenumab and eptinezumab, as well as several small molecule antagonists such as rimegepant and ubrogepant. Two approved diagnostic agents are analogs of CRF (corticorelin ovine triflutate peptide) and GHRH (sermorelin) for diagnosis of Cushing's disease or ectopic adrenocorticotropic hormone syndrome and growth hormone deficiency, respectively. Tesamorelin, another synthetic form of GHRH, was approved in 2010 to treat human immunodeficiency virus (HIV)-associated lipodystrophy.[15]

PTH analogs, teriparatide and abaloparatide, were approved in 2002 and 2017, respectively, for postmenopausal osteoporosis with similar side effects. However, abaloparatide binds to parathyroid hormone 1 receptor (PTH1R) with higher affinity and selectivity that resulted in greater bone density. No therapeutic agent from the adhesion subfamily has entered clinical trial to date. Although, adhesion GPCRs have shown coupling to heterotrimeric G proteins, the major challenge associated with this family is connecting G protein signals with biological activities. This subfamily was found to play functional roles in the immune, cardiovascular, respiratory, nervous, musculoskeletal, reproductive, renal, integumentary, sensory, endocrine, and gastrointestinal systems, with implications in neurological and neoplastic disorders. For instance, ADGRG1 and ADGRF1 are considered as potential drug targets due to their extensive pathogenetic involvement. Two ADGRG1/ADGRG5 modulators, dihydromunduletone and 3-α-acetoxydihydrodeoxygedunin developed via drug screening efforts, showed disease-related efficacy changes thereby calling for exploration of their activities in a pathological environment. However, associated drug resistance may not only hamper disease but also offer insights into potential mechanisms of such resistance and strategies to tackle it.

Classes C and F: Class C (glutamate) contains 22 receptors, which are further divided into 5 subfamilies including 1 calcium-sensing receptor (CaSR), 2 gamma-aminobutyric acid (GABA) type B receptors (GABAB1 and GABAB2), 3 taste 1 receptors (TS1R1–3), 8 metabotropic glutamate receptors (mGluR1–8), and 8 orphan GPCRs. The distinctive features of glutamate subfamily are their large ECD and obligated constitutive dimer for receptor activation. The structural information of ECD indicates the roles of conserved Venus fly trap (VFT) and cysteine-rich domain (CRD) on the ligand-binding site. Two conserved disulfide bonds between VFT domains stabilize the homodimers or heterodimers of class F GPCRs. The cryo-EM structures of the first full-length mGluR551 and more recently the GABABRs further revealed their assembly mechanism and overall architecture. To date, 16 drugs have been approved by the FDA targeting 8 class C GPCRs. As archetypal receptors, mGluRs mediate the stimulus of agonists such as glutamate and their malfunction are implicated in various diseases, including cancer, schizophrenia, depression, and movement disorders. Acamprosate, an antagonist of mGluR5, was launched in 2004 as an anti-neoplastic agent. In fact, mGluRs have been vigorously pursued as therapeutic targets and there are 15 drug candidates undergoing clinical trials at present for pain, migraine, Parkinson's disease, Fragile X syndrome, etc. Although allosteric modulators of class C have attracted significant development efforts involving 8 clinical trial stage compounds [2 positive (PAM) and 6 negative (NAM) allosteric modulators], the only success is cinacalcet, a small molecule PAM of CaSR approved in 2004 for hyperparathyroidism and calcimimetics.[16]

Only one class F GPCR (smoothed receptor SMO) has been validated as a drug target whose small molecule antagonists were approved as anti-neoplastic agents. Other 10 members of this class are all Frizzled receptors (FZD1–10), which mediate Wnt signaling and are essential for embryonic development and adult organisms. FZDs together with cognate Hedgehog and Wnt signal are associated with a variety of diseases such as cancer, fibrosis, and neurodegeneration. They share a conserved CRD in the extracellular part and ECD structures of SMO and FZD2/4/5/7/8 were determined. However, only SMO, FZD4, and FZD5 have TMD structures. Lack of full-length structures and complexity in signaling pathways impeded drug discovery initiatives. Linking of Wnt with extracellular CRD would activate downstream signaling, while the dimerization process and the interaction between CRD and TMD remain elusive. It is known that the downstream effectors of Wnt signaling consist of β-catenin, planar cell polarity, and Ca^{2+} pathways, whereas receptor activation involves in Wnt, Norrin, FZD, LDL receptor-related protein 5/6, and many other co-factors.64 Key breakthrough is thus required to advance our knowledge of these receptors.[17]

Medicinal chemistry of GPCR:

Agent type: Agents targeting GPCRs continue to expand in the past decades. Among them, exogenous small molecules, including traditionally developed synthetic organics, natural products, and inorganics, still dominate with a total percentage of 64%. Nevertheless, the proportion of small molecules declines since 2010. In addition to traditional ligand discovery, several new modalities appear, though currently at the stage of academic research. Covalent ligands, with the embedding of reactive moieties that can be covalently linked to receptors, significantly enhance the weak binding of optimized leads.

