Biochemistry

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Perspective

Olfactory Receptors as an Emerging Chemical Sensing Scaffold

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Cite This: *Biochemistry* 2023, 62, 187−195

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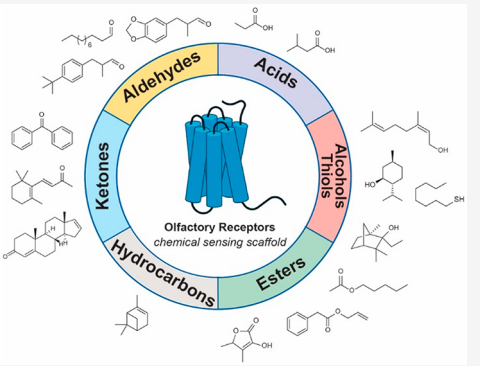
ABSTRACT: Chemical biosensors are an increasingly ubiquitous part of our lives. Beyond enzyme-coupled assays, recent synthetic biology advances now allow us to hijack more complex biosensing systems to respond to difficult to detect analytes, such as chemical small molecules. Here, we briefly overview recent advances in the biosensing of small molecules, including nucleic acid aptamers, allosteric transcription factors, and two-component systems. We then look more closely at a recently developed chemical sensing system, G protein-coupled receptor (GPCR)-based sensors. Finally, we consider the chemical sensing capabilities of the largest GPCR subfamily, olfactory receptors (ORs). We examine ORs' role in nature, their potential as a biomedical target, and their ability to detect compounds not amenable for detection using other biological scaffolds. We conclude by evaluating the current challenges, opportunities, and future applications of GPCR- and OR-based sensors.



INTRODUCTION

From monitoring blood glucose for diabetes management to detecting pathogenic viruses, biosensors are an integral part of our lives. Continuous glucose monitors rely on the enzymatic oxidation of blood glucose and measure the associated electron transfer to an electrode. Home pregnancy tests detect the presence of antibodies against human chorionic gonadotropin in urine. SARS-CoV-2 serological tests detect the presence of antibodies able to bind the SARS-CoV-2 spike protein in a nasal swab. More recently, the programmability of RNA has been exploited to detect pathogenic viruses, including Ebola[1] and Zika,[2,3] via RNA strand displacement. RNA and DNA-based detection has even reached attomolar sensitivity by coupling RNA target complementarity with CRISPR-CAS enzymology to detect COVID19.[2,4] The negative charge of RNA and the different single stranded conformations it can adopt have also been exploited to detect a variety of ions including mercury, lead,[5] and fluoride.[6] Similarly, DNAzymes have been developed for the detection of zinc[7] and lithium.[8]

Due to their size, and the nongenetically programmable recognition of their structural diversity, chemical small molecule detection remains a formidable challenge. Their small size (MW< 400 Da) results in a low availability of epitopes, making it difficult to raise or evolve antibodies against them. Although some small molecules are metabolites and have known or readily engineered enzymes to transform them into compounds that can be rapidly detected, other small molecules, such as secondary metabolites, are less amenable to direct or even coupled enzyme assays, and can only be detected via mass spectrometry. Nucleobases' ability to perform $\pi-\pi$ stacking makes nucleic acid-based aptamers particularly amenable at binding aromatic compounds, such as ampicillin, theophylline, xanthine, and adenosine triphosphate.[9] In biological systems, the remaining small molecules are detected via allosteric transcription factors (aTFs), histidine receptor kinases (HRKs), or G protein-coupled receptors (GPCRs).

