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GPCR-OKB: The G Protein Coupled Receptor - Oligomer Knowledge Base.

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Abstract

Background: With the substantial growth over the past few years of available data on G Protein-Coupled Receptor (GPCR) dimers/oligomers, the need for an effective system to organize this information electronically has never been greater. Based on an ontology derived from a community dialog involving experimental and computational colleagues, we developed the GPCR-Oligomerization Knowledge Base (GPCR-OKB).

Description: GPCR-OKB is an information system that supports browsing and searching for information about GPCR oligomers. The system offers information manually extracted from the literature, and curated according to the previously described GPCR-OKB ontology. GPCR-OKB is seamlessly connected to GPCR-DB, facilitating navigation back and forth between information about GPCR protomers and oligomers.

Conclusions: The GPCR-OKB system provides both experimental and computational information about GPCR oligomerization. Through data added regularly, GPCR-OKB is intended to support productive interactions between computational and experimental scientists working in the GPCR oligomerization field. This information system is freely accessible at http://www.gpcr-okb.org.

Background

G Protein-Coupled Receptors (GPCRs) are transmembrane (TM) helical proteins that transduce signals from the exterior to the interior of the cell via G-protein dependent and independent pathways [1]. Since they are the largest family of membrane proteins in mammalian genomes, and are implicated in many biological responses [2], it is not surprising that GPCRs also constitute the largest family of membrane protein targets for therapeutic compounds [3]. Although still a matter of controversy for some GPCR subtypes, the very close proximity of two or more receptors has been demonstrated unambiguously in artificial cell systems for several GPCRs using biochemical and biophysical techniques [4, 5]. The challenge now is to demonstrate that the majority of these receptor complexes exist and have functional significance in native tissue, although this information is already available for a few pairs [6].

Information supporting the existence of functionally relevant GPCR dimers/oligomers is described in multiple articles published in different journals. This creates a challenge to the researcher who is trying to gather experimental evidence and/or computational predictions about any given oligomer. Our previous work focused on the design of an ontology [7] to organize information about GPCR oligomerization, and its functional consequences in vitro and in vivo. The ontology was well received [8], demonstrating significant interest in an electronic system to access GPCR oligomer information. In this manuscript, we describe the GPCR-OKB system, which implements the GPCR-OKB ontology and an electronic front-end to information curated from more than 160 published articles (as of January 2010).

Construction and Content

We developed the GPCR-OKB system to store both experimental and computational information about GPCR oligomerization. This information is publicly accessible at http://www.gpcr-okb.org. The GPCR-OKB system supports both browsing and structured searches.

Content

The content provided in GPCR-OKB was extracted from 164 Pubmed articles published mainly during the past decade, and was recorded in the database as close to the raw experimental data as possible to preserve objectivity. For this reason, only original research papers were annotated; review articles were excluded from curation. The latter, however, were checked to ensure that data from referenced original research articles were included in GPCR-OKB. Information was organized according to the GPCR-OKB ontology [7].

Information captured

From the 164 articles processed, we extracted information about 125 GPCR oligomers. Information pertaining to the physiological relevance or to the *in vivo* existence of the oligomer, as well as details about phenotypic changes, proposed oligomerization interfaces, proposed activation mechanisms, discovery/characterization methods, and cell types were considered for inclusion into the GPCR-OKB system. Figure 1 presents the

broad types of information captured, and the relationships between them. These relationships are conveyed through web navigation in GPCR-OKB.

Software implementation

Web application. The GPCR-OKB web application was developed with the Grails web application framework. Information is stored in XML files that follow the GPCR-OKB XML schema (see http://www.gpcr-okb.org ->The Ontology ->Schema). The XML Schema was designed in agreement with the GPCR-OKB ontology [7]. XML files are versioned with a subversion source control system. The GPCR-OKB web application loads these XML files at startup, indexes text fields, and starts serving web pages.

Links with GPCR-DB. Data are exchanged between GPCR-DB [9] and GPCR-OKB to automate the creation of bidirectional navigation links. GPCR-DB exports information about protomers, which is loaded into GPCR-OKB. GPCR-OKB exports data about oligomers. All data exchanges are done in the XML format with an agreed upon XML schema.

Utility

The GPCR-OKB web user interface is organized around a gray navigation bar located at the top of each page. This navigation bar provides the “Home”, “Browse”, “Contributors”, “Disclaimer”, and “Help” categories (see Figure 2). Scroll down menus are located under these broad categories. An item called “Locating data” is provided

under “Help”, and provides quick pointers to help new users find their way around the system. “Abbreviations”, “Glossary” terms, and Frequently Asked Questions” can also be found under “Help”.