Photoactive ligands, developed by the introduction of photo-responsive groups to drug candidates, bring a new interdisciplinary field, photo pharmacology. Albeit in its infancy, it has already found in vivo applications.

In comparison, biologicals, such as peptides, antibodies, and metabolites, become more and more visible in the list. Particularly, the number of approved peptide drugs occupies approximately one third of the whole repertoire, with many more in different clinical stages as the pipeline—most of them target classes A and B GPCRs. Naturally occurring peptides have been continually discovered from plants, animals, fungi, and bacteria. Although they act as efficient chemical messengers to modulate cellular functions, these peptides suffer from unfavorable pharmacokinetic and pharmacodynamics properties, such as very short plasma half-lives and low plasma protein binding. Therefore, chemical modifications are required to promote the membrane permeability, brain penetration, and oral bioavailability. Available strategies include peptide cyclization, N-methylation, palmitoylation, unnatural amino acid insertion, peptide–small molecule conjugation, and peptide self-assembly. By the way, developing peptidic agents may offer a new approach to de-orphanize certain orphan GPCRs. MAbs represent a promising alternative in GPCR drug discovery. Over small molecules, mAbs possess obvious advantages of improved specificity, affinity, and other pharmacological properties. Thus they are being developed against cancers, inflammation, and metabolic disorders. To date, three GPCR-targeting mAbs were approved (mogamulizumab, erenumab, and eptinezumab) while bi-specific antibodies, nanobodies, antibody–drug conjugates, and antibody–peptide conjugation are also in the development stage. The emergence of many conceptually new molecular entities, such as RNA aptamer, provides not only powerful tool for biophysical study but also potential therapeutic candidates. Usually, aptamer has great molecular diversity and little immunogenicity. In addition, GPCRs are known to function by forming dimers (homodimers or heterodimers) and oligomers on the cell membrane. Therefore, strategies to induce receptor dimerization and/or oligomerization have received attention using scaffolds based on DNA (aptamer), small molecule, and physical stimuli.

GPCR pharmacology: The explosion of 3D GPCR structures and computational simulations has revealed the dynamic conformations between inactive, intermediate, and active states of GPCRs. The detailed structural information illustrated that cholesterol, ion, lipids, and water also participate in receptor activation. The flexibility of receptor-binding pocket endows the complex pharmacological mechanisms of ligand recognition and signal transduction. Biased signaling, allosteric modulation, and polypharmacology are helping us better understand how GPCRs bind to numerous ligands and how they transmit diverse signals to elicit physiological functions.[18]

