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# Screening the receptorome

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## Abstract

The term 'receptorome' is now being used to describe receptors, ion channels and transporters in the human genome that are potential drug targets. These proteins comprise a considerable fraction of the human genome, and include the G protein-coupled receptors, which are the targets for many medications. In this review, we summarize recent advances in the field, including the concept that the ultimate goal of drug discovery may not be the development of highly selective single-target drugs, the idea that potential side-effects can also be the goal of multi-target drug screening, and a discussion of the application of computational screening and public domain databases available to interested investigators.

## Keywords

receptorome, G protein-coupled receptors (GPCR), high-throughput screening, selectivity, salvinorin A, fen-phen, HERG, weight gain, database

## Screening the receptorome

We are in an era of unprecedented opportunity for drug discovery. These opportunities arise from a number of recent developments, advances and improvements in our understanding of ligand-receptor interactions. The recent completion of the sequencing of the human genome (Venter *et al*., 2001), and its almost complete annotation, has allowed a more complete appreciation of the repertoire of potential drug targets in the genome. As we learn more about the function of these molecular targets and how they are regulated, as well as how they participate in various biochemical pathways and intracellular processes, it is reasonable to expect that this new knowledge will also inform further developments in drug discovery. Additionally, the methods used to screen ligand-receptor interactions (binding and/or activation) have become increasingly miniaturized, allowing for high-throughput screening of large compound libraries and multiple receptors at a scale and pace previously unimagined. The continued 'de-orphanization' of so-called orphan receptors, for which no natural ligands are known, also contributes new potential molecular targets to our base of knowledge. In the case of G protein-coupled receptors (GPCRs) in particular, the recent publication of a crystal structure for bovine rhodopsin (Palczewski *et al*., 2000) has invigorated the field, with many molecular models of additional GPCRs based on this template now populating the literature. When combined with mutagenesis studies and judicious selection of test ligands, the modelling approach is extremely powerful in improving our understanding of ligand-receptor interactions. With this relative explosion of information, the means to manage such large quantities of information has become ever more critical. The development of biologically relevant databases of receptors, ligands and their interactions, especially those in the public domain, as well as other databases, (*e.g.* single nucleotide polymorphisms, gene expression databases, databases of interacting proteins), the linking and integration of these databases, and increasing use of *in silico* data mining methods further adds to opportunities in drug discovery.

We have recently coined the term 'receptorome' to describe that fraction of the human genome that encodes receptors, transporters and ion channels that can potentially serve as drug receptors (Kroeze *et al*., 2003a). Receptorome proteins probably constitute about 5% of the human genome (Venter *et al*., 2001). Of the classes of molecules in the receptorome, the G protein-coupled receptors (GPCRs) are of particular interest, for the following reasons: (1) GPCRs comprise a significant proportion (estimated at 3.7%) of the human genome (Roth *et al*., 2004b); (2) the blueprint for these receptors, i.e. seven transmembrane helices with three extracellular loops, three intracellular loops and extracellular N-terminus and an intracellular C-terminus, has been conserved through many phyla and through evolutionary time; thus, GPCRs are seen in bacteria, yeast, nematodes and other invertebrates, as well as in mammals (Fredriksson and Schioth, 2005); (3)

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GPCRs provide the means for transducing a variety of extracellular signals to intracellular responses, including photons, odorants, pheromones, hormones, peptides, biogenic amines, nucleotides and lipids; (4) GPCRs are the targets of many therapeutic medications, perhaps up to 50 or 60% of currently marketed drugs (Muller, 2000). In this review, we will describe some recent advances in the screening of the receptorome for novel targets of drug action, the discovery of novel agents that interact with members of the receptorome and issues that influence the best strategies for screening the receptorome.

The problem of conceptualizing 'screening the receptorome' is illustrated by diversity of the potential targets, as well as the availability of millions or more potential ligands in various chemical libraries. The GPCRs are of particular interest in drug discovery since they have already been a success story. The natural ligands for GPCRs are extremely diverse and include light, odorants, biogenic amines, peptides, hormones, and others. Many GPCRs still are categorized as 'orphan' receptors; whether these orphan receptors will provide additional targets for drug development remains to be seen. The diversity of this receptor group is increased by the existence of numerous single nucleotide polymorphisms (SNPs) (Iida and Nakamura, 2005; Iida *et al*., 2004), as well as splicing and editing variants; the exact number of these remains unknown for most, if not all, GPCRs, but the GPCR database (http://www.gpcr.org/7tm/mutation/index.html) has listed '7244 point mutations for 363 proteins extracted from 1454 articles'.