Locating Data

The information contained in GPCR-OKB can be browsed starting from any of the following concepts: “Oligomers”, “Protomers”, “Methods”, “Phenotypic Changes”, and/or “Evidence for Physiological Relevance”. For simplicity, GPCR-OKB only refers to GPCR oligomers, although the majority of published experimental studies cannot discriminate between dimers, tetramers, or higher-order oligomers. The “Search” tool at the top right side of each page (see Fig. 2) provides keyword searches across different types of information.

Search Tool

The Search tool is initially configured to search only oligomers. Selecting additional check boxes will also search protomers, methods, or restrict oligomer searches to specific types of oligomers. The “Question mark” icons are clickable to provide additional information about what the different options mean and how to use them. To search for a specific oligomer, the user specifies in the input box the name of the oligomer’s constituent protomers. For instance, if users are interested in retrieving information about an oligomer of the dopamine D2 receptor, they could enter "dopamine" or "D2" in the search box. Of note, GPCR-OKB searches are case-insensitive, and support the usual

boolean operators (e.g., “and”, “or”, “not”) as well as advanced options, such as searching by regular expression.

Oligomer names

Oligomer names are derived from the names of their constituent protomers, separated by a hyphen, in alphabetic and numerical order (Fig.2), following the recommendations of [10]. By browsing “Oligomers”, users can scroll through a list of GPCR oligomers listed in alphabetical order using this nomenclature, with the organism in which they were characterized and the GPCR family name specified. When the same combination of protomers has been studied in different species, GPCR-OKB lists these combinations as different entries (for instance, the A2A-mGLU5 oligomer has been studied in both the rat and human organisms and appears as (at least) two entries.

Oligomer categories

Users can limit their oligomer search to a fewer oligomers by checking the following options in the search tool (Fig. 2):

- "With Phenotypic Changes": users can limit their queries to retrieve only oligomers with demonstrated phenotypic changes with respect to the constituent protomers. For instance, these changes may refer to specific signaling or ligand recognition events. More detailed examples of these changes, and the description of the attributes used to characterize them for insertion into GPCR-OKB, have been reported previously in the literature [7].

- "With Proposed Interfaces": it restricts queries to retrieve only oligomers with published information about predicted interfaces of dimerization/oligomerization.
- "With In Vivo Evidence": it limits the search to Oligomers for which at least one of the following conditions is satisfied: 1) Evidence for physical association in native tissue or primary cells; 2) Knowledge of specific functional properties in native tissue; and/or 3) Information from knockout animals or RNAi technology. These conditions are NC-IUPHAR recommendations to recognize a *bona fide* functional GPCR oligomer, according to a recent report published in the literature [11].
- "Evidence for Physiological Relevance": it limits the search to oligomers for which at least two of the aforementioned conditions are satisfied.

Further cross-searches can be established by checking the “Find Protomer” and/or “Find Method” options.

Protomers

Protomers of oligomers stored in GPCR-OKB are also listed under “Browse” -> “Protomer”. No specific information about these protomers is stored in GPCR-OKB, but the database is linked to the latest release of GPCR-DB [9] for an easy access to updated information (e.g., sequences, alignments) about GPCR individual subunits, as described above. Protomers of oligomers can also be accessed by specifying the name of the constituent subunits of the oligomers in the input box of the search tool (Fig.2), and by checking the "Protomer" option.

Methods

The user can search the information contained in GPCR-OKB by specifying the computational/experimental procedures used to characterize/discover GPCR oligomers (i.e., the Methods). These include methods such as Fluorescence Resonance Energy Transfer (FRET), Bioluminescence Resonance Energy Transfer (BRET), Time Resolved FRET (TR-FRET), cross-linking, co-immunoprecipitation, Atomic Force Microscopy (AFM), and correlated mutation analysis. At the time of publication, GPCR-OKB includes information deriving from 14 different methods.

Mechanisms of Activation

GPCR-OKB also provides information in support of proposed activation mechanisms (e.g., trans- or cis-activation) of the oligomer. Specifically, where available in the literature, the user has access to information regarding structural changes upon oligomer activation, activating ligand, activated protomers, and/or GPCR/G protein stoichiometry.

