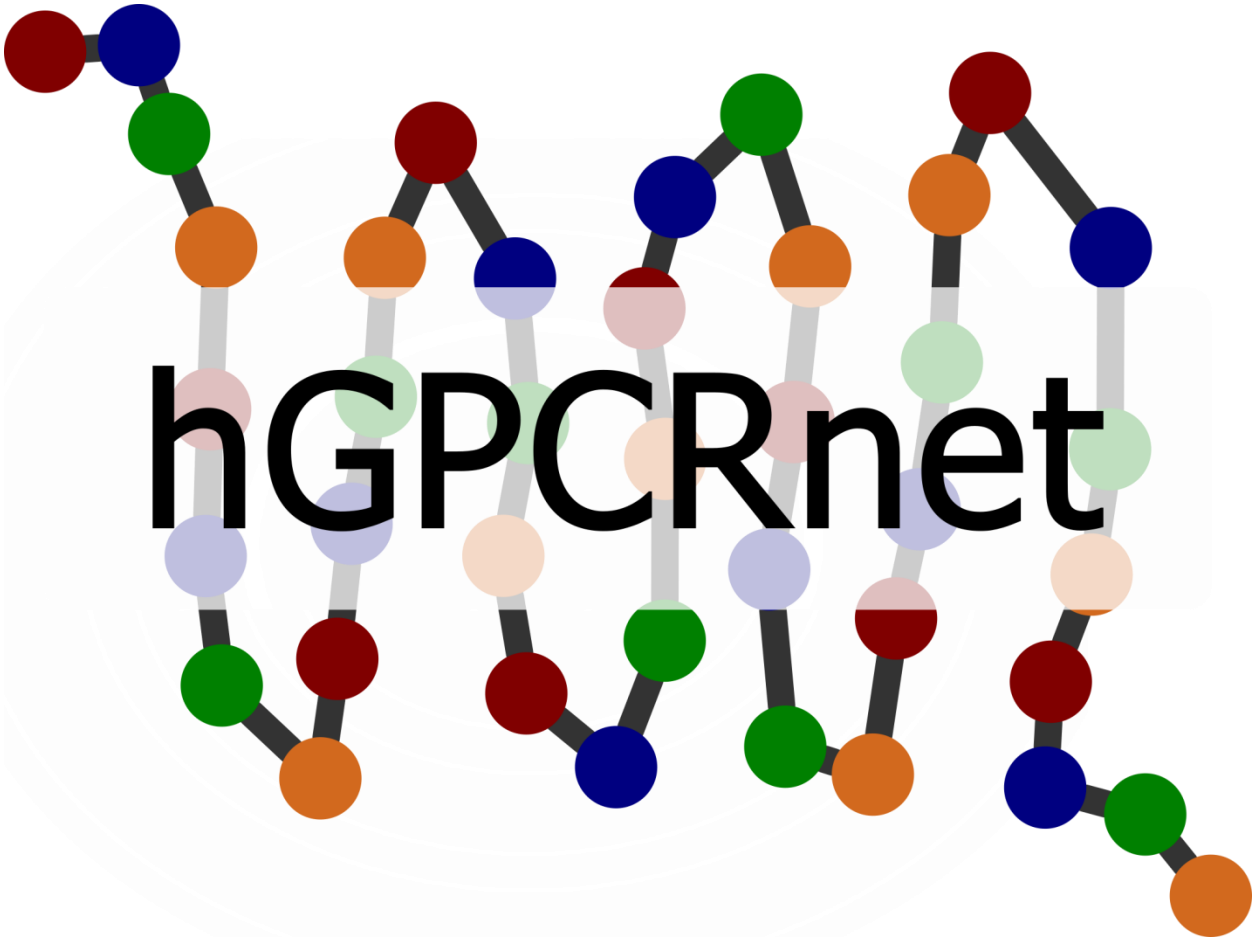
Manual



Contents

About

hGPCRnet: a freely available web application that allows users to explore the extended human GPCR network and cell-type-specific GPCR signaling pathways.

This application was created with Cytoscape.js (Version 3.3.0). Network files were generated through an automated process using Cytoscape (Version 3.7) and the py2cytoscape (0.7.0) Python library.

