An Online Biased Signaling Atlas

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Biased signaling is a paradigm in signal transduction whereby hormones, probes, or drugs bind the same receptor but engage different intracellular signaling pathways leading to distinct functional outcomes. Whereas there is a wealth of knowledge of bias signaling, it is scattered throughout literature and the vast majority of signaling pathways still lack a biased tool compound and functional annotation. Here, we provide an online Biased Signaling Atlas (https://BiasedSignalingAtlas.org) letting any researcher swiftly navigate 9,000 ligand bias datapoints and 640 functional/therapeutic annotations of signaling pathways, analyze ligand-bias relationships, download machine learning-ready data, select tool compounds, and calculate consistent bias values. We invite the global community to together advance biased signaling by depositing its data into the shared repository. This provides a common hub for the global research community to jointly explore the principals of signal transduction and to translate mapped molecular mechanisms to design drugs with better efficacy and safety.

'Biased signaling' is a molecular mechanism allowing specific ligands to accomplish functional selectivity through a single receptor by steering its signaling into different intracellular pathways (Fig. 1). This allows physiological processes to be modulated differentially by alternative endogenous ligands. For example, the chemokine[1], opioid[2], PACAP[3], protease-activated[4], serotonin[5], and PTH[6] receptor systems have evolved multiple endogenous agonists eliciting biased signaling (relative to the principal endogenous ligand).

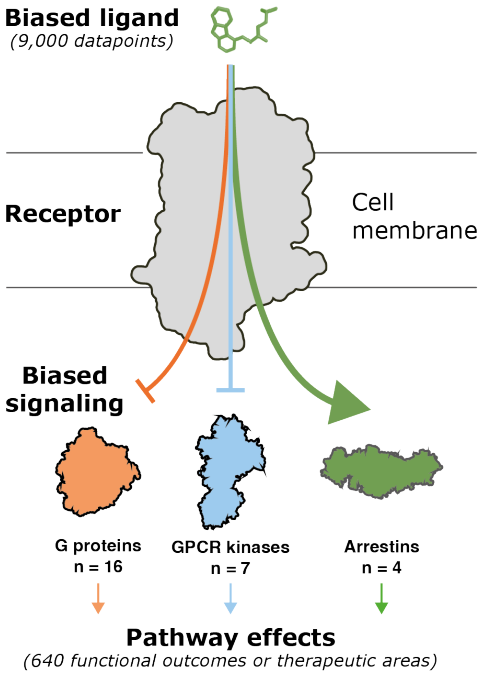

Fig. 1 | Biased signaling. A biased ligand steers receptor signaling towards one out of several pathways leading to a distinct physiological or therapeutic effect. Text in italics denote data curated from 269 scientific articles.

Furthermore, drugs can exhibit biased signaling letting them favor therapeutic over adverse signal pathways[7,8]. Hence biased signaling opens new opportunities to increase drug efficacy and safety and to target each pathway separately. One-third of drugs[9] and two-thirds of hormones modulate G protein-coupled receptors (GPCRs). For GPCRs, biased signaling is classically studied for the six transducer families: the G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$ G protein families, GPCR kinases and arrestins[10] (Fig. 1). However, recently the scope has been greatly expanded to bias among any of the 27 specific transducer subtypes – 16 G proteins, 7 GRKs and 4 arrestin proteins – which can differ in their functional outcome[11-15]. For example, a new study has demonstrated that the selective Adenosine A_1 receptor agonist benzyloxy-cyclopentyladenosine preferentially activates G_{ob} among and achieves analgesia while avoiding sedation, bradycardia, hypotension or respiratory depression[16].

Ligand bias entails very complex pharmacology, as it must be separated from experimental system bias, compares two pathways and two ligands, and confers physiology-bias, pathway-bias, or benchmark-bias depending on the reference ligand used[10]. For example, there is controversy whether the drug Oliceridine (approved by FDA in 2020) is a biased ligand at the μ-opioid receptor[17]. Recent community guidelines strive to improve reproducibility of ligand bias studies[10], but tremendous work lays ahead to identify lacking pathway-selective probes/drugs and map therapeutically relevant signaling pathways. These challenges require consolidated efforts across the global signaling and drug discovery communities, but they lack a common platform to integrate, overview, and analyze the mounting multifaceted data. Here, we provide an online Biased Signaling Atlas (Fig. 2-4) that allows any researcher to tap into present data, address several scientific questions (Table 1) and deposit data for wider dissemination in the community hub.

Biased Signaling Atlas access and sections

The Biased Signaling Atlas features retrieval of biased ligand and pathway effect data, analysis of ligand-bias relationships, study design, bias calculation, and data deposition. It is available via its web site (https://BiasedSignalingAtlas.org) and the related field hubs, GPCRdb[18], GproteinDb[19] and ArrestinDb[20]. The Biased Signaling Atlas spans five sections GUIDELINES, BIASED LIGANDS (for transducer families/subtypes), PATHWAY-PREFERRING LIGANDS, PATHWAYS, and DATA DEPOSITION comprising in total 21 different data and tool pages (Fig. 2) described in the following sections.

Guidelines and bias calculation

To aid those entering the biased signaling field or seeking advice on how to design and report complex ligand bias experiments, the first section, GUIDELINES provides a page '*Community guidelines paper*' referencing recent community guidelines for GPCR ligand bias from the authoritative International Union of Basic and Clinical Pharmacology[10]. A key study design consideration is to choose a reference ligand that can support the given scientific question. Biased signaling in the context of physiology, a pathway, or a compound of special interest requires a reference that is an endogenous agonist, pathway-balanced ligand, and user-selected drug/tool compound, respectively. Help to select the optimal reference ligand for bias is provided in a page '*Reference ligand selection'* with links to relevant ligand resources.