Discussion

The information contained in GPCR-OKB, all linked to the corresponding original research papers, pertains specifically to GPCR oligomers. As such, the database is unique since there are no other available resources on GPCRs that provide the large amount of pharmacological, biophysical, and biochemical studies included into GPCR-OKB. Also unique to this system is the expert scrutiny that the information added to GPCR-OKB undergoes, given the quality control it receives by the scientific committee involved in

the database development and population. Notably, the information chosen for inclusion into GPCR-OKB was tailored to the specific needs of experimental and computational investigators working in the GPCR oligomerization field. Thus, GPCR-OKB is expected to be very useful to the scientific community as a whole. While specialized on GPCR oligomers, GPCR-OKB maintains bi-directional links with GPCR-DB that allow easy navigation to information about protomers as well as oligomers.

We are aware of two other databases under development that provide information about GPCR oligomerization. The GRIP-DB system [12-13] is a mainly web-based facility that offers predictions about protein-protein interaction interfaces for GPCR oligomerization. The gpDB relationship database [14-15] also provides some information about GPCR oligomerization. However, it is mainly dedicated to disseminating and storing the knowledge pertaining to GPCR interactions with partner G-proteins and effector molecules. Despite its uniqueness in providing detailed information on GPCR oligomers verified by experts (e.g., effects of oligomer specific ligands, phenotypic changes, evidence for physiological/clinical relevance, proposed mechanisms of activation, etc.), GPCR-OKB was not designed to be a universal resource on GPCRs, but to interconnect with existing databases (e.g., GPCR-DB) to enhance its value.

In the future, the GPCR-OKB will be updated on a regular basis to enrich the original dataset used for its first release in January 2010. As new information on GPCR oligomers emerges in the literature, it is possible that the relational scheme of GPCR-OKB will also need to be updated to offer better features to the users. The latter are encouraged to provide their feedback by email to help us implement improved future versions of GPCR-OKB.

Conclusions

We present the GPCR-OKB system, a web-enabled knowledge base focused on GPCR oligomers. GPCR-OKB was designed for the benefit of the scientific community working in the GPCR field, and is intended to advance and disseminate knowledge on GPCR oligomerization. The information system, which is first of its kind, relies on a published ontological scheme that resulted from a community dialog between experimental and computational investigators working on GPCR oligomerization. GPCR-OKB builds on its ontology to integrate curated experimental and computational information about GPCR dimers/oligomers. The information is reported in an objective and neutral manner to encourage productive and substantiated communication among the domain experts. GPCR-OKB is expected to be very useful for molecular biologists conducting experiments, but will also benefit computational investigators interested in studying the implication of structure and dynamics of GPCR dimers/oligomers on receptor function.

Availability and requirements

GPCR-OKB is publically accessible at http://www.gpcr-okb.org. The system has been tested with a variety of web browsers and has no specific requirements. However, Internet Explorer version 7 and below are discouraged due to known problems with CSS layouts.

Authors' contributions

GK curated the majority of the data, and helped draft the manuscript. KD developed the web application software. JS was involved in the first phase of curation. MC-A helped

with data curation, user interface design, and application testing. LS participated in web application development, and helped to finalize the manuscript. BV implemented links between GPCR-DB and GPCR-OKB. MB, LD, JAJ, SRG, MJL, GM, RN, KP, MP, JPP, and GV are members of the scientific committee scrutinizing system implementation and contents. They also contributed helpful comments/suggestions to improve the final manuscript. FC oversaw the construction of the GPCR-OKB web application, the interface with GPCR-DB, and helped write the manuscript. MF conceived the study, coordinated and personally contributed to database interface design and improvement, and helped write the manuscript. All authors read and approved the final manuscript.

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Figure captions

Figure 1. Types of information supported and navigation patterns. The main concept of GPCR-OKB is an Oligomer. Phenotypic Changes, Physiological Relevance, In vivo Evidence, Mechanisms of Activation, and Study, all are associated with each Oligomer. Oligomerization Interface and Methods concepts are linked to specific experimental/computational studies conducted on any given oligomer.

Figure 2. Sample oligomer page at GPCR-OKB. A section of the webpage annotating 5-HT2A–mGLU2 oligomer is illustrated alongside the different concepts associated with this oligomers (e.g., oligomer IUPHAR-approved and full names, Protomers, Studies, *In vivo* evidence, and Phenotypic Change). Additional concepts (not pictured) associated to each oligomer are Physiological Relevance and Mechanisms of Activation.