Publication

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The extended human GPCR network (hGPCRnet): cell-type-specific analysis of GPCR signaling pathways
In preparation

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Scientific questions

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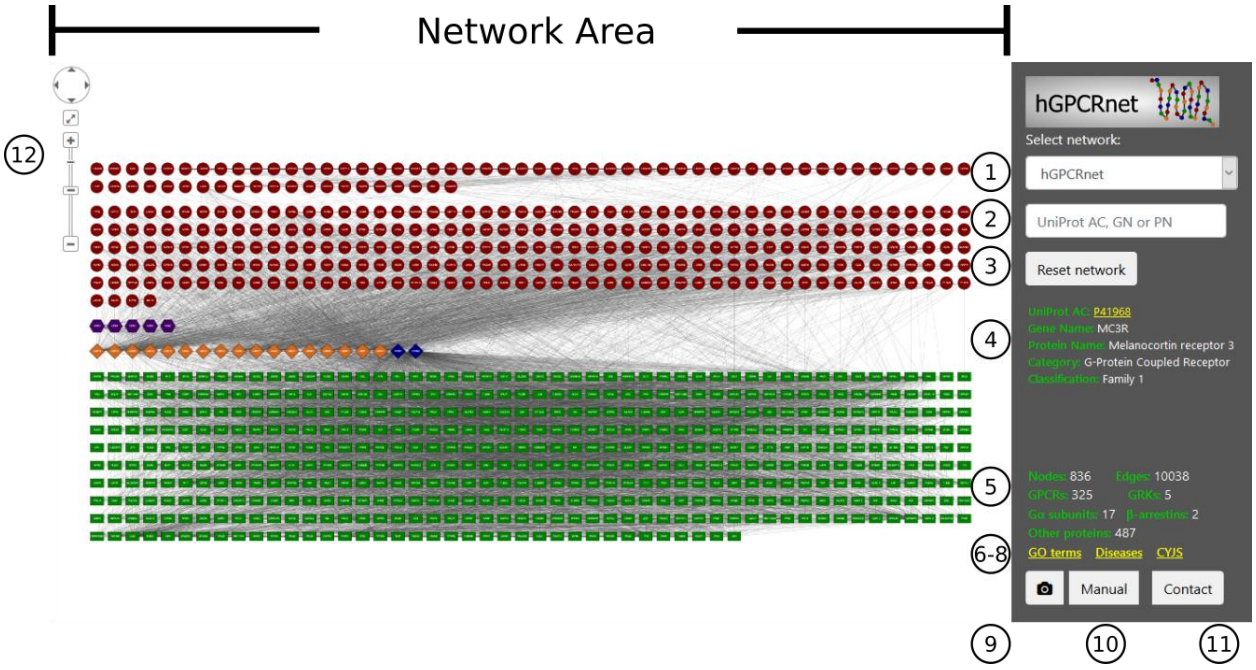
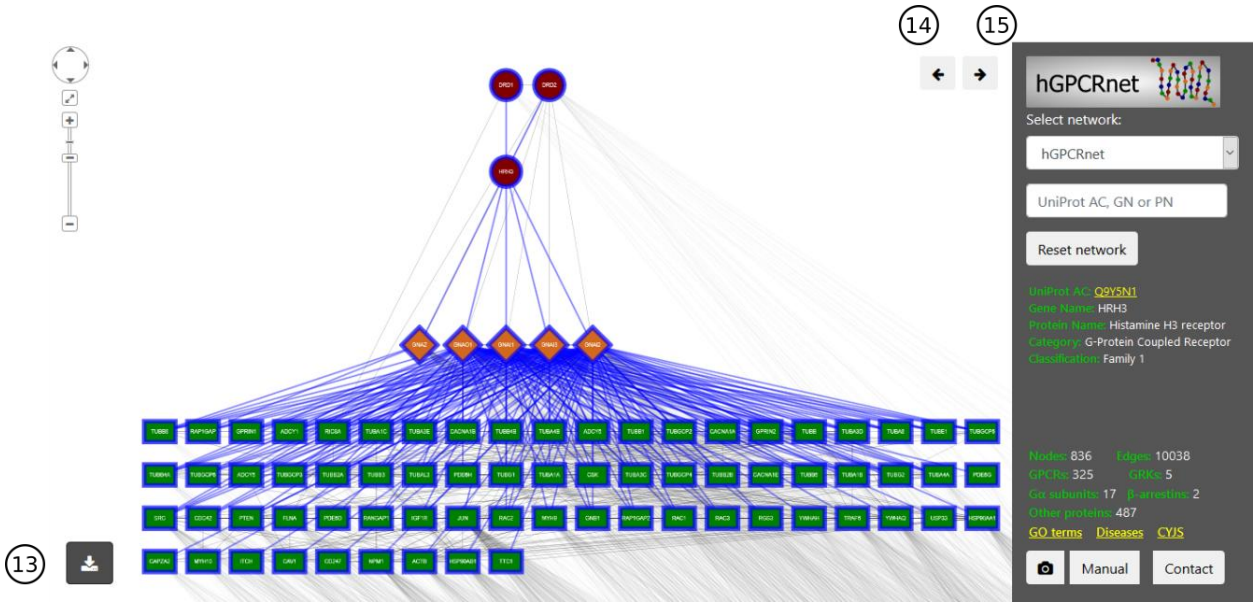
Quick overview