Allosteric transcription factors (aTFs) bind metabolites, sugars, antibiotics, natural products, and respond to some wavelengths of light.[10−14] Upon activation, aTFs undergo a conformational change, resulting in the transcriptional activation or repression of target genes (Figure 1A). The large availability of sequenced genomes and the reduction in cost of DNA synthesis, now allows genome mining for aTFs to respond to a user-specified chemical.[15] Interestingly, aTFs cannot be simply generated by mixing and matching ligand binding and DNA-binding domains as the allosteric response is often lost,[16] but promiscuous or generalist[17] aTFs, particularly those involved in antibiotic resistance, have proven to be highly engineerable to changes in substrate specificity, including the generation of sensors for secondary metabolites.[18−20] Currently, one of the major applications of aTFs is the dynamic monitoring of intracellular metabolites for optimal carbon flux control toward improved chemical production.[21] An exciting recent application of aTFs is the study of the vertebrate microbiome *in vivo* by detecting external metabolites (for example, lactate) and cues (oxygen, pH),[22] which could be

Special Issue: Protein Engineering

Received: August 22, 2022
Revised: October 14, 2022
Published: November 1, 2022

https://doi.org/10.1021/acs.biochem.2c00486
Biochemistry 2023, 62, 187−195

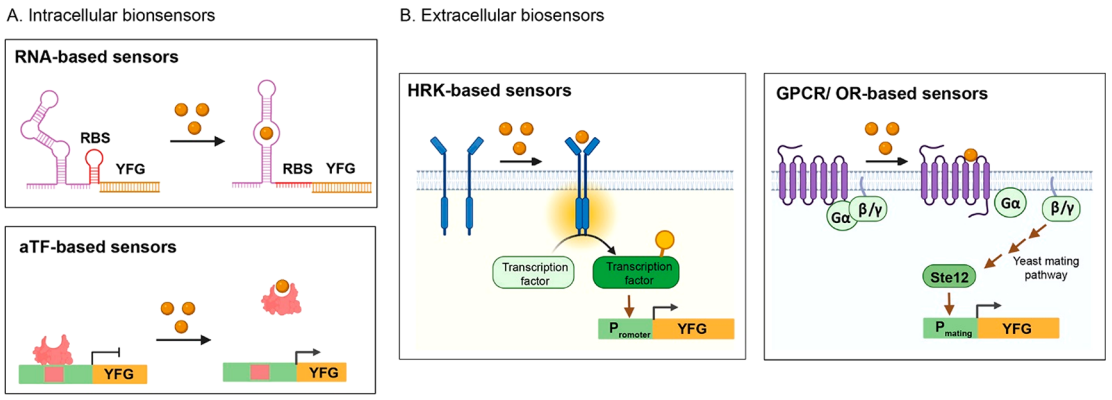


Figure 1. Types of biological sensors. A. Intracellular biosensors detect intracellular chemicals or chemicals capable of crossing the plasma membrane. Top: Example of an RNA-based sensor. The analyte (yellow sphere) binds to the RNA aptamer (purple) leading to a conformational change that unmasks the ribosomal binding site (RBS, red hairpin) enabling translation of your favorite gene (YFG). Bottom: Example of an allosteric transcription factor (aTF)-based sensor. A repressor aTF binds upstream of YFG impeding transcription. Binding of the analyte to the aTF results in a conformational change that releases aTF from the DNA enabling transcription of YFG. B. Extracellular sensors detect chemicals on the outside of the cell. Left: Example of a histidine receptor kinase (HRK)-based sensor. The analyte binds the HRK leading to the phosphorylation of an intracellular transcription factor that binds to the promoter (green) resulting in the expression of YFG. Right: Example of a G protein-coupled receptor (GPCR)/ olfactory receptor (OR)-based sensor in yeast. The analyte binds the GPCR on the yeast cell surface triggering release of G proteins ($G_{\alpha\beta\gamma}$) that go on to activate the yeast mating pathway, leading to the activation of a mating transcription factor (Ste12) that binds to a mating pathway promoter (green), resulting in the transcription of YFG.

applied to localize therapeutic bacteria to specific biological niches. Going forward, ever more sophisticated applications will be enabled by engineering aTFs with a high dynamic range, low background, and limited cross reactivity, such as the Marionette sensors,[23] to enable multilayer control of genetic circuits.