For the reporting, a key consideration is how to quantify ligand bias factors, as it can be challenging to make correct calculations across ligands and pathways leading to inconsistent or even incorrect literature reports[21,22]. Therefore, the GUIDELINES section also provides an accessible spreadsheet template to calculate ligand bias factors using predefined formula and a minimum set of pharmacological parameters – potency, and efficacy, or signal transduction coefficient (Emax and EC_{50}, or τ/K_A values defined in[10]).

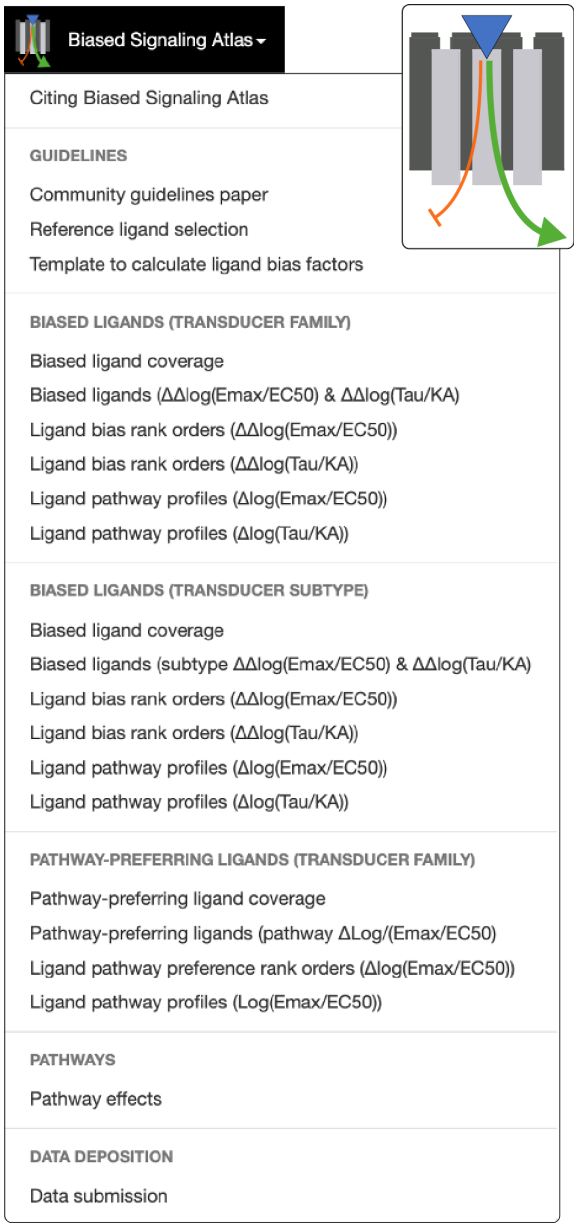

Fig. 2 | Data and tools in the Biased Signaling Atlas. The different sections and pages in the Biased Signaling Atlas, and its logotype.

Biased ligands

The BIASED LIGANDS (TRANSDUCER FAMILY / SUBTYPE) sections present biased ligands for transducer families (e.g., $G_{i/o}$ vs. $G_{q/11}$) and subtypes (e.g., G_{i1} vs. G_o), respectively. These data and tools build on a unique dataset of 8,956 ligand-receptor-pathway activities curated from 212 literature reports. The ligand bias data is greatly expanded by incorporating publications that, although not presented as biased signaling studies, contain the minimum information to calculate bias – a ligand with potencies and efficacies at two pathways. To increase the comparability of results, all bias comparisons are restricted to the same or similar cell lines and pathway transducer/downstream levels (Methods and Tables S1-5) and have consistent bias factors using the predominant quantification models (relative activity ($\log(Emax/EC_{50})$) or operational model $\log(\tau/K_A)$). By using alternative reference ligands from the same study, the atlas uniquely covers different types of bias: *i)* pathway-bias (vs. pathway-balanced ligand), *ii)* physiology-bias (vs. endogenous agonist), and *iii)* benchmark-bias (vs. user-selected drug/tool compounds) (*mid* and green in Fig. 3). This enables different studies investigating physiological, therapeutic, and experimental aspects, respectively of biased signaling.