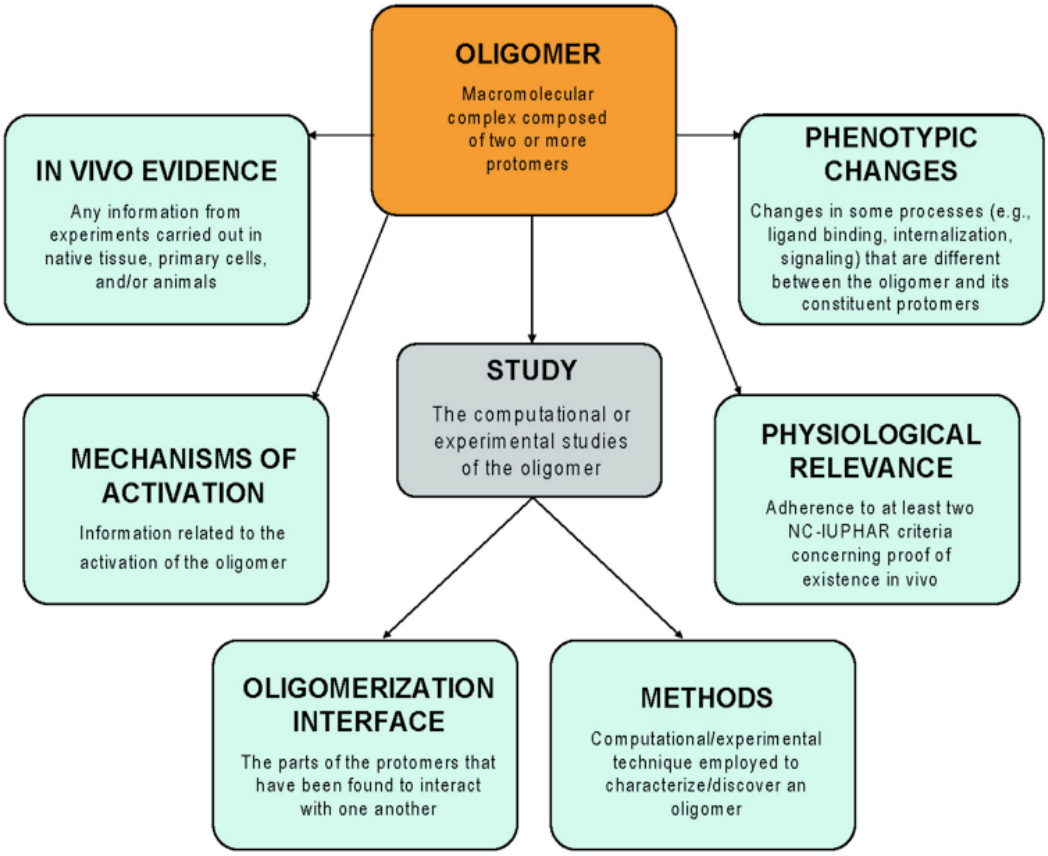
OLIGOMER
Macromolecular complex composed of two or more protomers
IN VIVO EVIDENCE
Any information from experiments carried out in native tissue, primary cells, and/or animals
PHENOTYPIC CHANGES
Changes in some processes (e.g., ligand binding, internalization, signaling) that are different between the oligomer and its constituent protomers
STUDY
The computational or experimental studies of the oligomer
MECHANISMS OF ACTIVATION
Information related to the activation of the oligomer
PHYSIOLOGICAL RELEVANCE
Adherence to at least two NC-IUPHAR criteria concerning proof of existence in vivo
OLIGOMERIZATION INTERFACE
The parts of the protomers that have been found to interact with one another
METHODS
Computational/experimental technique employed to characterize/discover an oligomer