Likewise, the numbers of potential ligands in 'chemical space' is overwhelming; some estimates of the number of possible carbon-based compounds with molecular weights under about 500 daltons are as high as $10^{60}$ (Bohacek *et al*., 1996). The number of such small compounds actually present in the human body is a vanishingly small fraction of these, probably in the order of a few thousand (Dobson, 2004). These numbers imply that the odds of success in unfocused screening of random chemical libraries of millions of compounds on hundreds of potential targets are extremely low, and the cost in terms of effort and expense would be enormous. Numerous approaches have been taken to improve these odds and lower these costs, and we will illustrate some of these below.

## Selective vs non-selective drugs

The search for selective compounds that had only one molecular target was a 'holy grail' of drug discovery for many years. However, many compounds previously thought to be selective, once they had been screened against additional targets, have been found not to be as selective as first imagined. Additionally, many highly effective and useful compounds have high affinities for multiple targets, and would never have reached the market if only highly selective compounds had been chosen for further drug development. For example, the Ki database (http://pdsp.case.edu) has affinity values at 79 different targets for clozapine, and 30 of these are in the sub-micromolar range. Indeed, it may be the combination of targets, rather than one target in isolation, that makes particular compounds so effective such as clozapine (Roth *et al*., 2004a).

An interesting counter-example is given by the hallucinogen salvinorin A, a highly selective kappa-opioid receptor agonist isolated from the 'magic mint' *Salvia divinorum*, a member of the sage family. Multiplexed screening of 48 different targets in the receptorome has shown high affinity at only one target, the kappa-opioid receptor (Roth *et al*., 2002; Sheffler and Roth, 2003; Chavkin *et al*., 2004; Yan and Roth, 2004). That the biological effects of salvinorin A are mediated by the kappa-opioid receptor is supported by the observation that kappa knockout mice given salvinorin A show the same behaviour as those given vehicle alone, but that wild-type mice have an increased tail-flick latency in response to salvinorin A (Pinter *et al*., submitted). We have argued that comprehensive screening of plant-derived psychoactive compounds may provide further insight into the neurochemistry of many CNS diseases, may extend our understanding of the 'chemistry of human consciousness', and may thus lead to novel treatments for a variety of mental illnesses (Roth *et al*., 2004b; O'Connor and Roth, 2005).

## Counter-screening for side effects

The availability of comprehensive receptorome screening for potential lead compounds and drug candidates also makes possible 'counter-screening' these compounds for their potential to induce undesirable side effects, while precluding the need for costly and time-consuming ADMET studies.

### *Phen-fen and 5-HT$_{2B}$ receptors*

In the 1990s, a combination of phentermine and fenfluramine ('Phen-fen') was used as a diet drug due to its anorectic actions, and the relative lack of side effects seen in initial clinical trials. However, as phen-fen became more commonly used, there were case reports suggesting that use of the drug could be leading to valvular heart disease and pulmonary hypertension. Our group and others subsequently discovered, following a comprehensive screening of the receptorome, that these complications of phen-fen use, as well as other drugs that cause similar symptoms (e.g. pergolide, cabergoline, MDMA, ergotamine, dihydroergotamine and methysergide and its metabolite methylergonovine), were likely to be due to their high agonist activity at serotonin 5-HT$_{2B}$ receptors (Fitzgerald *et al*., 2000; Rothman *et al*., 2000; Setola *et al*., 2003; Setola *et al*., 2005), which are highly expressed in human heart valves. This has led to the suggestion that drugs with high 5-HT$_{2B}$ agonist activity not be used in clinical practice, and that potential lead candidates in drug development be 'counter-screened' at the 5-HT$_{2B}$ receptor (Rothman *et al*., 2000).

### *Atypical antipsychotics, obesity and H1 histamine receptors*

Health-threatening weight gain is a common side effect of many medications currently in use for psychiatric disorders, including

