

Study of G protein coupling receptors

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Abstract. G-protein coupled receptor is a type of cell membrane receptor that plays a crucial role in cellular signaling and is involved in various physiological processes in the human body. They are involved in transmitting signals from the extracellular environment to the inside of the cell, which ultimately leads to various cellular responses. The basic structure of a GPCR consists of a single polypeptide chain that crosses the cell membrane seven times, forming seven transmembrane helices. These helices create a pocket or cleft on the cell surface that acts as the binding site for specific ligands, such as hormones, neurotransmitters, or other signaling molecules. Overall, GPCRs are essential components of cellular communication and play vital roles in maintaining homeostasis and regulating various physiological functions in the human body. This review presents a comprehensive analysis of GPCR structure, mechanism, and physiological relevance, offering a novel framework for comprehending GPCR biology.

Keywords: GPCR, Structure, signal transduction, physiological.

1. Introduction

G-Protein Coupled Receptors (GPCRs) are a diverse and ubiquitous family of cell membrane receptors that play fundamental roles in cellular signaling and mediate a wide range of physiological processes. GPCRs represent one of the largest and most versatile superfamilies of proteins, with their dysregulation being associated with numerous diseases. Understanding the structure, activation mechanisms, and physiological significance of GPCRs is crucial for unraveling their complex functions and exploring their therapeutic potential.[1]

GPCRs are characterized by a conserved seven-transmembrane helical domain that spans the cell membrane. This unique structural motif forms a ligand-binding pocket on the extracellular side, allowing GPCRs to interact with a diverse array of ligands, including hormones, neurotransmitters, peptides, and small molecules. Ligand binding induces conformational changes in the receptor, activating intracellular signaling pathways through interactions with G-proteins.[2]

Upon activation, GPCRs couple to heterotrimeric G-proteins, which consist of α , β , and γ subunits. The binding of an activated GPCR to the G-protein leads to the exchange of GDP (guanosine diphosphate) for GTP (guanosine triphosphate) on the α subunit, triggering its dissociation from the $\beta\gamma$ subunits. Both the α and $\beta\gamma$ subunits can then modulate the activity of downstream effector molecules, such as enzymes or ion channels, initiating a cascade of intracellular signaling events.

The physiological importance of GPCRs cannot be overstated. These receptors are involved in vital processes including sensory perception, neurotransmission, hormonal regulation, immune response, cardiovascular function, and many others. Consequently, GPCR dysfunction or dysregulation can contribute to various disorders, ranging from neurological and psychiatric disorders to metabolic diseases and cancer.[1,2]

Advancements in GPCR research have led to significant breakthroughs in drug discovery and development. A large proportion of clinically approved drugs target GPCRs, either as agonists to enhance receptor activity or as antagonists to block receptor signaling. Detailed knowledge of GPCR structure and function has enabled the design of more selective and effective therapeutics.[3]

In this review, we aim to provide a comprehensive overview of GPCR biology, covering topics such as receptor structure, ligand binding, signaling mechanisms, and their involvement in physiological processes. Additionally, we will highlight the clinical implications of GPCR research and the potential for therapeutic interventions targeting these receptors. By delving into the intricacies of GPCR function, this review seeks to contribute to a deeper understanding of these remarkable receptors and their significance in human health and disease.[2,4,5]

2. The structure of GPCR (Figure 1)

GPCRs are integral membrane proteins composed of seven transmembrane α -helical domains. These transmembrane domains divide the receptor into three outer membrane loops, three inner membrane loops, and the N-terminal extracellular domain (N-end) and C-terminal intracellular domain. The extracellular loops of the receptor are commonly subject to glycosylation modifications. Notably, the outer membrane loops contain two conserved cysteine residues that form disulfide bonds, contributing to the stabilization of the receptor's spatial structure..^{4,6} Photosensitive channel proteins, also known as channel retinoids, and GPCRs share a common structural feature, comprising seven transmembrane helices. Liposin receptors, similar to GPCRs, possess seven transmembrane domains; however, they traverse the membrane in an inverted orientation, with the N-terminal end located inside the membrane and the C-terminal end positioned outside the membrane. Unlike GPCRs, liposin receptors do not engage in interactions with G proteins.⁷ The initial models of G-protein-coupled receptor (GPCR) structures were derived from their limited resemblance to bacterial rhodopsin, which had been elucidated through electron diffraction and X-ray crystallography experiments. These studies provided a structural template that served as an analogy for GPCRs. It wasn't until the year 2000 that the crystal structure of the first mammalian GPCR was successfully determined and made available in the protein database.⁸ In 2007, the first human G-protein-coupled receptor (GPCR) structure was unveiled. However, a subsequent publication shortly thereafter presented an improved and higher-resolution depiction of the same receptor. The GPCR in question, the β -2 adrenergic receptor, exhibits significant similarity to the bovine rhodopsin, despite their distinct compositions in the second extracellular loop. This dissimilarity is noteworthy as the second extracellular loop forms a lid-like structure that covers the ligand-binding site. Consequently, the structural variation poses challenges in establishing a homologous model of GPCR structure based on rhodopsin conformation alone.[9]