Polypharmacology: Ligand binding to multiple targets leads to antagonism, additive, or synergism pharmacological responses that could be positive or negative based on the mechanism of action. The paradigm of one drug vs. multiple targets has outpaced the time and cost associated with the conventional therapy. Polypharmacology thus emerges to study acceptable degree of specificity toward multiple targets, interconnected signaling pathways that result in clinical benefit or cross-reactivity that may cause adverse events. T2DM, obesity, cancer, and Alzheimer's disease are major indications for GPCR modulators.4 These polygenic diseases are not completely treatable by a single agent, while desirable efficacies may be achieved for certain respiratory conditions, central nervous system (CNS) disorders, and cardiovascular diseases through modulators directed against β2AR, DRD2, and AGTR1, respectively. It was shown that 5-hydroxytryptamine receptor 2 (5-HT2) binds to selective inverse (ritanserin) and highly promiscuous (ergotamine) agonists but the interaction with ergotamine is broad. This feature allows the development of pan serotonin receptor modulators to treat different diseases. For instance, zolmitriptan as an anti-migraine drug is also used for hyperesthesia via binding to off-target site, and lorcaserin (Belviq) is used to treat obesity while its therapeutic potential for depression, schizophrenia, and drug addiction is being investigated. However, off-target activity, hallucinations, and cardiac valvulopathy related to 5-HT2A and 5-HT2B modulation should be carefully monitored. Atypical antipsychotics are mainly targeting both dopamine and serotonin receptors, usually as antagonist for DRD2 and antagonist or inverse agonist for 5-HT2A. Exemplified by clozapine and aripiprazole, haloperidol, amoxapine, and asenapine4 display a diverse spectrum of receptor interaction. Additionally, carazolol, a member of aminergic division exerts its effects by interacting with multiple adrenergic receptors as inverse agonist or allosteric antagonist. Istradefylline combined with L-DOPA/dopamine simultaneously target A2AR, DRD1 and DRD2 in animal model of Parkinson's disease. Amitryptyline, a tricyclic compound targeting muscarinic and histamine H1 receptors, is used to treat depression and non-selective muscarinic receptor antagonists are trialed for bladder dysfunction.4 Lorazepam, indicated for anxiety due to interaction with GABAAR, is also an allosteric modulator of the proton-sensing GPCR (GPR68)134 and has been repurposed to treat pancreatic cancer. 6′-Guanidinonaltrindole (6′-GNTI) is an agonist with higher selectivity for δ/κ-opioid receptor heterodimer but not homodimer. Importantly, 6′-GNTI is an analgesic that offers additional benefit. In cardiovascular diseases, β blockers decrease catecholamine-induced heart rate elevation via interaction with valsartan (AT1R-mediated signaling). It is of note that mono-, dual-, and tri-agonists for the glucagon family of receptors (GLP-1R, GCGR, and GIPR) have been developed and trialed for weight loss and glucose control.

Successful outcome will determine whether unimolecular polypharmacology is a practical approach to translate safety and efficacy of multiple agents into a single molecule.

Biased agonism: Activated GPCRs can recruit multiple transducers (such as heterotrimeric G proteins, GPCR kinases, and β-arrestin) and consequently produce distinct biological responses. Ligands that preferentially engage one signaling pathway over others are regarded as bias and may show improved therapeutic outcomes.

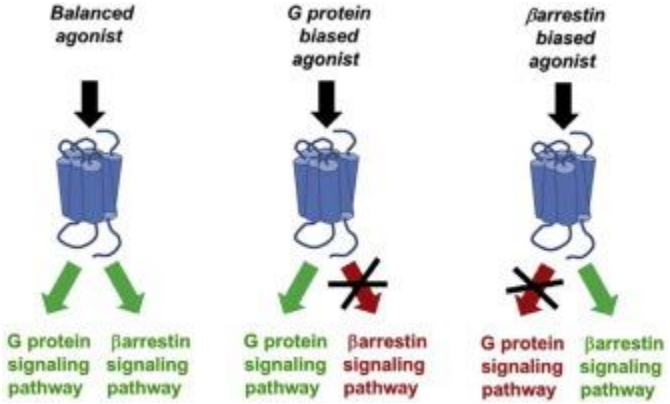


Figure- 13: Biased Agonism

Biased signaling that has been applied to drug discovery involve AT2R, μ-OR, κ-OR, β-adrenergic receptors, DRD2, CTR, CCR, and adenosine receptors. μ-OR is the best studied receptor for biased agonism. Compounds that stimulate Gαi coupling and cAMP production but not β-arrestin recruitment are preferable to retain analgesia and reduce opioid-related side effects. This G protein bias was also demonstrated with widely used drug tramadol, whose active metabolite, desmetramadol, elicited maximum cAMP production without affecting β-arrestin 2 recruitment compared to fentanyl and morphine. Safety profile is improved with less adverse effect such as respiratory depression. Another μ-OR-biased ligand, oliceridine (TRV, OlinvoTM), passed phase III clinical trial but did not get the FDA approval for safety concerns. The NDA for oliceridine was resubmitted and a new counterpart, TRV, is not only suitable for oral administration but also safer due to reduced dependency. A fourth μ-OR-biased ligand, PZM, cross-reacts with κ-OR and failed to reduce respiratory depression in C57BL and CD-1 mice. Whether this relates to its residual but marked effect on β-arrestin 2 recruitment, as opposed to oliceridine whose action is negligible, remains to be further studied. Similar situation occurred with κ-OR as well whose agonists possess analgesic property and have a low risk of dependence and abuse but with adverse effects such as sedation, motor dysfunction, hallucination, and dysphoria. G protein-biased agonists of κ-OR, including RB-64, mesyl salvinorin B, triazole 1.1, diphenethylamines and LOR, were reported to minimize the adverse effects in preclinical settings. One of such, nalfurafine, was approved in Japan (2015) as an anti-pruritic agent for patients with chronic liver diseases.