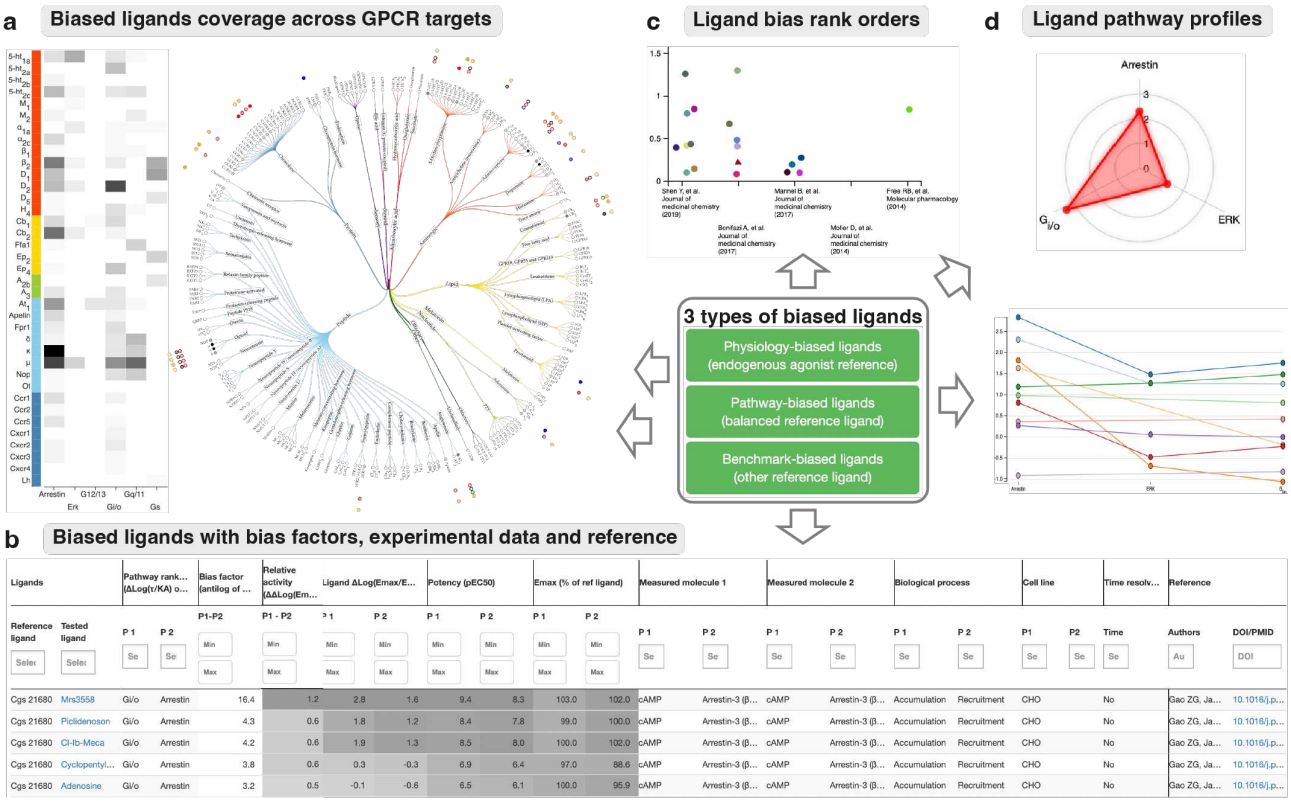

Fig. 3 | Biased ligand data, analysis, and visualization. a, The *Biased ligand coverage* page shows the distribution of physiology-biased or pathway-biased ligands across GPCRs and pathways as interactive heatmap and circular tree representations. **b**, The *Biased ligands* browser presents ligands with quantitative or qualitative bias values, underlying pharmacological parameters, experimental details, and original references. **c**, The *Ligand bias rank orders* tool visualizes the relative bias of ligands as a scatter plot. **d**, The *Ligand pathway profiles* visualizes the relative pathway responses of ligands as line charts or radar plots.

The '*Biased ligand coverage'* page (Fig. 3A) shows the distribution of physiology-biased or pathway-biased ligands (bias factor >5) across GPCRs and pathways. The top of the page displays biased ligand counts, which is a tabulation of the overall numbers of biased ligands and GPCRs with a biased ligand, respectively across the human GPCR classes. Currently, this spans 180 pathway-biased ligands for 234 distinct receptors and 388 physiology-biased ligands for 223 GPCRs across the classes A, B1 and C. This is followed by visualization of the biased ligand coverage for each GPCR class and pathway as either a heatmap or a circular tree which further classify receptors into families sharing endogenous ligands (as a coloring option and tree branches, respectively). To facilitate use in publications and presentations, these can be downloaded as images with the option of interchanging between receptor names from UniProt[23] or the International Union of Basic and Clinical Pharmacology (www.guidetopharmacology.org/nomenclature.jsp).

The *'Biased ligands'* browser (Fig. 3B) presents ligands with quantitative or qualitative bias values. To aid the selection of any receptor of interest with a minimum number of biased ligands, the preceding receptor selection page lists the number of pathway-biased and physiology-biased ligands. Currently, the receptors with the largest numbers of pathway-biased ligands (relative to a pathway-balanced reference ligand) are the Adenosine A_3, δ-opioid, Cannabinoid 1, Dopamine D_2 and μ-opioid receptors (27, 25, 21, 11, and 8 such ligands, respectively). Furthermore, the receptors with the largest numbers of physiology-biased ligands (relative to an endogenous reference ligand) are the μ-opioid, κ-opioid, Dopamine D_2, β_2-adrenoceptor, and Cannabinoid 2 receptors (43, 42, 40, 34, and 25 such ligands, respectively). To aid researchers in selecting ligands for new studies based on validation and availability, the *Biased ligands* browser provide reference and laboratory counts and vendors for tested biased ligands. The browser then details consistent and structured bias information (pathway rank orders, bias factors, specific pathways, operational model bias, and relative activity bias) together with the supporting underlying pharmacological parameters (ligand $\Delta\text{Log}(E_{max}/EC_{50})$ values, ligand $\Delta\text{Log}(\tau/K_A)$ values, potency (pEC_{50}) and Emax (% of reference ligand)). Finally, it lists key experimental details (measured molecules, biological process, cell lines, and time resolution) with a reference to the original paper for further information.

Ligand bias rank orders (Fig. 3C) visualizes the relative bias of ligands as a scatter plot. Similarly, *Ligand pathway profiles* (Fig. 3D) visualizes the relative pathway responses of ligands (without a reference ligand and hence not bias) as line charts or radar plots. Both tools support two complementary scientific use cases – comparison of different ligands from the same study or of one ligand across publications – and image download.