The structural studies reveal that the extracellular region of GPCRs is responsible for ligand binding, triggering subsequent conformational changes in the intracellular region. Notably, a significant conformational change observed is the outward displacement of the intracellular loop located between the fifth and sixth transmembrane helices. This alteration is evident in the activated state of the β 2 adrenergic receptor complexed with the G protein, where the G protein α subunit binds to the cavities created by the aforementioned conformational movements.[6,10]

G protein-coupled receptors (GPCRs) constitute a diverse group of membrane protein receptors. A characteristic shared among these receptors is the presence of seven transmembrane α -helices in their three-dimensional structures. The binding sites for both the C-terminal region of the receptor and the G protein (Guanine nucleotide-binding protein) are situated on the intracellular loop (the third intracellular loop) of the receptor's peptide chain, starting from the N-terminal end. GPCRs can be classified into six distinct types, and their gene sequences exhibit no homologous relationships among each other..[11]

Class A (or Class I): This class, also known as Class A or Class I GPCRs, includes receptors that exhibit structural similarities to the bovine rhodopsin. They are involved in diverse physiological processes and are the most extensively studied and well-known GPCRs.

Class B (or Class II, Secretin Receptor Family): Class B GPCRs, also referred to as Class II GPCRs, belong to the Secretin Receptor Family. These receptors are involved in regulating hormone secretion and play crucial roles in endocrine and metabolic functions.

Class C (or Class III, Metabotropic Glutamate Receptor): Class C GPCRs, also known as Class III GPCRs, include the Metabotropic Glutamate Receptors. These receptors are mainly associated with synaptic transmission in the central nervous system and are involved in processes such as learning, memory, and neuroplasticity.

Class D (or Class IV, Fungal Mating Pheromone Receptor): Class D GPCRs, also referred to as Class IV GPCRs, are specific to fungal species. They play a crucial role in mediating the mating process and are involved in detecting and responding to pheromones released by other mating partners.

Class E (or Class 5 Cyclostyleptide Receptors): Class E GPCRs, also known as Class 5 Cyclostyleptide Receptors, are a relatively newly identified class of GPCRs. They are characterized by their sensitivity to cyclostyleptides, a type of cyclic peptide.

Class F (or Category 6, Fury/Smooth Family): Class F GPCRs, alternatively referred to as Category 6 GPCRs, belong to the Fury/Smooth Family. These receptors are involved in various processes, including smooth muscle contraction, and are targets for therapeutic interventions in conditions such as asthma and hypertension. By classifying GPCRs into these different categories, researchers can better understand their diverse functions and develop more targeted approaches for drug discovery and therapeutic interventions. A recent study has introduced a novel classification system for G protein-coupled receptors (GPCRs) known as GRAFS. This updated classification system provides a refined framework for categorizing GPCRs based on their structural and functional characteristics. In parallel, bioinformatics-based studies have employed a predictive approach called pseudo-amino acid composition to assess the classification of GPCRs. By utilizing a comprehensive set of amino acid sequences derived from G protein-coupled receptors, researchers have successfully predicted the potential function and classification of these receptors across various organisms. These advancements in classification and prediction methodologies contribute to a deeper understanding of the functional diversity and evolutionary relationships among G protein-coupled receptors. They offer valuable insights into the potential roles and implications of these receptors in various biological processes.