Carvedilol, known as a β1 and β2 adrenoceptor blocker, was found to be biased toward β-arrestin recruitment, G protein-coupled receptor kinase activation, and ERK1/2 phosphorylation. Joining its rank included alprenolol, bucindolol, and nebivolol, all are used to treat hypertension and congestive heart failure. In the case of β3 adrenoceptor, CL316243 is cAMP-biased, whereas L748337 and SR59230 are ERK/p38 phosphorylation-biased. Interestingly, CL316243 was also tested for treatment of obese mice. However, none of them have advanced to the clinic. In contrast to μ-OR, arrestin bias is desirable for AT1R to improve cardiac performance. Nonetheless, clinical development of AT1R modulators either resulted in a phase IIb trial failure (TRV027) in 2017 or never reached to clinical stage (SBpa, SVdF, SI, sarmesin, saralasin, and SII). Of note is that biased molecules may show species preference. For instance, CL is more active in mice than in humans, whereas nalfurafine works better in humans vs. rodents.

Allosteric modulation: In recent years, studies on allosteric GPCR modulators have gained unprecedented momentum. An allosteric modulator is a ligand binding to a position other than the orthosteric site but can modify responses of a receptor to stimulus. Allosteric modulators that enhance agonist-mediated response are called PAMs, while those attenuate the response are called NAMs. This phenomenon is very common such that the Allosteric Database 2019 records 37520 allosteric modulations on 118 GPCR members, covering all four classes. Allosteric modulation is advantageous in terms of (i) using highly druggable pockets. In some cases, it is easier to design ligands at an allosteric site than the orthosteric site, such as class B GPCRs with orthosteric pockets wide open. For example, both PAM and NAMs binding to the same position at the TMD of GLP-1R were reported; (ii) improving selectivity.