antipsychotic and antidepressant drugs (Wirshing *et al*., 1999; Wetterling, 2001). In addition to weight gain, related symptoms may include diabetes and diabetic keto-acidosis, hypertension and hyperlipidaemia (reviewed by Kane *et al*., 2004). Using the weight gain data provided in a published extensive meta-analysis (Allison *et al*., 1999), and a receptorome screen of a large number of typical and antipsychotic drugs, we discovered, by using discriminant functions analysis to separate weight-gain inducing drugs from weight-gain neutral drugs, that a likely molecular culprit for this weight gain was the H1-histamine receptor, with additional statistical contributions to this correlation with weight gain coming from α1A adrenergic and 5-$HT_{2C}$ serotonin receptor affinities (Kroeze *et al*., 2003a). Interestingly, additional compounds that have similar pharmacological profiles, i.e. high H1-histamine receptor affinity, including mirtazepine, imipramine, amitryptiline and mianserin, have also been reported to induce weight gain (reviewed by Kroeze *et al*., in press). Thus, the combination of receptorome screening with clinical weight gain data has provided useful clues to identify compounds that may carry a weight gain liability, although the biochemical mechanism or pathways that contribute to such weight gain remain unknown. Counter-screening of potential lead compounds for H1 histamine receptor affinity will be a useful and economical means to avoid compounds with potential to cause significant weight gain and other metabolic sequelae. In addition, considerable effort is being expended to discover targets for appetite suppression in humans, including various peptide (melanocortin) receptors among others, as well medications for the treatment of obesity, an increasingly important health problem, especially in the developed world.

### *HERG potassium channels*

Blockade of the HERG (Human EtheR-a-Go-go) related voltage-gated potassium channel can result in prolongation of the QT interval in the cardiac action potential, resulting in a fatal arrhythmia known as 'torsade de pointes' and many drugs currently on the market can block the HERG channel (reviewed by De Ponti *et al*., 2002; Recanatini *et al*., 2005)). While the 'gold standard' for measurement of HERG blockade remains patch-clamping, including its more high-throughput manifestations (Kiss *et al*., 2003; Guo and Guthrie, 2005; Guthrie *et al*., 2005), this method still has insufficient throughput for truly high-throughput applications such as the screening of large compound libraries. The use of membrane potential dyes and a fluorescence microplate reader has increased the throughput of HERG blockade assays considerably (Baxter *et al*., 2002), and can be supplemented with the more theoretical *in silico* pharmacophore prediction methods (Cavalli *et al*., 2002; Ekins *et al*., 2002; Pearlstein *et al*., 2003; Aronov and Goldman, 2004; Bains *et al*., 2004; Testai *et al*., 2004; Cianchetta *et al*., 2005; Sanguinetti and Mitcheson, 2005).

As can be seen from the preceding, comprehensive focused receptorome screening can allow both for the identification of molecular targets and for the identification of potential to cause side effects. This combination promises to be fruitful in future drug development efforts, especially when combined with more high-throughput technologies and *in silico* data mining, as outlined below. The ability to perform unbiased, discovery-based whole-receptorome screens strikingly parallels the widely-used whole-genome screens for gene expression afforded by microarray and SAGE (serial analysis of gene expression) methodologies.

## High-throughput screening

GPCRs transduce extracellular stimuli to intracellular responses via the coordinated action of a variety of proteins and intracellular messenger pathways. Many, if not all, of these pathways can be used in a variety of high-throughput assays (see review by Armbruster and Roth, 2005). The problem of specificity of receptor-G protein coupling can be overcome, for example, by the use of chimeric or 'promiscuous' G proteins (recently reviewed by Kostenis *et al*., 2005), which is particularly useful in the case of receptors whose coupling specificity remains unknown, including 'orphan' receptors. Truly high-throughput assays can be developed when the assays can be carried out on a miniaturized scale, and indeed, cell-based β-lactamase reporter cell assays can now be carried out in a 3456-well format in a total assay volume under 2 μl, allowing for the assay of 150 000 compounds per day (Kornienko *et al*., 2004). Additional assays have been successfully scaled down to the 1536-well format, including cAMP assays (Weber *et al*., 2004) and calcium mobilization assays (Hodder *et al*., 2004). More routinely, however, 96-well and 384-well formats are used, with a corresponding decrease in throughput. This throughput is still much higher than conventional radioligand-binding assays, lacks the disadvantages of the use of radioactivity and affords the opportunity to distinguish agonists, partial agonists, inverse agonists and antagonists. For example, in calcium mobilization assays, agonists can be distinguished from antagonists in 'dual-addition' experiments, in which test compounds are added first, and known agonists are added in a second addition; in such experiments, test compounds that are agonists activate a calcium response after the first addition, and compounds that are antagonists inhibit the agonist response after the second addition (Fig. 1).