Pathway-preferring ligands

The PATHWAY-PREFERRING LIGANDS section describes each ligand's activity across pathways and contains the same four data and tool resources as the two *Biased ligands* sections (above). However, ligand pathway-preference differs from ligand bias by using no reference ligand and therefore cannot distinguish bias introduced by the ligand or system (biological, biosensor, experimental setup etc.)[10]. A common use case for ligand pathway-preferences is selection of a reference ligand for ligand bias studies. Here, the ligand browser has a special utility as it provides information to reduce the impact of system bias by focusing on same or near-identical systems and assays. Another advantage of this browser, over often used mere fold potency measures, is that it presents relative activities ($\Delta\log(E_{max}/EC_{50})$) accounting for differing efficacies, which can substantially influence signaling responses. An alternative uses case is rank ordering of ligands when it is not possible to define a fixed reference point because a suitable reference ligand is lacking. This type of ranking is possible through the bias rank order and ligand pathway profile tools.

Pathway effects

The PATHWAY EFFECTS section provides a functional annotation of signaling pathways spanning physiological responses, therapeutic effects, or adverse effects (Fig. 4). So far, the featured browser covers 638 pathway effects (outcomes or therapeutic areas) from 57 literature reports with diverse experiments whereof a subset has used a ligand. These data may inform rational drug design targeting 'on-pathways' while avoiding 'off-pathways' analogous to how 'on-target' and 'off-target' proteins mediate therapeutic and adverse effects, respectively. However, this represents only a fraction of known G protein[24] and arrestin couplings[20] (GPCR kinases lack systematic profiling) – necessitating new data deposition as more pathways gain functional characterization (below).

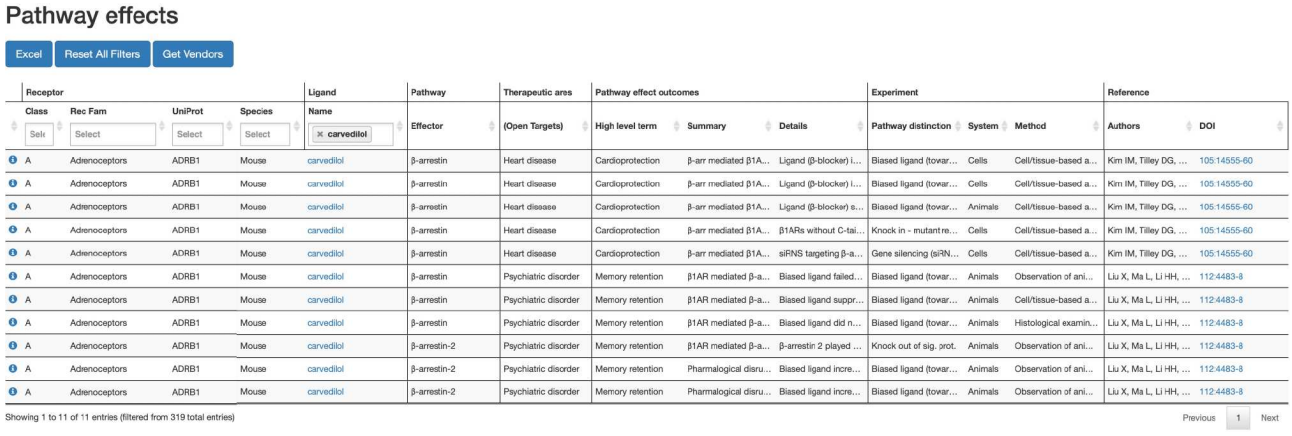

Fig. 4 | Pathway effects browser. The *Pathway effects* browser provides functional outcomes, therapeutic relevance, experimental details, and original references for further information. Which receptors are capable of signaling through which transducer, and hence have the theoretical capacity to elicit bias (compared to a single transducer receptor), can be answered by browsing the coupling pages of the GproteinDb[19], and ArrestinDb[20].

Data deposition

Our annotation of pathway effects is the most comprehensive to date and covers in total 29 GPCRs with pathway effects (see the online atlas for details). Given that there are 108 GPCR with approved drugs, at least 66 additional receptors in clinical trials, and 224 non-olfactory GPCRs with broad untapped therapeutic potential[9] it would be extremely valuable for the biased signaling community to expand the coverage (and database deposition) of pathway effect outcomes and therapeutic areas. Via the DATA DEPOSITION section, we invite all researchers to submit ligand-pathway-activity and pathway-effect measurements via standardized spreadsheets. Integration in a consolidated hub will help the field to jointly progress the characterization of ligands and pathways across laboratories with complementary techniques e.g., measuring signaling at the transducer level, downstream or *in vivo*. It will also support the community to better assess reproducibility of published studies and conduct more conclusive meta-analyses. Furthermore, it offers advantages to authors and journals through consistent formatting of supplementary materials and increased publication exposure.

Insights on the biased signaling landscape of GPCR and pathway targets

We analyzed our unique datasets of 8,956 ligand-receptor-pathway activities and 319 pathway effects to gain an overview of which GPCR and pathway targets have been most extensively studied in literature (Fig. 5). It should be noted that the numbers of pathway-biased ligands and their GPCR targets presented herein are likely underestimated. Both numbers are substantially higher for physiology-bias: 32 compared to 12 receptors and up to 40 compared to 23 maximum no. ligands (Fig. 5a-b). There is also a larger number of publications with a physiological agonist (n = 78) than pathway-balanced (n = 74) reference ligand. We use a rather stringent definition of pathway-biased ligands: pathway preference (ΔLog(τ/KA) or ΔLog(Emax/EC_{50}) value) in the range from -0.2 to 0.2, and >=90% Emax in both compared pathways. For the field to identify more pathway-ligands, we recommend future studies to define and use a pathway-balanced reference ligand – even when their study concerns physiology-bias or benchmark-bias against a reference drug or tool compound.