Histidine receptor kinases (HRKs) detect extracellular signals on the cell surface and use phosphorylation to relay the signal to ultimately activate a transcription factor (Figure 1A). Thus, HRK-based sensors are also called two-component systems (TCSs). HRKs tend to bind small molecules that are complementary to the mostly intracellular signals detected by aptamers and aTFs. In addition to small molecules, including antibiotics and metabolites, HRKs bind larger compounds, such as peptides and oligosaccharides, and respond to nonchemical cues, including a wider range of light wavelengths, pH and temperature.[24] Some of the most unique applications of TCSs have enabled the optogenetic control of biological systems. Most recently, to provide reversible metabolic switches for the dynamic regulation of metabolic pathways,[25] and to control chemical biosynthesis, providing insight into longevity in the model organism *Caenorhabditis elegans*.[26]

G protein-coupled receptors (GPCRs) are seven transmembrane cell surface proteins that couple to trimericG-proteins to ultimately phosphorylate a transcription factor resulting in the activation of genomic targets (Figure 1B). GPCRs are a particularly flexible chemical detection scaffold, binding compounds as small and with as few functional groups as menthol (MW 156) to compounds as large as the cyclic peptide hormone somatostatin (MW 1637). Beyond small molecules, GPCRs can detect light (rhodopsin) and protons (GPR65). Unlike other chemical detection scaffolds, GPCRs are only expressed in eukaryotes, with humans expressing ~800 GPCRs. Indeed, GPCRs are the entry point to control a number of physiologically important pathways, thus they are a key pharmacological target, with ~30% of FDA approved drugs modulating their activity.[27]

In this perspective, we review GPCR-based sensors with an emphasis on olfactory receptors (ORs), the largest family of GPCRs. In the GPCR-based sensors section, we focus on yeast-based sensors and provide a thumbnail sketch of their development. In the OR-based sensors section, we present their development and latest applications. Although ORs are the main biosensor of exogenous chemicals in animals, they remain an underexplored biosensor scaffold. A key limitation to the widespread use of ORs for chemical detection is the fact that most ORs are orphan, that is, they have no known ligands that have been identified as activating them. We delve into potential reasons for the paucity of OR deorphanization and put forth technological advances for their rapid deorphanization. Finally, we look ahead to potential future applications of these technologies.

G PROTEIN-COUPLED RECEPTOR-BASED SENSORS

GPCRs transduce chemical detection on the cell surface by coupling to G-proteins on the inside of the cell. Specifically, GPCRs signal via four G-protein families ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$),[28] resulting in changes in secondary messenger concentration that can be detected using commercial sensors for cAMP ($G_{\alpha s}$ and $G_{\alpha i}$), Ca^{2+} or inositol-1,4,5-triphosphate ($G_{\alpha q}$, G_{oq}). G-protein independent activation requires GPCR phosphorylation followed by β-arrestin recruitment, leading to the simultaneous activation of kinase pathways and receptor internalization.[29] Applications of GPCR-based sensors in mammalian cells have been recently reviewed.[30] Mammalian cells express numerous GPCRs on their cell surface (for example, HEK293 expresses 75[31]). The fact that all GPCRs couple via a small subset of G-proteins, increases the likelihood of crosstalk between GPCR signaling pathways, making it difficult to engineer GPCR-based sensors in mammalian cells, as sensors should have a specific predictable output. For sensor development applications, GPCRs are expressed in eukaryotes with a limited number of endogenous GPCRs, such as the yeast *Saccharomyces cerevisiae*.