Yeast-based assays for GPCR function have also been developed, and have the advantage that results are not confounded by the presence of endogenous GPCRs, as in mammalian cell systems. In these assays, activation of a mammalian GPCR expressed in genetically-engineered yeast leads to activation of the MAPK pathway and thus to reporter gene expression (reviewed by (Pausch, 1997; Dowell and Brown, 2002), with an interesting recent example of the application of this technology in the screening of odorants at 17 olfactory receptors (Minic *et al*., 2005)).

Notwithstanding the current 'state-of-the-art' in high-throughput screening, as well as future developments, the volume of the 'chemical space' that could be screened for receptor interactions and the number of potential targets, including their variants, argue for a focused rather than random screening of the receptorome, and this will undoubtedly be aided by *in silico* approaches, as illustrated below.

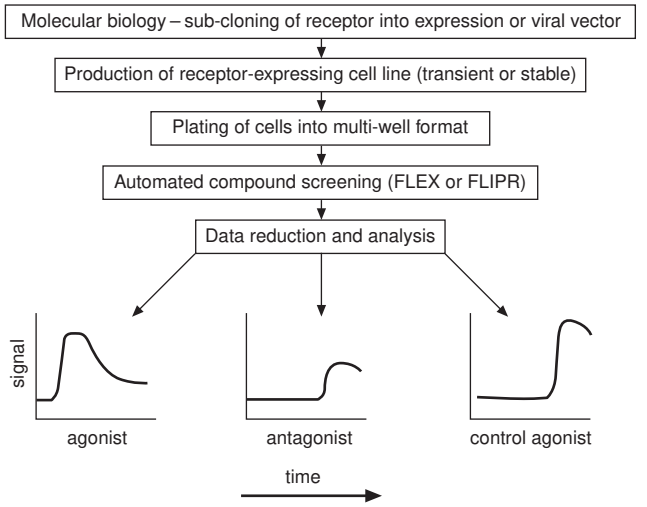


**Figure 1** Steps in the general procedure for high-throughput screening of compounds for agonist or antagonist activity. Test compounds are added in the first addition, and a control agonist is added in the second addition.

## Computational screening, databases and information management

The increasing dissemination of biological, as well as chemical, information, especially in the public domain, and the increasingly high output of the assays used to produce this information, provides both opportunites and challenges for students of the receptorome (Bajorath, 2001; Stahura and Bajorath, 2002). The tools for predicting or modelling drug-receptor interactions are becoming increasingly informative, and may be either drug- or receptor-based (see review by Lyne, 2002). Even in the absence of empirically-determined structures for most GPCRs, and in the case of orphan GPCRs, even in the absence of credible models, *in silico* approaches have been used to predict ligand–receptor interactions (see, for example, Bock and Gough, 2005). Operationally, the usefulness of a compound may be limited by its solubility and permeability, and computational methods to predict these parameters have been in existence for some time (reviewed by Lipinski *et al*., 2001).

One of the most useful resources for mining the receptorome is the NIMH-PDSP website (http://pdsp.case.edu), which currently (November 2005) has over 37 000 $K_i$ values in both searchable and downloadable formats. As illustrated above, data provided by this resource has been very useful in identifying potential molecular culprits for drug-induced side effects (e.g. H1-histamine receptor affinities and weight gain), and as the database grows, is likely to become increasingly useful for many purposes. A particularly useful feature of the PDSP-$K_i$ database is the linkage of its data with the PubMed database, and various data mining tools are also provided (http://kidb.bioc.cwru.edu/dataMining/). Additional bioinformatic resources likely to be useful in receptorome mining or *in silico* screening have been reviewed by us elsewhere (Kroeze *et al*., in press). The proteochemometric approach (Lapinsh *et al*., 2001; Lapinsh *et al*., 2002a; Lapinsh *et al*., 2002b; Wikberg *et al*., 2003; Freyhult *et al*., 2005; Lapinsh *et al*., 2005) that uses receptor sequence information, but not putative receptor structures, has also been useful in identifying ligands for certain receptors.

## Conclusion

One of the hallmarks of the post-genomic era is the widespread availability of bioinformatic data, which has allowed the identification of most members of the receptorome. In combination with additional databases, for example SNP databases (e.g. http://www.ncbi.nlm.nih.gov/SNP/), databases of potential ligand structures (e.g. those available at http://ligand.info/) and databases of $K_i$ values like the NIMH-PDSP database (http://pdsp.case.edu), we have an unprecedented opportunity to use this information to understand human disease and to develop potential drug treatments for these diseases. Integration and linkage of this information will be invaluable in reaching these goals, and will help inform focused receptorome screening and drug design as essential tools to be fully exploited.

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