Fig. 5 | Biased signaling landscape of GPCR and pathway targets. a-b, GPCRs with the largest numbers of pathway-biased and physiology-biased ligands, respectively. **c**, The most studied ligand-GPCR pairs (no. annotated pathway activities). **d**, GPCRs with the largest characterization of pathway effects (no. annotated data points). **e**, Distribution of annotated data across therapeutic areas (all areas annotated). **f**, Therapeutic areas of specific GPCR-pathway pairs with the highest accumulated information in literature (experimental data points). **a-f**, GPCR names are shown as UniProt[23] entry name / Guide to Pharmacology[25] receptor name (official protein name from the nomenclature committee of the International Union of Basic and Clinical Pharmacology as listed).

Which GPCRs have the largest number of biased ligands?

We investigated the top ten GPCRs with pathway-biased and physiology-biased ligands (Fig. 5a-b) revealing 16 distinct receptors, whereof the adenosine A_3, cannabinoid CB_2, dopaminergic D_2, and serotonin 5-HT_{2C} have both types of biased ligands. These receptors span in total nine receptor families defined by shared endogenous agonists: the acetylcholine (M_5), adenosine (A_3), adrenoceptor (β_2), apelin, angiotensin (AT_1), cannabinoid (CB_1, CB_2), chemokine (CCR1, NOP), dopamine (D_2), opioid (δ, κ, μ), serotonin (5-HT_{1A}, 5-HT_{2C}) receptor families – and GPR84 which is an 'orphan' receptor lacking a cognate physiological ligand. Notably, these receptors are targets of approved drugs except Apelin, CCR1, and GPR84 whereof the last two have clinical agents in phase 2[9]. The Apelin receptor has no clinical agents, but plays a key role in early development such as gastrulation, blood vessels formation, and heart morphogenesis[26]. CCR1 is responsible for affecting stem cell proliferation and antagonists are currently investigated in clinical trials for rheumatoid arthritis and chronic obstructive pulmonary disease[27]. GPR84 is an understudied receptor expressed on the

surface of immune cells currently being targeted by clinical agents in phase II trials for idiopathic pulmonary fibrosis[28].

Which ligands have been most extensively investigated for bias?

Seven out of 10 ligands with the largest number of pathway activities activate the μ-opioid receptor. This includes endogenous agonists (endomorphin-1, met-enkephalin), natural abused substances (morphine), approved medicines (buprenorphine, fentanyl, loperamide), and tool compounds (DAMGO). The exceptionally high pursuit of biased ligands for the μ-opioid receptor is due to its wide prescription for pain treatment and the often-lethal adverse effect of breathing suppression, leading to an 'opioid crisis' in the US (buprenorphine is used to treat opioid use disorder)[29]. Two additional ligands, aripiprazole and quinpirole target the D_2 receptor. Aripiprazole is an atypical antipsychotic used in the treatment of schizophrenia and bipolar disorder as well as an add-on treatment in major depressive disorder, tic disorders and irritability associated with autism (https://www.drugs.com/monograph/aripiprazole.html). Quinpirole is a psychoactive drug and tool compound modulating locomotor activity and obsessive-compulsive disorder[30]. The last ligand, CP55,940 is a synthetic tool compound stimulating the cannabinoid CB_1 receptor inducing effects similar to the naturally occurring Tetrahydrocannabinol (THC), one of the psychoactive compounds in cannabis. In all, this shows a focus of ligand bias investigations towards highly validated drug targets and clinical drugs or tools.

Which GPCR-pathways pairs been mapped to therapeutic or adverse effects?

Our annotation of 57 original reports (including those cited by major reviews[31-34]), so far, covers in total 29 distinct GPCRs that are predominantly drug targets (see the online atlas for details). As for ligand-GPCR pairs (Fig. 5c), the highest number of experiments (n=49) is observed for the μ-opioid receptor (Fig. 5d). Of the therapeutic areas, a majority of data points fall within cardiovascular disease 53% followed by analgesia/pain therapy 22%, and psychiatric disorder 16% whereas the four remaining areas comprise only 1-5% of data (Fig. 5e). This trend is mirrored in the triaging of GPCRs, pathways, and therapeutic areas (Fig. 5f). Here, cardiovascular disease spans five out of ten most prevalent receptor pathways (AT_1-arrestin, β_1-arrestin, LPA_1-arrestin, LPA_2-arrestin, and Apelin-$G_{i/o}$ family), whereas the analgesia/pain therapy and psychiatric disorder have two such pairs each (μ-arrestin and μ-G protein, and D_2-arrestin and β_1-arrestin, respectively) and nutritional or metabolic disease one GPCR-pathway entry GLP1-G_s family). The top-ranked angiotensin AT_1 receptor (37 experimental data points) is targeted by a number of antagonist drugs indicated for hypertension, diabetic nephropathy and congestive heart failure (https://go.drugbank.com/polypeptides/P30556).