GPCR-based sensors in yeast have been engineered by coupling mammalian GPCRs to the yeast-mating pathway, a

https://doi.org/10.1021/acs.biochem.2c00486
Biochemistry 2023, 62, 187−195

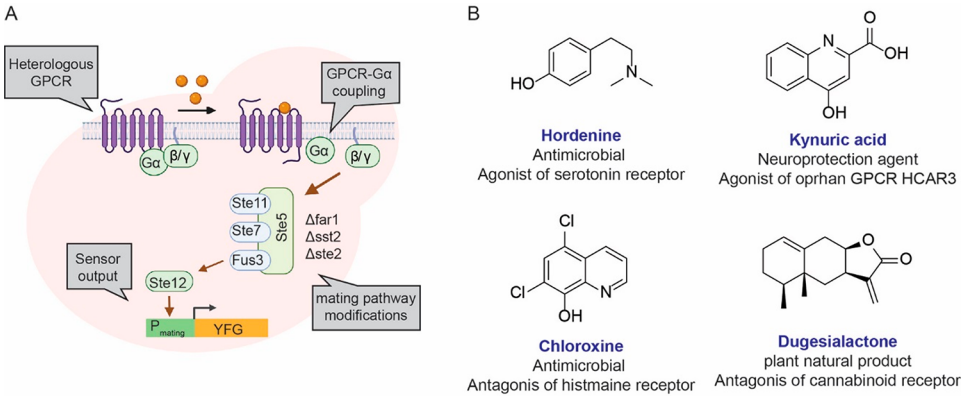


Figure 2. G protein-coupled receptor (GPCR)-based sensors in yeast and recently identified ligands via this technology. A. GPCR-based sensor schematic with nodes for sensor optimization highlighted. A summary of sensor generation and optimization strategies can be found in Table 1. B. Recently identified ligands using GPCR-based sensors in yeast. Information obtained from refs 33−36.

mitogen-activated scaffolded protein kinase cascade, ultimately resulting in transcription factor activation leading to expression of reporter genes, such as auxotrophic markers, fluorescence or luminescence reporters[32] (Figure 2A). A major application of GPCR-based sensors in yeast has been the discovery of ligands for medically important GPCRs. Recent examples include the discovery that antimicrobials activate serotonin receptor 4 in colon cells, leading to colon cell motility;[33] the identification of kynurenic acid, a neuroprotection agent, as a ligand for the orphan GPCR HCAR3;[34] the identification of a new histamine receptor 2 blocker scaffold, 8-hydroxyquinoline, that does not decompose into the carcinogen *N*-nitrosodimethylamine for the development of improved antiacids;[35] and the discovery of new ligands for the cannabinoid receptor[36] (Figure 2B). A second fertile area has been the use of GPCR-based sensors in yeast for the development of point-of-care diagnostics, such as the detection of fungal pathogens,[37] and cannabinoids in artificial bodily fluids.[36] Finally, GPCR-based sensors have been used for metabolic engineering applications, including the quantification of microbially produced serotonin[38] and melatonin.[39] Recent technical improvements to GPCR-based sensors in yeast encompass the evaluation of different promoters and vector copy numbers to modulate the sensor response,[39] the biosynthesis of cholesterol to improve human GPCR trafficking,[40] and the introduction of CRISPR landing pads in the genome for rapid sensor construction.[41]

■ OLFACTORY RECEPTOR-BASED SENSORS

Dogs can smell explosives, disease stage, and even plant invasive species.[42] Sharks use smell to navigate, localize food and predators, and mate.[43] In humans, smell is not only used to detect chemicals in the environment, but ORs are also expressed ectopically—that is, outside the olfactory tissue—where they drive specific biological processes including sperm chemotaxis, muscle regeneration, lipolysis, and blood pressure regulation.[44−48] Olfactory receptors are even overexpressed in several cancers,[49−54] including colorectal and prostate cancer, leukemia, and melanoma. Indeed, 55 ORs are highly expressed ectopically in 18 different tissues (Figure 3A). Taken together, this hints at the possibility of ORs playing a role not only as biomarkers for disease, but also as potential therapeutic targets.