Figure 1

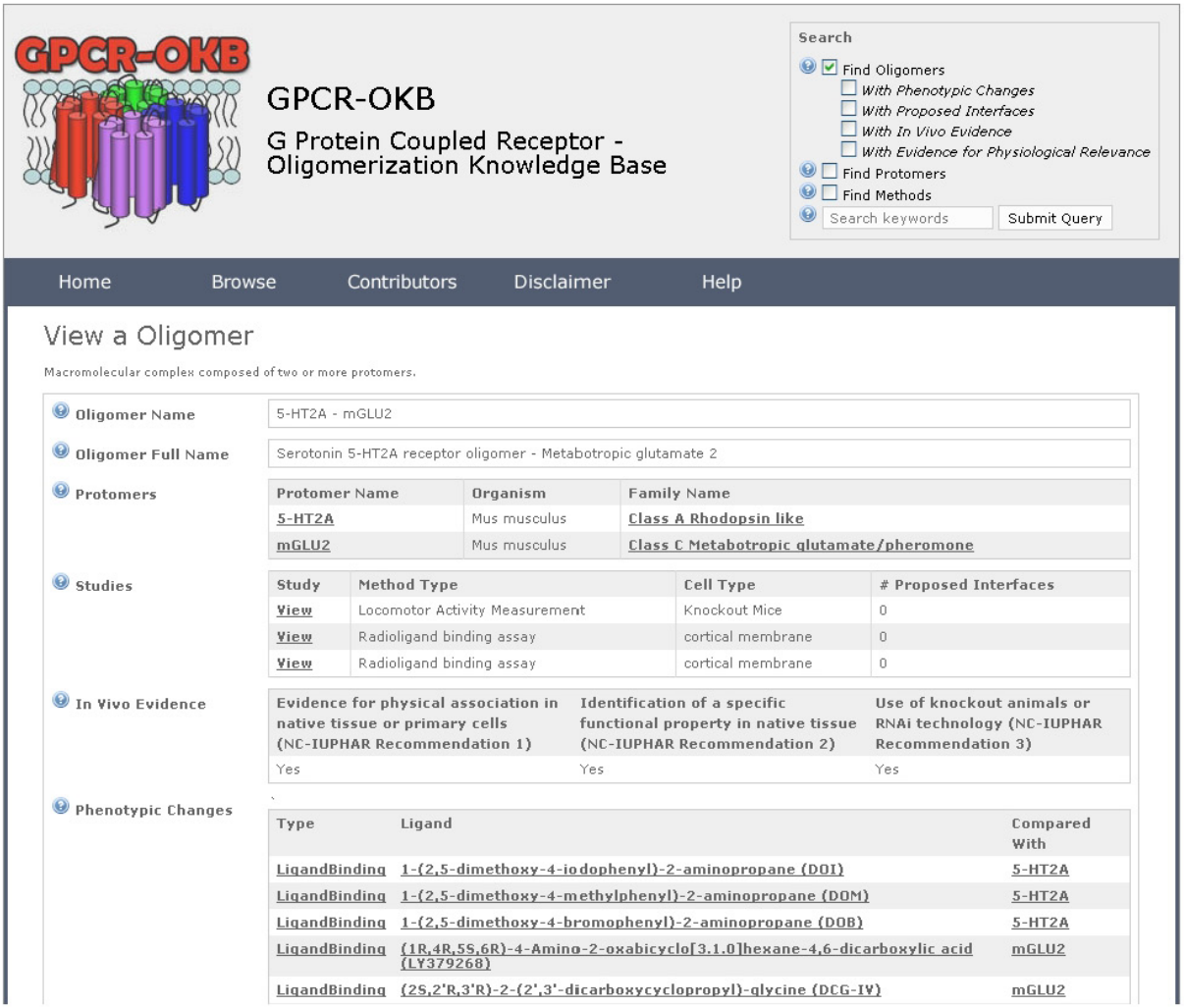
GPCR-OKB
GPCR-OKB
G Protein Coupled Receptor -
Oligomerization Knowledge Base
Search
Find Oligomers
With Phenotypic Changes
With Proposed Interfaces
With In Vivo Evidence
With Evidence for Physiological Relevance
Find Protomers
Find Methods
Search keywords
Submit Query
Home
Browse
Contributors
Disclaimer
Help
View a Oligomer
Macromolecular complex composed of two or more protomers.
Oligomer Name
5-HT2A - mGLU2
Oligomer Full Name
Serotonin 5-HT2A receptor oligomer - Metabotropic glutamate 2
Protomers
Protomer Name
Organism
Family Name
5-HT2A
Mus musculus
Class A Rhodopsin like
mGLU2
Mus musculus
Class C Metabotropic glutamate/pheromone
Studies
Study
Method Type
Cell Type
Proposed Interfaces
View
Locomotor Activity Measurement
Knockout Mice
0
View
Radioligand binding assay
cortical membrane
0
View
Radioligand binding assay
cortical membrane
0
In Vivo Evidence
Evidence for physical association in native tissue or primary cells (NC-IUPHAR Recommendation 1)
Identification of a specific functional property in native tissue (NC-IUPHAR Recommendation 2)
Use of knockout animals or RNAi technology (NC-IUPHAR Recommendation 3)
Yes
Yes
Yes
Phenotypic Changes
Type
Ligand
Compared With
LigandBinding
1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)
5-HT2A
LigandBinding
1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM)
5-HT2A
LigandBinding
1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB)
5-HT2A
LigandBinding
(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268)
mGLU2
LigandBinding
(2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)-glycine (DCG-IV)
mGLU2

Figure 2