Discussion

Despite that at least 53 ligand-receptor systems have been found to engage multiple endogenous agonists for the same receptor(s)[35], biased signaling in different physiological states or tissues are predominantly unexplored. Furthermore, whereas biased signaling has huge potential as a new mechanism-of-action for the design of more potent and safer drugs[7,8], so far Tirzepatide is the only approved drug described to have pathway-bias[36] (Oliceridine had too, but this has been contradicted[17]). The Biased Signaling Atlas offers a timely and critical community hub to consolidate present knowledge and enable collaboration on the characterization of ligands and pathways. Advances in one of these domains will catalyze the progress in other. New pathway-biased ligands will unlock the characterization of pathway function and therapeutic relevance. When we have learnt which pathways to target to achieve desired therapeutic effects, more resources will be invested in screening the bias of existing and new ligands.

The Biased Signaling Atlas will enable scientists from diverse disciplines, including pharmacologists, molecular biologists, cell biologists, clinicians, drug developers, medicinal and computational chemists, bioinformaticians, and data scientists, to address diverse questions (Table 1). Some typical tasks include finding reference ligands for bias experiments, quantification of ligand bias, navigation of biased ligands, assessing the experimental support for bias, prioritization ligands to be tested for bias, exploring pathway function and therapeutic relevance, and disseminating published results. Finally, the Biased Signaling Atlas can also serve as an excellent tool for teachers and students to explore and understand biased signaling mechanisms and relationships at the chemical and biological levels. We anticipate that the Biased Signaling Atlas will be a very valuable scientific resource and learning platform enabling biomedical research.

Table 1 | Questions answered by the different resources in the Biased Signaling Atlas.

Resource	Questions answered
Reference ligand selection	Which is, and where can I buy, the best reference ligand for my bias experiments?
Template for calculation of ligand bias factors	How to correctly calculate ligand bias?
Biased ligand coverage	Which receptors and pathways have biased ligands reported?
Biased ligands	Which biased ligands exist for my receptor pathway and how strong are their experimental evidence?
Ligand bias rank orders	How consistent is the relative bias of my ligand of interest across multiple studies? How does different experimental setups or parameters affect the bias?
Ligand pathway profiles	What are the relative responses of ligands across pathways and publications?
Pathway-preferring ligands	What is the pathway-preference of ligands not yet assessed against a reference ligand and which of them should be prioritized for future bias studies?
Pathway effects	Which receptor pathways have been functionally mapped, so far? Which pathways have suggested therapeutic benefits and what is the basis?
Data deposition	How do I increase the exposure when publishing ligand bias and pathway effects? How do I compare my data to that already stored in the biased signaling hub?

The only previous resource, BiasDB has only tentatively biased ligands, 727 activity datapoints (8% of those in the Biased Signaling Atlas), not been updated since 2020, and never been published in a peer-reviewed journal despite a pre-print from 2019[37]. Furthermore, it lacks quantitated bias factors, pharmacological parameters, key experimental information, and does not distinguish pathway-bias, physiology-bias, and benchmark-bias (must define a pathway-balanced, endogenous, and user-selected drug/probe reference ligand, respectively). We are committed to the long-term development of the Biased Signaling Atlas and will actively engage the community in data deposition, as we did previously for over 35,000 ligand site mutations stored in the GPCRdb[18,38].

Data availability

All data is available in the online Biased Signaling Atlas (https://BiasedSignalingAtlas.org) and GitHub (https://github.com/protwis/gpcrdb_data). Documentation is available at http://docs.biasedsignalingatlas.org.

Code availability

All open-source code can be obtained from GitHub (https://github.com/protwis/protwis) under the permissive Apache 2.0 License (https://www.apache.org/licenses/LICENSE-2.0).

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Author contributions

Conceptualization, D.E.G.; Methodology, A.J.K., and D.E.G.; Data Curation, A.S.H, E.G., K.H., L.D., and S.G.; Validation: A.J.K., D.E.G., and K.H.; Writing – Original Draft, D.E.G.; Writing – Review & Editing, all authors; Visualization, D.E.G., Funding Acquisition, D.E.G.; Software, A.J.K., A.M., and J.C.; Supervision, A.J.K., A.S.H, D.E.G., and K.H.

Competing interests

The authors declare no competing interests. After completing their contribution to this study, A.M. and S.G. moved to Novo Nordisk A/S.

Methods

Ligand bias data annotation. To facilitate unambiguous annotation and consistent formatting of ligand bias data, we designed a standardized spreadsheet for data deposition with controlled vocabulary terms, validation of cell values (e.g., integer or range only), example data and explanations. This is available in the DATA DEPOSITION section of the Biased Signaling Atlas. For publications with multiple, complementary experiments on the same ligand-receptor-pathways combination, we added a label to discriminate the experiments allowing for multiple bias factors to be calculated from the same publication, for example using different cell lines or pathway levels (transducer or downstream). Singleton pathway lacking a comparative pathway, e.g., due to no other pathway measured using a similar cell line or pathway levels (see below), were not annotated. Some special cases, including inverse agonism and non-quantitative terms, were annotated with dedicated terms and presented using quantitative bias terms in the *Biased ligand* browsers (Extended Data Table 1).

Reducing system bias by requiring similar cell lines and pathway levels. To reduce system bias (defined in[10]), we only calculate bias across pathways that have been measured using 'similar' cell lines. Cell lines were considered similar if they originate from the same species and organ or tissue (Tables S2-3). To further limit system bias, we only calculated bias across pathways that have been measured on the same "Pathway level", which was defined as either 'transducer' or 'downstream' based on the data 'Measured molecule 1/2' and 'Measured process' (Tables S4-5). In brief, if any of the measured molecules is or involves a receptor-binding transducer (a Gα, arrestin, or GRK), the pathway level was defined as 'transducer' and otherwise as 'downstream'.