Discovered in 1991 by Buck and Axel,[55] ORs are at the heart of chemical detection in animals. Humans have ∼400 functional ORs with which they can detect ∼10 000 smells.[56] Rats have the largest number of ORs, about 1200,[57] while mice have ∼1000,[58] and dogs ∼800.[57] Briefly, in the human nasal olfactory tissue, each olfactory sensory neuron (OSN) expresses a different OR on their cilia.[59] Humans have about 25 cilia per OSN, while dogs have hundreds of cilia per OSN enabling detection of lower chemical concentrations.[42] Chemical binding to the OSN triggers activation of $G_{\alpha olf}$, which stimulates cAMP accumulation causing cAMP-gated Ca^{2+} channels to open. The influx of Ca^{2+} triggers the opening of a Ca^{2+}-gated Cl^- channel, and the Cl^- efflux, which is the main carrier of transduction current to the brain.[60] Unlike nonsensory GPCRs that are highly selective for their ligands, ORs tend to bind a family of chemicals, and each chemical tends to be recognized by multiple ORs. The low-selectivity of ORs is what enables the combinatorial code to detect thousands of smells.[56]

The large number of ORs, and the variety of chemicals they bind, presents a treasure trove for the development of chemical biosensors. ORs tend to bind very small chemicals (<200 MW), mostly terpenes, small chain esters, acids, and aldehydes. One can envision using ORs with known ligands to streamline the generation of both biological and electronic sensors. A current limitation to this vision is the fact that the vast majority of ORs have no know ligands. Of the 350 functional human ORs,[61] only 15%, or ∼54 ORs, have known ligands[62−77] (Figure 3B).

A key challenge with developing OR-based sensors is the difficult functional expression of ORs outside the OSN. In mammalian cells, OR-based sensors have been generated by coexpressing ORs and $G_{\alpha olf}$ in HEK293 or HEK293T-derived Hana3A that stably express accessory proteins, such as receptor-transporting proteins[78] and receptor expression enhancing proteins[79] to promote OR cell surface expression. In this system, OR activation results in cAMP increases that can be linked to cell luminescence. Alternative cell lines include HeLA/Olf, where cAMP increases results in a Ca^{2+} influx that can be measure via fluorescence.[80] More recently, the ability of ORs to functionally express ectopically has led to the use of human prostate carcinoma to generate OR-based sensors.[81]

A major application of OR-based sensors in mammalian cells has been OR deorphanization, with much of our knowledge about OR-ligand pairs coming from these assays. Deorphanization of ORs has a generally low hit rate. In a recent OR deorphanization campaign in mammalian cells, 18 ORs were deorphanized by screening 394 human ORs against 73