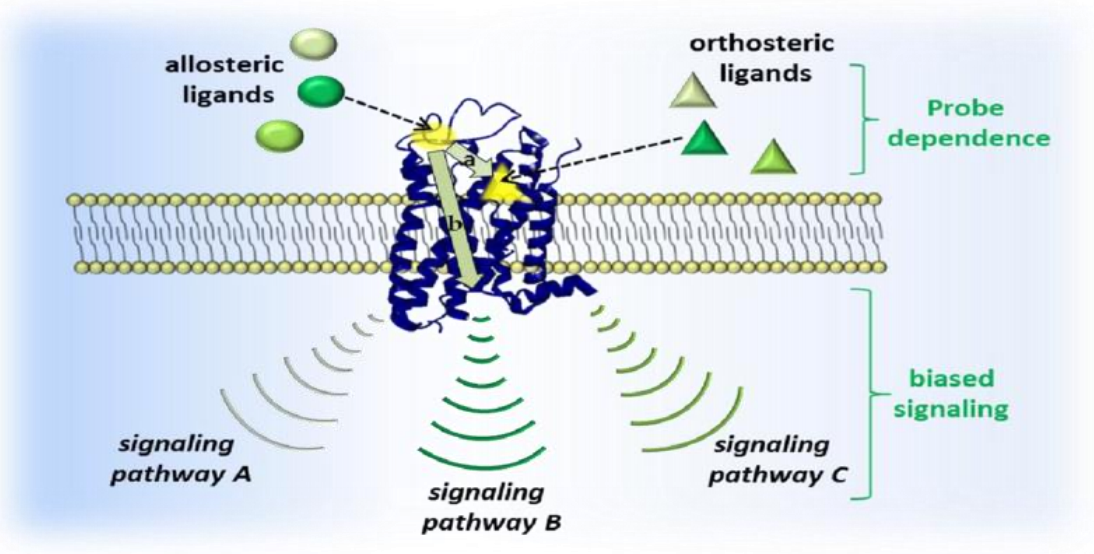


Figure- 14: Allosteric modulation

The orthosteric site and cognate ligand are often highly conserved, making it hard to discover very selective orthosteric binders. Meanwhile, non-conserved allosteric sites would be a better choice evidenced by discovery of many subtype selective allosteric modulators of acetylcholine and cannabinoid receptors; (iii) introducing signal bias. Allosteric modulators with biased signaling were developed for prostaglandin F2α receptor and chemokine receptor CXCR4. Albeit still as an emerging concept, allosteric modulators have exhibited a great potential with some compounds being marketed or in clinical trials. However, developing allosteric modulators of GPCRs remains challenging—molecules recorded in the ASD largely concentrate on two subfamilies, the mGluRs (8 members, 17,115 modulations), and mAChRs (5 members, 7666 modulations), accounting for nearly 2/3 of the total number. Some individual receptors also contribute a significant proportion, such as CB1 (1948 modulations), GABAB (1286 modulations), and follicle-stimulating hormone receptor (1233 modulations). Excluding these "easy cases," allosteric modulators are few in number. Furthermore, the structural diversity of the allosteric modulators is quite low, for many derivatives would be included soon after a parent compound is identified. The difficulty in developing allosteric modulators is partly due to the limitation of detecting allosteric behavior: Not every newly discovered active compound could be tested for its effect on binding affinity or EC50 of an orthosteric agonist, therefore some allosteric modulators were not correctly identified. For instance, BPTU in P2RY1, the first GPCR NAM solved in complex structure, was not considered allosteric until the structure was obtained. To make things worse, NAMs may weaken the binding of an endogenous ligand thus behaving like a competitor, such as NDT9513727 in C5AR1170. The most effective way to Identify "the binding site of an allosteric modulator on a GPCR is solving the complex structure. Crystallography is an effective technique, while rapidly deployment of cryo-EM has started to deliver its promise. To date, 17 GPCRs have reported structures in complex with allosteric modulators. Detailed analysis of complex structures before October 2018 was reported previously, and here we focus on insights provided by newly published results. The most unusual allosteric-binding sites on GPCRs are at the lipidic interface embedded in cell membrane. Five different positions were identified by crystal structures: UP12, UP34, LOW34, LOW345, and LOW67. Four of them were recently reviewed.1 The LOW34 site was reported in 2019 for ORG27569 in CB1.

ORG27569 attracted much attention for its distinctive function: increasing the binding of orthosteric agonist CP55940 but making it act as inverse agonist.165 Many attempts were made to locate the binding site of ORG27569 by mutagenesis but the results are conflicting: one study showed that the effect of ORG27569 on CP55940-induced [35S]GTPγS binding was disturbed by mutations to multiple residues at the orthosteric site, leading to a hypothesis that ORG27569 stays in the same pocket close to CP55940. Another study found that ORG27569 reduced the binding of a fluorescence-labeled orthosteric antagonist, and the effect was only disturbed by mutations at the lipidic interface close to the cytoplasmic end of CB1. Besides, it was reported that the functions of ORG27569 were also affected by breaking a disulfide bond at the N-terminus or by constitutive active/inactive mutations at the cytoplasmic interface. The crystal structure exhibited that the position of ORG27569 is considerably overlapped with a cholesterol captured in another intermediate state, consistent with the site located by the fluorescence-labeled orthosteric antagonist. At this site, the higher selectivity to CB1 over CB2 could be explained. Interestingly, ORG27569 is the only allosteric modulator at lipidic interface forming no hydrogen bond to the receptor. There have been three more complex structures of allosteric modulators at lipidic interface since October 2018, all obtained by crystallography. Two are β2AR, with a NAM AS408 or a PAM Cmpd-6FA. Both allosteric modulators bind

to the LOW345 site The NAM stays at a position very similar to NAMs in C5AR1 but the PAM is close to ICL2 and only partially overlaps with PAMs of FFAR1, showing a complex regulation nature at this site. The other complex structure is full-length GLP-1R with PF-06372222, a NAM previously used to co-crystallize with GLP-1R TMD.[19]