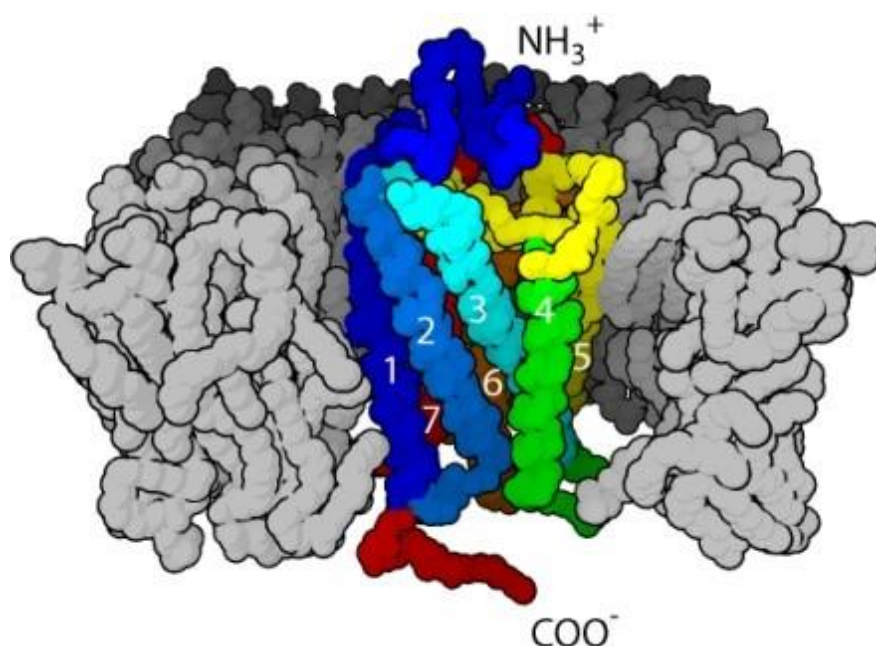


Figure 1. The structure of GPCR.

3. The signal transduction mode of GPCR (Figure 2.)

GPCRs engage in various downstream signaling pathways upon ligand binding. Activation of GPCRs leads to conformational changes that resemble the characteristics of a guanine nucleotide exchange factor (GEF), facilitating the exchange of guanosine diphosphate (GDP) bound to the G protein with guanosine triphosphate (GTP). The α subunit of the G protein, upon binding GTP, becomes active, while the β and γ subunits remain associated but separate from the α subunit. This process enables the G protein, particularly the active α subunit, to participate in subsequent signaling events. The specific downstream pathways depend on the type of α subunit, with two prominent pathways involving the second messengers cyclic adenosine monophosphate (cAMP) and phosphatidylinositol. Overall, GPCRs modulate diverse signaling cascades by activating G proteins and initiating subsequent intracellular signaling processes. This intricate mechanism contributes to the versatility and specificity of GPCR-mediated cellular responses.[11]

Within the intracellular region of the cell, there exists a binding site for G proteins. G proteins are composed of three subunits: α , β , and γ . In the resting state, the G protein is bound to guanosine diphosphate (GDP). Upon receptor activation, the GDP-bound α subunit associates with the $\beta\gamma$ complex in the cytoplasm. With the assistance of magnesium ions (Mg^{2+}), GDP is exchanged for guanosine triphosphate (GTP) in the cytoplasm. This leads to the dissociation of the GTP-bound α subunit from the $\beta\gamma$ complex, resulting in the activation of downstream effector proteins. Meanwhile, the ligand separates from the receptor. The α subunit possesses GTPase enzyme activity, which catalyzes the hydrolysis of GTP to GDP. The α subunit, along with the $\beta\gamma$ complex, reforms the inactive G protein trimer with GDP. This process restores the original resting state, ready for subsequent activation upon ligand binding.^[12] G-protein-mediated signaling pathways can be classified into three main categories, each comprising four subclasses of G proteins. The classification of these subclasses is based on the sequence homogeneity of the mediated signaling. Within each subclass, there are multiple proteins, which are encoded by different genes or may undergo alternative splicing, resulting in slight variations in their signaling characteristics. However, they can generally be categorized into these four classes. The classification is primarily defined based on the α subunit types, as there are minimal fundamental differences in signal transduction characteristics among different β - γ subunit combinations.^[13]