https://doi.org/10.1021/acs.biochem.2c00486
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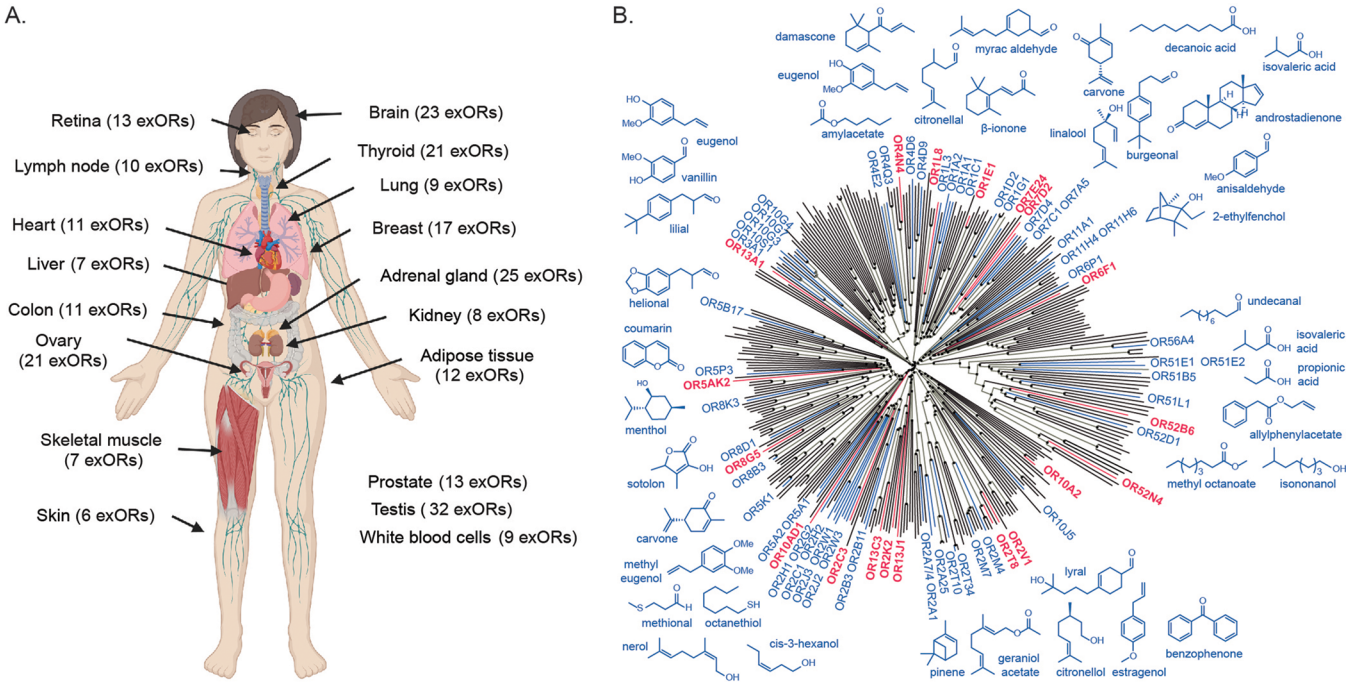

Figure 3. Ectopically expressed human olfactory receptors (ORs) and their ligands. A. Location of 55 ORs highly expressed ectopically in 18 different tissues. Information obtained from refs 46−48. B. Human ORs and their ligands. Blue: Human ORs with known ligands. Black lines: Human ORs with no known ligands. Red: Ectopically expressed human ORs with no known ligands. Information about OR ligands collected from citations 62−77. Sample OR ligands shown.

chemicals.[71,82] In addition to the large chemical space that high-throughput screens need to cover for OR deorphanization, a major challenge is the fact that it is not known whether an OR has been functionally expressed in the system unless a chemical is found to activate it. As with any mammalian-based assay, the slow doubling time (24 h), the necessity for fresh transformations, and long chemical incubation times (12 h) limits the ease of use and throughput of these sensors.

With a shorter doubling time and chemical incubation times, and an overall ease of use, OR-based sensors in yeast have opened the door to applications beyond OR deorphanization. Akin to GPCR-based sensors in yeast, OR activation is linked to the yeast mating pathway ultimately resulting in cell fluorescence[83] or luminescence.[84] In the latest OR deorphanization campaign, 2 ORs were deorphanized by screening 7 ORs against 57 chemicals.[72] The higher OR deorphanization success rate in yeast compared to mammalian cells could be attributed to the chemoinformatics-based approach to selecting ligands for high-throughput screening, favoring odorants over larger chemicals, and the availability of a large synthetic biology toolbox in yeast that allows greater control over OR copy number, transcription, and translation. Of note, as with OR deorphanization in mammalian cells, it is not known whether an OR has been functionally expressed or successfully coupled to the yeast machinery unless a chemical is found to activate it. Table 1 lists strategies to successfully generate GPCR- and OR-based sensors in yeast with citations to the most recent uses thereof. Of note, often multiple strategies are used simultaneously. Not all strategies work for all GPCRs, thus trial and error is needed to identify the strategies that work best for the GPCR at hand. Beyond deorphanization, OR-based sensors have been used for metabolic engineering applications by detecting microbially produced medium-chain fatty acids directly in the microbial broth, distinguishing strains with different levels of fatty acid production.[85]