Even around the position of orthosteric ligands (among the helices and facing extracellular side), another ligand may occupy the space not taken by the endogenous ligand and act as an allosteric modulator. The very abundant PAMs/NAMs of mAChRs function in this mechanism. PAM LY2119620 in M2R (with the orthosteric agonist iperoxo and stabilized by a nanobody) was the first allosteric modulator to obtain complex structure with a class A GPCR. Recently, LY2119620 was also observed in protein complexes of M2R with G protein or arrestin by cryo-EM. CCR5 is a chemokine receptor and an important anti-HIV drug target. A marketed inhibitor, maraviroc, has long been recognized as a NAM of CCR5. There were hypotheses that small molecule NAMs, chemokine, and the HIV-binding protein have separate binding sites. However, structures of CCR5 in complex with maraviroc, chemokine analog antagonist, or HIV envelope glycoprotein show that these ligands highly overlap in CCR5 pocket. Therefore, the noncompetitive behavior of maraviroc may be due to a very extensive interface of peptidic CCR5 agonist, thus a small molecule cannot diminish the binding even with this much collision. The results illustrate that allosteric behavior is not equal to totally separated binding positions, because partially overlapped sites with different key interactions are also allowed. The last case of allosteric modula or in extracellular pocket is PAM TT-OAD2 of GLP-1R. This small molecule agonist only slightly collides with the endogenous peptide, consistent with its behavior that only partially displaces an orthosteric probe. The cytoplasmic interface, where a GPCR interacts with intracellular partners, including Gα and β-arrestin, contains pockets suitable for drug design. So far, four small molecules have been validated by crystallography to bind at this position. The targets are three chemokine receptors (CCR2, CCR7, and CCR9,) and β2AR. These ligands are all NAMs and proximately share the same binding site (TM1, TM2, TM6, TM7, ICL1, and H8). Their binding position does not overlap with Gα, therefore they may stabilize the inactive state by blocking conformational changes required for receptor activation. This site is generally non-conserved in the GPCR superfamily, thus targeting here may provide some selectivity. Additionally, many nanobodies at the cytoplasmic interface were also developed for several receptors, including AGTR1, β1AR, β2AR, and SMO. Multi-domain regulation is an interesting topic in allosteric modulator discovery. Class C GPCRs use ECDs to recognize their cognate ligands, leaving the classic pocket of TMD for allosteric modulating. This is the major reason why this class has a large number of allosteric modulators. In the case of mGluRs, both PAMs and NAMs have been widely reported, but only NAMs obtained complex structures—there is no solved active state structure. The full-length structures of mGlu5 displayed how the binding of orthosteric agonist to ECD triggers the change of interaction between two monomers, but the conformational change of TMD remains elusive.

SMO in class F is also a multi-domain receptor. The first reported ligand of SMO cyclopamine (an antagonist causing birth defects) binds to the classic TMD pocket shared by several other antagonists with different chemical scaffolds and an agonist (SAG). ALLO-1, an antagonist identified as allosteric modulator not competitive to cyclopamine, was recently found to bind at a deeper position in the pocket by photo-affinity labeling combined with mass spectrometry (MS). SMO has another pocket in ECD that interacts with steroids, including cholesterol and 20(S)-hydroxycholesterol. Since cholesterol has been the most favored candidate of SMO endogenous ligand, the ECD pocket is treated as orthosteric making the TMD pocket allosteric. However, newly obtained structures demonstrated that cholesterol or its analog can also bind to TMD pocket, leaving the question open for which is the true orthosteric site.[20]

Disease indication: GPCRs are involved in many human diseases and specific drug intervention is one of the most celebrating achievements in the pharmaceutical industry. Among all available drugs targeting GPCRs, HRH1, DRD2, M1R, and ADRA1A are the most frequently addressed for indications such as hypertension, allergy, pain, and schizophrenia, and 33% of them have >1 indication with an overall average of 1.5. Although CNS diseases are still popular accounting for 26% of all approved indications, development focuses have now been shifted to T2DM, obesity, multiple sclerosis, smoking cessation, short bowel syndrome, and hypocalcemia. Repurposing of existing drugs for new indications also emerged to supplement discovery efforts.

Conclusion: Recent scientific and technological advancements in GPCR biology have provided an enormous amount of information that will benefit our current and future efforts in rational drug design. Integration and refinement of massive data by artificial intelligence is a clear direction to guide both virtual and experimental screening of efficacious therapeutic agents with new scaffolds and of novel chemotypes for all classes of GPCRs. However, as described in this review, factors that influence GPCR drug discovery include, but not limited to, therapeutic target, chemical diversity, mechanism of signaling, ligand-binding site, mode of action, clinical indication, polypharmacology, etc. Future opportunities may arise from: (i) de-orphanization of orphan GPCRs to provide novel targets; (ii) new indication for drug intervention via discovery and/or repurposing efforts; (iii) development of lead compounds targeting classes B2 and F GPCRs to address unmet medical needs; and (iv) validation of polypharmacology may lead to improved drug therapies.

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