Functional selectivity, also known as ligand bias, plays a significant role in the activation of specific G-protein-coupled receptor (GPCR) subtypes. While a particular ligand can activate multiple G proteins, there is usually a preferred isomer that activates the $G\alpha$ subunit first. However, if the preferred isomer fails to activate the $G\alpha$ subunit effectively, an alternative isomer can be activated. Additionally, feedback pathways can lead to receptor modifications, such as phosphorylation, which can alter the preference of G protein activation. The $G\alpha$ and $G\alpha i/o$ subunits are involved in the adenosine (cAMP) signaling pathway, where adenylyl cyclase (AC) catalyzes the conversion of ATP to cAMP. Mammals have ten different AC gene products, each with slight differences in tissue distribution or function. The $G\alpha$ G protein directly activates this process, while interaction with the $G\alpha i/o$ subunit inhibits AC-mediated cAMP production. Consequently, GPCRs coupled with $G\alpha$ and $G\alpha i/o$ subunits exhibit opposing effects on cAMP production, as cAMP is considered a second messenger involved in signaling pathways, such as protein kinase A (PKA). The $Gq/11$ pathway activates phospholipase C-beta ($PLC\beta$), which catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ acts on IP₃ receptors on the endoplasmic reticulum (ER) membrane, leading to the release of Ca^{2+} from the ER. Meanwhile, DAG diffuses along the membrane, activating membrane-bound serine/threonine-specific protein kinase C (PKC). As PKC is also activated by increased intracellular Ca^{2+} levels, both pathways can interact and signal through the common secondary effector PKC. Elevated intracellular Ca^{2+} can also bind and activate proteins like calcineurin, which in turn activates the rho GTPase. Once bound to GTP, rho can further activate and regulate various proteins on a cellular level, including rho kinase (ROCK). It is worth noting that many GPCRs associated with $G\alpha_{12/13}$ subunits often show simultaneous coupling with $G\alpha q/11$ subunits, indicating an overlap and shared signaling pathways between these G protein subclasses.^[14,16]

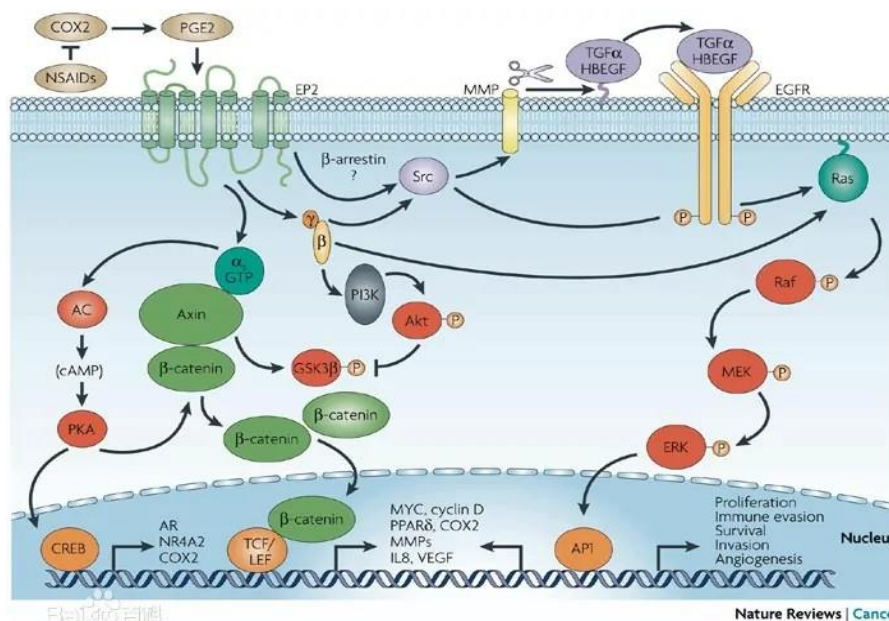


Figure 2. The signal transduction mode of GPCR.

4. The physiological role of GPCR

Recent research has elucidated the presence of G-protein-coupled receptors (GPCRs) exclusively in eukaryotic organisms, where they play crucial roles in diverse cellular signal transduction processes. GPCRs facilitate the binding of various chemicals present in the cell's external environment, initiating a cascade of intracellular signaling pathways that ultimately induce changes in the cellular state. A wide range of ligands have been identified that interact with GPCRs, including odorants, ferromones, hormones, neurotransmitters, and chemokines, among others. These ligands can be small molecules or larger biological macromolecules, such as sugars, lipids, peptides, or proteins. Notably, certain specialized GPCRs possess the ability to respond to non-chemical stimuli. For example, in photosensitive cells, GPCRs can be activated by retinol, a derivative of vitamin A, upon exposure to light. This unique characteristic enables these receptors to contribute to processes related to vision and light perception. The intricate involvement of GPCRs in diverse signaling pathways highlights their essential role in cellular communication and regulation. Continued research in this field promises to unravel further insights into the mechanisms underlying GPCR activation and signaling, opening avenues for the development of novel therapeutic strategies and drug discovery.[7]