1. Select network to visualize. Default option is the hGPCRnet; other options are 77 cell type specific networks.
2. Search for a protein of interest using UniProtKB Accession number, Gene name or Protein name.
3. Click button to reset the network to its original state.
4. Node information displayed after the cursor passes over a node.
5. Current network statistics.
6. Display overrepresented GO terms for Biological Process in the current network.
7. Display diseases (DisGeNET) overrepresented in the current network in a new tab.
8. Network file in CYJS format for import into Cytoscape.
9. Take a screenshot of the current network view.
10. View the manual for the application.
11. Contact information.
12. Zoom IN or OUT, move around the network or fit network to view.



Once a signaling pathway (or neighborhood) or a comparison has been displayed additional buttons appear:

13. A button to download a simple text file containing the nodes (proteins) found in the currently displayed signaling pathway (or neighborhood) or comparison.

14. Previous button. Repeat the previous selection/comparison made within the current network.

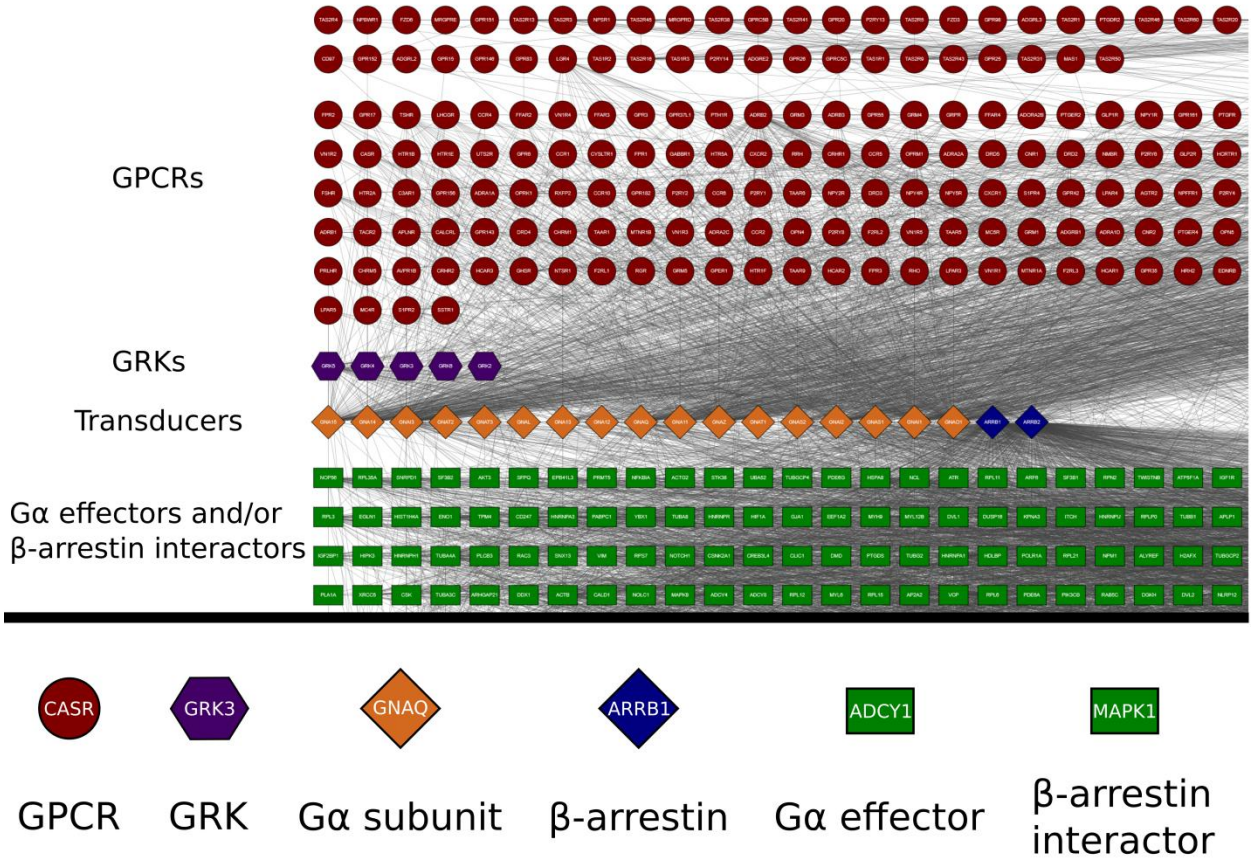
15. Next button. Activated once the previous button has been used to move forward again.