Assignment of missing effector families. Several publications had measured G protein-dependent signaling without reporting which specific G protein family was investigated. In these cases, we assigned a G protein family manually if GPCR-G protein data suggested an unambiguous primary G protein family. Firstly, the GEMTA G protein coupling dataset by the Bouvier laboratory[13] was used by identifying GPCR-G protein pairs for which the log(Emax/EC_{50}) was at least 10-folder higher than the second strongest G protein family (using the max value to aggregate G protein subtypes). Secondly, we used Guide to Pharmacology entries with a single "primary transducer" G protein family.

Calculation of ligand bias factors. We filtered out publications lacking quantitative activities (EC_{50} and Emax, log(τ/Ka) or Δlog(τ/Ka) values). For publications containing both log(τ/Ka), and EC_{50} and Emax data, the ligand bias factors were calculated using the operational model (ΔΔLog(τ/KA)) rather than the relative-relative activity model (ΔΔLog(Emax/EC_{50})). If there were more than two pathways, the multiple bias factors were calculated in order of decreasing bias.

Calculation of pathway-bias (using a pathway-balanced ligand as reference). We filtered out publications lacking a pathway-balanced reference ligand – here defined as having a pathway preference (ΔLog(τ/KA) or ΔLog(Emax/EC_{50}) value) in the range -0.2 - 0.2, and >=90% Emax in both two compared pathways. For publications containing multiple pathway-balanced ligands, we used the most pathway-balanced ligand as the reference (pathway-preference closest to 0).

Calculation of physiology-bias (using an endogenous ligand as reference). We filtered out publications lacking an endogenous ligand. For publications containing multiple endogenous ligands, the reference ligand for physiology-bias was selected based on three criteria (in order of priority): principal ligand chosen over secondary, highest potency (pEC_{50}) or highest affinity (pKi) – all derived from the Guide to Pharmacology database[25]. Bias factors were calculated, as described in[10].

Calculation benchmark-bias (using a user-selected ligand as reference). "Benchmark-bias" uses as reference ligand any ligand of interest, for example a drug, or tool compound[10]. For the *Biased ligand* browsers, we implemented user-based selection of a reference ligand and calculation of bias factors on the fly.

Definition and presentation of non-quantitative ligand bias, including "modality bias". "Modality bias" is when a ligand stimulates one pathway but is a neutral antagonist or inverse agonist for another[10]. In these cases, inverse agonism was denoted by adding a minus sign before the potency (pEC_{50}) value, and the bias defined as "Modality bias", in the biased ligand browsers. Furthermore, some ligand-receptor-pathway datapoints are qualitative (not quantitative). When such an activity of a secondary pathway had been annotated as "Low activity", we show this term in the biased ligand browser and use the term "High bias" to denote the bias for the strongest pathway relative to the secondary pathway. When an activity of a secondary pathway had been annotated as "No activity", we show this term in the biased ligand browser and use the term "Full bias" to denote the bias for the strongest pathway relative to the secondary pathway.

Pathway effect data annotation. To facilitate unambiguous annotation and consistent formatting of pathway effect data, we designed a standardized spreadsheet for data deposition with controlled vocabulary terms, validation of cell values (e.g., integer or range only), example data and explanations. This is available in the DATA DEPOSITION section of the Biased Signaling Atlas. For pathway effect outcomes, we assigned a "High level term" and a therapeutic area using terms from the Experimental Factor Ontology (https://www.ebi.ac.uk/ols/ontologies/efo).

Coding framework. We used a Django Framework[39] and the packages BioPython[40], NumPy[41], SciPy[42] and Scikit-learn (http://scikit-learn.org). The backend calculates signaling bias data based on the receptor and bias type selection made by the user. Subsequently, JavaScript functions parse the data in the browser and present the visualization options and data browsers to the user. For all data browsers (biased ligands, pathway preference, and pathway effects) we applied the DataTables.js (https://datatables.net) module in conjunction with yadcf.js (https://yadcf-showcase.appspot.com) which supports sorting and filtering. The visualizations were written in JavaScript and graphical representations of line charts, scatterplots, heatmaps and phylogenetic trees were custom developed using the D3.js framework (https://d3js.org).

Extended Data Tables

Extended Data Table 1 | Special cases in biased ligand activity annotation and bias presentation.

Special case	Activity annotation in data deposition spreadsheet	Bias definition in *Biased ligand* browsers
Inverse agonism in one pathway	-pEC$_{50}$ (negative potency)	"Modality bias"
Neutral antagonism in one pathway	"No activity"	"Modality bias"
Secondary pathway activity is reported as low activity	"Low activity"	"High bias"
Secondary pathway activity is reported as EC$_{50}$ > X	"No activity"	"Full bias"

Extended Data Table 2 | Tissue/organ information used to define similar human cell lines.

Cell line name	Tissue/organ	Morphology
Human neutrophils	Blood	Neutrophils
U2OS	Bone	Epithelial
SH-SY5Y	Bone marrow	Epithelial
1321N1	Brain	Glial
HeLa	Cervix	Epithelial
HT-29	Colon	Epithelial
HCAEC	Coronary artery	Endothelial
HCF	Heart	Fibroblast
Flp-In-293	Kidney	Epithelial
HEK	Kidney	Epithelial
HEK-293	Kidney	Epithelial
HEK-293/EBNA	Kidney	Epithelial
HEK-293A	Kidney	Epithelial
HEK-293FT	Kidney	Epithelial
HEK-293S	Kidney	Epithelial
HEK-293SL	Kidney	Epithelial
HEK-293T	Kidney	Epithelial
HEK-293T/17	Kidney	Epithelial
HEK-293TR	Kidney	Epithelial
HEK-A2B	Kidney	Epithelial
HTLA	Kidney	Epithelial
HLF	Lung	Fibroblast
PC-3	Prostate	Epithelial
WM266	Skin	Epithelial
HUASMC	Umbilical cord	Smooth muscle
HUVEC	Umbilical cord	Endothelial
HUVSMC	Umbilical cord	Smooth muscle
T24	Urinary bladder	Epithelial
Human Saphenous Vein	Vein	-

Extended Data Table 3 | Tissue/organ information used to define similar non-human cell lines.