G-protein-coupled receptors (GPCRs) play a pivotal role in numerous physiological processes, exemplified by the phenomenon of photosensitivity. A prominent example is found in the retina, where a diverse class of GPCRs exhibits photosensitivity. These receptors possess the remarkable ability to convert electromagnetic radiation signals into chemical signals within cells, a process known as photoisomerization. The intricate mechanism underlying this process involves the covalent binding of the visual protein, opsin, with the cofactor retinal. When exposed to light, retinal undergoes a molecular isomerization from a "cis" to a "trans" configuration. This isomerization event triggers a cascade of reactions that culminate in the activation of the G protein coupled to the visual protein. Consequently, downstream signaling processes are initiated, leading to visual perception. In addition to photosensitivity, GPCRs are also involved in olfaction. Within the nasal cavity, the olfactory epithelium and olfactory receptors are distributed, encompassing a wide array of olfactory receptor types. These receptors have the ability to detect and differentiate various odor molecules, facilitating the perception of smell. The roles of GPCRs in photosensitivity and olfaction exemplify their significance in sensory processes. The intricate signaling mechanisms and specific distribution of these receptors enable organisms to perceive and respond to environmental cues, contributing to their

survival and overall well-being. Continued research in this field will further enhance our understanding of GPCR-mediated sensory processes and their implications in human health and disease.[9] Behavioral and emotional regulation: G-protein-coupled receptors (GPCRs) play a crucial role in regulating behavior and mood in the mammalian brain. Various neurotransmitter receptors, including serotonin, dopamine, γ -aminobutyric acid (GABA), and glutamate receptors, belong to the GPCR family. These receptors modulate neurotransmission and contribute to the regulation of behavioral and emotional processes.

Regulation of the immune system: GPCRs are involved in the regulation of the immune system. Many chemokines, important signaling molecules in immune responses, function through GPCRs. Additionally, GPCRs such as leukocyte interleukin receptors and histamine receptors are implicated in inflammatory and allergic reactions, participating in the modulation of immune responses.

Regulation of autonomic nervous system: The GPCR signaling pathway plays a role in regulating the activity of the sympathetic and parasympathetic branches of the autonomic nervous system. In vertebrates, this regulation affects various physiological functions, including blood pressure, heart rate, and digestion. GPCRs are involved in controlling the activity of neurons responsible for sensory perception and the parasympathetic system, contributing to the self-regulation of these physiological processes.

Cell density regulation: Recently, a GPCR with lipid kinase activity was discovered in certain bacteria, such as *Pseudomonas aeruginosa*. This receptor is involved in the regulation of quorum sensing, a mechanism by which bacteria communicate and coordinate behavior in response to changes in cell density. The GPCR helps regulate the induction of specific behaviors and gene expression in bacteria in response to cell density changes.

These examples highlight the diverse roles of GPCRs in various regulatory processes, ranging from behavior and immune responses to autonomic nervous system control and bacterial cell density regulation. Understanding the intricate functions of GPCRs in these contexts can provide valuable insights into the underlying mechanisms of physiological and pathological processes.[16]

5. Conclusion

G-Protein Coupled Receptors (GPCRs) represent a fascinating and essential class of cell membrane receptors that regulate a plethora of physiological processes. Through their diverse ligand-binding capabilities and intricate signaling mechanisms, GPCRs play pivotal roles in sensory perception, neurotransmission, hormone regulation, immune responses, and cardiovascular function, among other vital functions.

This comprehensive review has provided valuable insights into GPCR biology, highlighting their structural characteristics, activation mechanisms, and physiological significance. By examining the intricate interplay between GPCRs, G-proteins, and downstream signaling pathways, we have gained a deeper understanding of how these receptors transmit extracellular signals to elicit specific intracellular responses. It is worth noting that the clinical importance of GPCRs as therapeutic targets. Numerous drugs currently in clinical use modulate GPCR activity, underscoring the significance of understanding GPCR function for the development of effective pharmacological interventions. Further research in this field holds the potential to revolutionize drug discovery and personalized medicine, leading to more precise and targeted therapies.