OUTLOOK

An exciting future avenue of GPCR-based sensors in yeast is their potential for multiplex combinatorial activation. Akin to the work with aTFs in yeast,[86] orthogonal activation of multiple GPCRs in a single cell would enable the engineering of complex genetic circuitry and cell computation. Unlike aTFs that have mainly relied on antibiotics and sugars for circuit activation, GPCR-based circuitry could use hormones, neurotransmiters, or pharmaceuticals for activation. For instance, multiplex detection of pharmaceuticals could open the door to the development of "therapeutic sentinels" that continuously report on the pharmaceutical concentration in the body. If the sensor is introduced in a yeast engineered to produce the desired pharmaceutical, then the cell could in addition produce the therapeutic on-demand, maintaining optimal dosage concentration.

In principle, multiplex activation of GPCRs in yeast should be possible. Mammalian cells express numerous GPCRs on the cell surface, always ready to detect multiple inputs, integrate them and elicit the appropriate response. The entry point is regulated by GPCR-G protein interaction, with $G_{\alpha s}$-, $G_{\alpha q}$-, $G_{\alpha i}$-, or $G_{\alpha o}$-coupled GPCR interacting with their respective G proteins eliciting different secondary messengers (cAMP, Ca^{2+}, inositol triphosphate, deacylglycerol) and activating specific transcription factors or channels. A key challenge in yeast will be to mimic these entry points, in particular the distinct GPCR-G protein interaction. Most GPCR-based sensors in yeast have relied on the yeast mating pathway to transduce the GPCR signaling. Toward GPCR multiplex activation, orthogonal signaling cascades will need to be engineered in yeast. Potential starting points include refactoring mammalian

https://doi.org/10.1021/acs.biochem.2c00486
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Table 1. Strategies to Generate and Optimize GPCR- and OR-Based Sensors in *Saccharomyces cerevisiae*

nodes for sensor optimization	strategy	description	ref
heterologous GPCR	improved expression	promoter strength, gene copy number, codon optimization, Kozak sequence	39,72,83,103
	improved membrane insertion	mating factor secretion signal	36,40
		introduce first amino acids of rhodopsin into coding sequence	104
		swap N-terminus with that of Ste2	40,105
	chemical chaperone	partial ligands to aid protein folding	105,106
	membrane composition	cholesterol incorporation to mimic mammalian membrane	40
	accessory proteins	receptor transporter proteins, odor binding proteins	104
	reduced degradation	removal of intracellular loops	107
	chimeras	embed ligand binding domain in well expressed GPCR	87
GPCR/Gα coupling coupling to yeast mating pathway	alternative Gα	swap C-terminal amino acids of GPA1 with those of human Gα	34,36,108
		replace GPA1 with human Gα	84,105
yeast machinery	mating pathway modifications	deletion of key mating pathway genes	multiple, for example 39
		modulate expression of mating pathway genes	39
	sensor output	synthetic transcription factors/promoters	39,83,109
	reporter	fluorescence, luminescence, auxotrophic, colorimetric	multiple, for example36

signaling cascades in yeast, including the mammalian adenylate cyclase/cAMP cascade, which was successfully transplanted to yeast for the generation of a dinitrotoluene sensor;[87] the Tango system that leverages the recruitment of β-arrestin upon GPCR activation/phosphorylation to cleave a transcription factor that is tethered to the GPCR;[88] and the ChaCha system, where β-arrestin recruitment to the GPCR releases a CRISPR-cCas9 trasncription regulator.[89]