Cell line name	Species	Tissue/organ	Morphology
COS-7	*Cercopithecus aethiops*	Kidney	Fibroblast
CHO	*Cricetulus griseus*	Ovary	Epithelial
CHO-K1	*Cricetulus griseus*	Ovary	Epithelial
Mouse brainstem	*Mus musculus*	Brain	Neuronal
Mouse striatal neurons	*Mus musculus*	Brain	Neuronal
N2a	*Mus musculus*	Brain	Neuronal
STHdhQ111/Q111	*Mus musculus*	Brain	Neuronal
STHdhQ7/Q7	*Mus musculus*	Brain	Neuronal
MEF	*Mus musculus*	Fibroblast	Fibroblast
NIH3T3	*Mus musculus*	fibroblast	Fibroblast
C2C12	*Mus musculus*	Muscle	myoblast
MIN6B1	*Mus musculus*	Pancreas	beta cells
AtT20	*Mus musculus*	Pituitary gland	-
Rat locus ceruleus neurons	*Rattus norvegicus*	Brain	Neuronal
INS-1 832/3	*Rattus norvegicus*	Pancreas	beta cells
Sf9	*Spodoptera frugiperda*	Ovary	Epithelial

Extended Data Table 4 | Measured molecules and processes defined as transducer pathway level.

Measured molecule 1	Measured molecule 2	Measured process	Pathway level motivation
Arrestin (any)	Clathrin	Binding/coupling	Measured molecule is a transducer
Arrestin (any)	ProLink-tagged endosome	Internalization	Measured molecule is a transducer
Arrestin (any)	Receptor	Recruitment	Measured molecule is a transducer
Arrestin (any)	CAAX	Recruitment	Measured molecule is a transducer
Arrestin (any)	mem-linker-citrine-SH3	Recruitment	Measured molecule is a transducer
Arrestin (any)		Recruitment	Measured molecule is a transducer
GRK (any)		Phosphorylation	Measured molecule is a transducer
GRK (any)	Receptor	Recruitment	Measured molecule is a transducer
GRK (any)	Gβγ	Recruitment	Measured molecule is a transducer
GTPγS		Activation	GTPγS binds to Gα when activated
Gα (any)	Receptor	Binding/coupling	Measured molecule is a transducer
Gα (any)	Gβγ	Binding/coupling	Measured molecule is a transducer
Gα (any)	Gβγ	Dissociation	Measured molecule is a transducer
Gα (any)	p115-RhoGEF	Recruitment	Measured molecule is a transducer
Gα (any)	p63-RhoGEF	Recruitment	Measured molecule is a transducer
Gβγ	Receptor	Binding/coupling	Measured molecule is receptor
p63-RhoGEF	CAAX	Recruitment	Recruitment of molecule 1 is to Gα though molecule 2 is a membrane bound protein
PDZ-RhoGEF	CAAX	Recruitment	Recruitment of molecule 1 is to Gα though molecule 2 is a membrane bound protein
RabGAP	CAAX	Recruitment	Recruitment of molecule 1 is to Gα though molecule 2 is a membrane bound protein
Receptor	Antibody	Internalization	Measured molecule is receptor
Receptor	ProLink-tagged endosome	Internalization	Measured molecule is receptor
Receptor	FYVE	Internalization	Measured molecule is receptor
Receptor		Internalization	Measured molecule is receptor
Receptor	Radiolabeled ligand	Internalization	Measured molecule is receptor
Receptor	KRas	Internalization	Measured molecule is receptor

Extended Data Table 5 | Measured molecules and processes defined as downstream pathway level.

Measured molecule 1	Measured molecule 2	Measured process	Pathway level motivation
[32P]GTP		Activation	Not transducer
Akt (protein kinase B)		Phosphorylation	Not transducer
Alkaline phosphatase		Signaling	Not transducer
Arachidonic acid (AA)		Accumulation	Not transducer
Ca2+		Accumulation	Not transducer
cAMP		Accumulation	Not transducer
cGMP		Accumulation	Not transducer
CREB	Antibody	Phosphorylation	Not transducer
DAG		Accumulation	Not transducer
ERK		Phosphorylation	Not transducer
GIRK		Activation	Not transducer
GIRK1/2 (Kir3.1/2)		Activation	Not transducer
GIRK2 (Kir3.2)	Gγ	Binding/coupling	Not transducer
GIRK4 (Kir3.4)		Activation	Not transducer
GSK3β		Phosphorylation	Not transducer
Inositole Phosphates (IP/IP1/IP3)		Accumulation	Not transducer
Phosphoinositide (PI)		Accumulation	Not transducer
PKC		Phosphorylation	Not transducer
PLCβ3		Phosphorylation	Not transducer
RhoA	Antibody	Activation	Not transducer
Serum responce element (SRE)	Receptor	Signaling	Not transducer
Serum responce element (SRE)		Transcription	Not transducer

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