As we continue to unravel the complexities of GPCR biology, it is evident that these receptors represent a promising frontier for future research and innovation. By harnessing our knowledge of GPCR structure, function, and signaling, we can deepen our understanding of human health and disease, opening new avenues for therapeutic strategies.

In conclusion, GPCRs occupy a central position in cellular signaling and are integral to the maintenance of physiological homeostasis. This review has shed light on their significance and provided a framework for comprehending their diverse roles. By further investigating GPCR biology,

we can unlock their full potential for advancing human health and improving patient outcomes in various disease contexts.

References

- [1] Ritter, S. L., & Hall, R. A. (2009). Fine-tuning of GPCR activity by receptor-interacting proteins. *Nature reviews. Molecular cell biology*, 10(12), 819–830. <https://doi.org/10.1038/nrm2803>
- [2] Stevens, R. C., Cherezov, V., Katritch, V., Abagyan, R., Kuhn, P., Rosen, H., & Wüthrich, K. (2013). The GPCR Network: a large-scale collaboration to determine human GPCR structure and function. *Nature reviews. Drug discovery*, 12(1), 25–34. <https://doi.org/10.1038/nrd3859>
- [3] Hauser, A. S., Attwood, M. M., Rask-Andersen, M., Schiöth, H. B., & Gloriam, D. E. (2017). Trends in GPCR drug discovery: new agents, targets and indications. *Nature reviews. Drug discovery*, 16(12), 829–842. <https://doi.org/10.1038/nrd.2017.178>
- [4] http://www.360doc.com/content/17/1130/17/35667241_708686423.shtml
- [5] https://baike.baidu.com/pic/G%E8%9B%8B%E7%99%BD%E5%81%B6%E8%81%94%E5%8F%97%E4%BD%93/9495289/0/0823dd54564e9258c4793c639c82d158cdbf4ee9?fr=lemma&fromModule=lemma_content-image&ct=single#aid=1&pic=6a600c338744ebf8f2410dc2def9d72a6059a71f
- [6] Rajagopal, S., Rajagopal, K. & Lefkowitz, R. J. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nature Rev. Drug Discov.* 9, 373–386 (2010).
- [7] Kobilka, B. K. & Deupi, X. Conformational complexity of G-protein-coupled receptors. *Trends Pharmacol. Sci.* 28, 397–406 (2007).
- [8] Chien, E. Y. et al. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* 330, 1091–1095 (2010).
- [9] Shimamura, T. et al. Structure of the human histamine H1 receptor complex with doxepin. *Nature* 475, 65–70 (2011).
- [10] Rask-Andersen, M., Masuram, S. & Schiöth, H. B. The druggable genome: evaluation of drug targets in clinical trials suggests major shifts in molecular class and indication. *Annu. Rev. Pharmacol. Toxicol.* 54, 9–26 (2014).
- [11] Christopoulos, A. et al. International Union of Basic and Clinical Pharmacology. XC. Multisite pharmacology: recommendations for the nomenclature of receptor allosterism and allosteric ligands. *Pharmacol. Rev.* 66, 918–947 (2014).
- [12] Solari, R., Pease, J. E. & Begg, M. Chemokine receptors as therapeutic targets: why aren't there more drugs? *Eur. J. Pharmacol.* 746, 363–367 (2015).
- [13] Cromie, K. D., Van Heeke, G. & Boutton, C. Nanobodies and their use in GPCR drug discovery. *Curr. Top. Med. Chem.* 15, 2543–2557 (2015)
- [14] Wootten, D., Miller, L. J., Koole, C., Christopoulos, A. & Sexton, P. M. Allostery and biased agonism at class B G protein-coupled receptors. *Chem. Rev.* 117, 111–138 (2017).
- [15] Law, V. et al. DrugBank 4.0: shedding new light on drug metabolism. *Nucleic Acids Res.* 42, D1091–D1097 (2014).
- [16] Ahlgren, N. A., Ren, J., Lu, Y. Y., Fuhrman, J. A. & Sun, F. Alignment-free d2* oligonucleotide frequency dissimilarity measure improves prediction of hosts from metagenomically-derived viral sequences. *Nucleic Acids Res.* 45, 39–53 (2017).