OR deorphanization is a necessary step toward a plug-and-play approach to the development of biosensors for chemicals that are difficult to detect using other biological scaffolds. Although purely experimental approaches to OR deorphanization are the state of the art, they are too slow, with a small number of ORs deorphanized per campaign leaving still ∼85% of human ORs orphan. Today, virtual screening approaches to OR deorphanization are limited by the lack of OR structural information. For GPCRs with known crystal structures with bound ligands,[90−92] such as the dopamine[90] or melatonin[92] receptors, virtual docking of large chemical libraries followed by experimental validation of a reduced subset has proven successful at identifying new ligands. In the case of GPCRs with known ligands and high sequence similarity to known structures (∼30%), multiple homology models together with the ligand structure have been used to identify potential binding sites prior to virtual compound docking.[93,94] Nevertheless, virtual docking using homology models remains risky.[95] Mammalian ORs have no known crystal structures (insect ORs are ion channels[96]), and share only ∼20% sequence identity to structures in the Protein Data Bank, rendering OR virtual docking a high-risk proposition. OR crystallization remains a challenge due to their poor membrane trafficking, resulting in low receptor abundance, and the lack of high-affinity ligands (OR ligands bind in the μM range[97]) to stabilize these receptors. The recent development of artificial intelligence algorithms for protein structure prediction, such as AlphaFold,[98] may enable OR virtual screening and deorphanization in the future.

Machine learning (ML) approaches have the potential to accelerate OR deorphanization by leveraging both computational and experimental data. Although ML approaches have been widely implemented in chemosensory research, that is to predict the perception response to an odorant, ML approaches to predict OR-ligand pairs have been limited to expanding the number of ligands for ORs with known ligands,[99−101] or making OR-ligand predictions without experimental validation.[99] Other computational approaches have been applied to the identification of alternative ligands for ORs with known ligands, but not to the more difficult challenge of OR deorphanization. For example, folding recognition methods for OR structure modeling followed by molecular dynamics simulation docking starting with a docked known ligand.[97] A current challenge is the nonstandardized literature data for OR-ligand pairs to train ML algorithms. Data on OR-ligand pairs has been acquired using different systems (different cell lines and conditions), and often extrapolating OR-ligand pairs in rat or mouse to humans. Although OR orthologs sometimes bind the same chemical this is not always the case.[102] What is likely needed is standardized OR-ligand data acquired for ORs from the same species, using the same assay conditions to provide ML algorithms with high-quality comparable data to appropriately featurize each OR and ligand. Trained on high-quality data, such algorithms should be able to deorphanize ORs from the experimentally tested organism and potentially, the model could be tweaked with extra data points to deorphanize ORs from different species as well.

CONCLUSIONS

The rapid generation of aTFs, TCS-, and GPCR/OR-based sensors to detect different cues foreshadows their ever-expansive use either directly by the consumer or as a tool for biotechnology and biomedical applications. As a field, the challenge has moved from the generation of chemical biosensors with no defined application, to developing biosensors that are uniquely suited to the application at hand. That is, detecting the user-specified cue at the concentration needed with the appropriate linear and dynamic range in the prespecified environment (for example, host organism). These parameters can be used as a starting point to choose the most appropriate biosensor scaffold to be constructed and optimized.

https://doi.org/10.1021/acs.biochem.2c00486
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Although several steps are necessary to unravel the expanse of OR-ligand pairs, these discoveries will support applications beyond chemical sensing to the identification of the role of ectopically expressed ORs in different tissues, and even aid research in human olfaction. Effectively bringing together *in silico*, experimental, and machine learning approaches to address the OR deorphanization challenge will ultimately allow the routine generation of OR-based sensors.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work as supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM124871. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Figures 1, 2A, and 3A, and Table of Content graphic were partially generated using biorender.com.

ABBREVIATIONS

GPCR, G protein-coupled receptor; OR, olfactory receptor; HRK, histidine receptor kinase; aTF, allosteric transcription factor; TCS, two component system; cAMP, cyclic adenosine monophosphate

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https://doi.org/10.1021/acs.biochem.2c00486
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