Mouse controls:

- *Move the cursor* over a node to display its information
- *Left Click* on a node to display its signaling pathway if it is a GPCR (to display its neighborhood otherwise)
- *Right Click* on a second node to compare its signaling pathway (or neighborhood) to that of a currently selected node
- *Click and Drag*
  - on a **node** to move it around the network
  - on the **background** to move the entire network
- *Scroll* to zoom IN/OUT

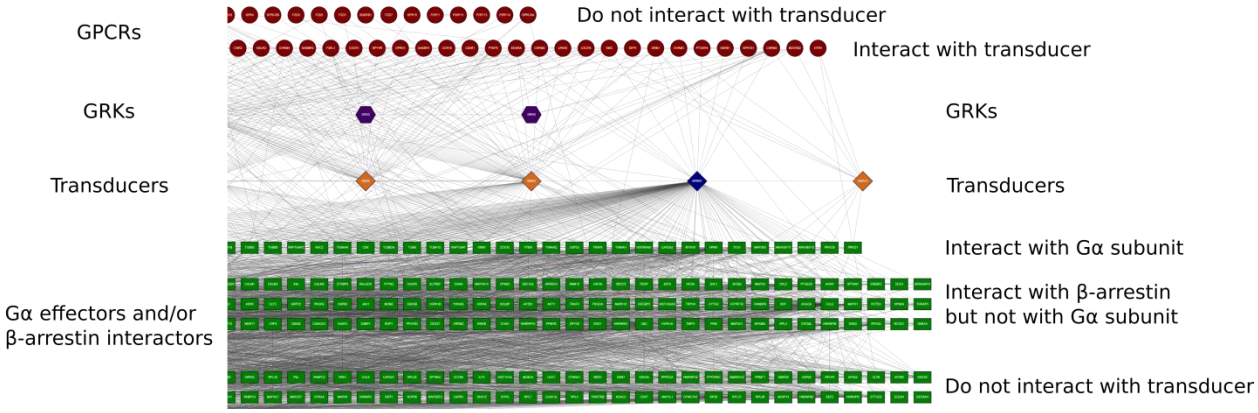
Network description

There are four distinct node styles according to the protein's category. GPCRs are colored red with an oval shape, Gα subunits and β-arrestins are both shaped as diamonds and are colored orange and blue respectively and the remaining proteins (Gα effectors, β-arrestin interactors) are rectangular and green. Every node is labeled with the Gene name attributed to the protein according to UniProtKB.



The hGPCRnet consists of proteins involved in GPCR signaling represented as nodes and the interactions between them represented as edges. The nodes are placed in three layers illustrating the hierarchy underlying the signaling network and how information flows. In the top layer are the GPCRs, typically located on the cell surface and responsible for receiving signals. A distinct upper sublayer of GPCRs consists of those GPCRs that do not interact with Gα subunits or β-arrestins. Right bellow the GPCR layers is the GRK layer, GRKs phosphorylate activated GPCRs causing an increase in the affinity between the now phosphorylated GPCR and β-arrestin, ultimately ceasing signaling via G-proteins. The next layer represents the transducers, Gα subunits and β-arrestins, which upon activation by GPCRs regulate a variety of

proteins in order to formulate the cellular response. The number of transducers is limited and so they act as bottlenecks in the network mediating and controlling the majority of the information flow. Lastly is the layer of Gα effectors and β-arrestin interactors whose function is ultimately regulated.



Cell type specific networks consist of proteins expressed in a specific cell type and all interactions between them. These are structured similarly to hGPCRnet, with some modulations in the third layer. Specifically, the layer of Gα subunit effectors/β-arrestin interactors is split into three sublayers. The top one has Gα subunit effectors/β-arrestin interactors interacting with Gα subunits present in the network. The middle sublayer has Gα subunit effectors/β-arrestin interactors interacting with β-arrestin (if present). Lastly, the bottom sublayer consists of Gα subunit effectors/β-arrestin interactors that do not interact with any of the transducers found in this cell type.

Interacting with the network

Basic

To navigate around the network you may *Click and Drag* on an empty area of the network or click on the *circle with arrows* found on the upper left side. In order to *zoom IN or OUT* of the network simply use the mouse wheel or the *+ and – buttons* on the upper left side. Right above the zoom controls is the button that can be used to *pan the network*, meaning the network view will zoom so that all nodes are simultaneously visible.

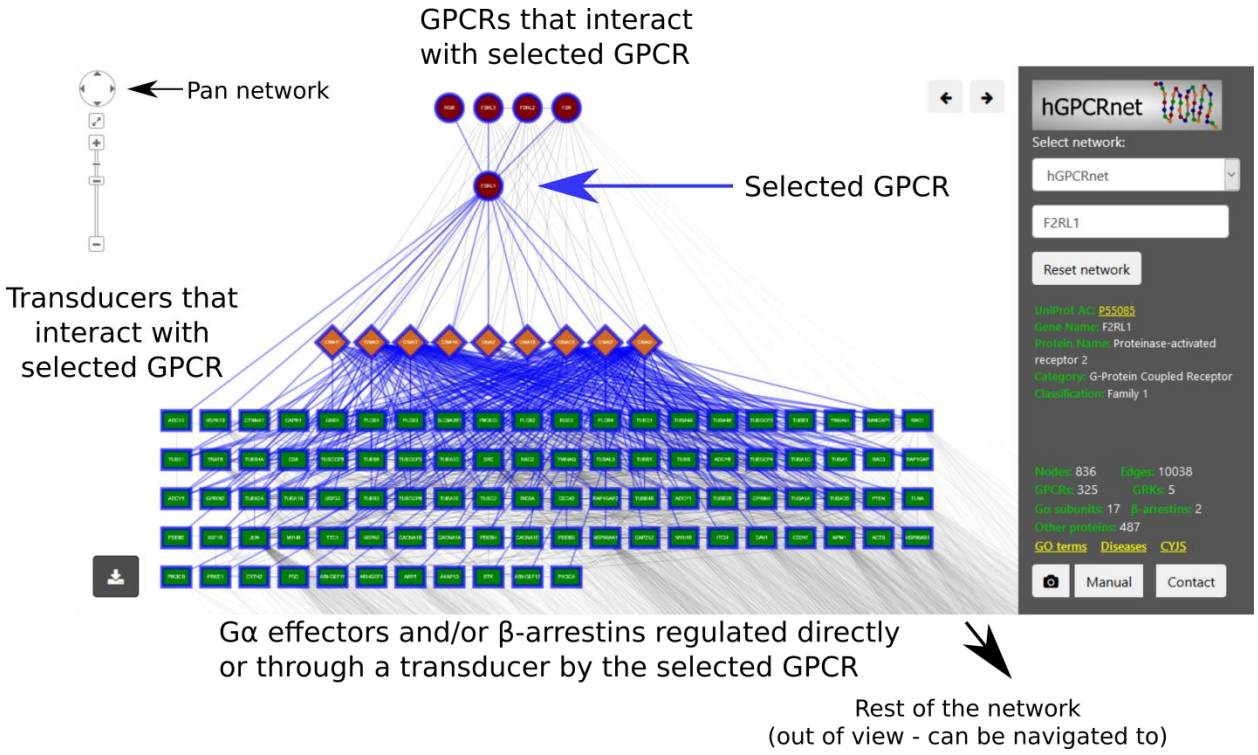
Hover over a node with the mouse to display the *Node Information*, these include the *Protein name*, the *UniProtKB Accession number* that links to the protein's UniProtKB entry page, the *Gene name*, the *Category* this node belongs in (G-Protein Coupled Receptor, G-protein coupled Receptor Kinase, Gα subunit, β-arrestin, Gα effector, β-arrestin Interactor) and its *Classification* (family or functional class).

Search for a protein of interest using the Accession number, Protein name or Gene name, as provided by UniProtKB. While typing, a number of suggestions will appear displaying the UniProtKB Accession and Gene name. Then *Click* on the appropriate suggestion. Once a suggestion has been selected, the view will zoom IN on the corresponding node and its information will be displayed.

To move a single node, *Click and Drag* on it across the view. To move multiple nodes: First select the nodes by *holding the Ctrl key* pressed and then *Click and Drag* to create a rectangle. All nodes in the rectangle when the mouse button is released will be selected. Repeat as necessary to include all desired nodes, finally move the nodes by *Click and Drag* on any of the selected nodes. To clear the node selection, simply click on an empty area in the network.

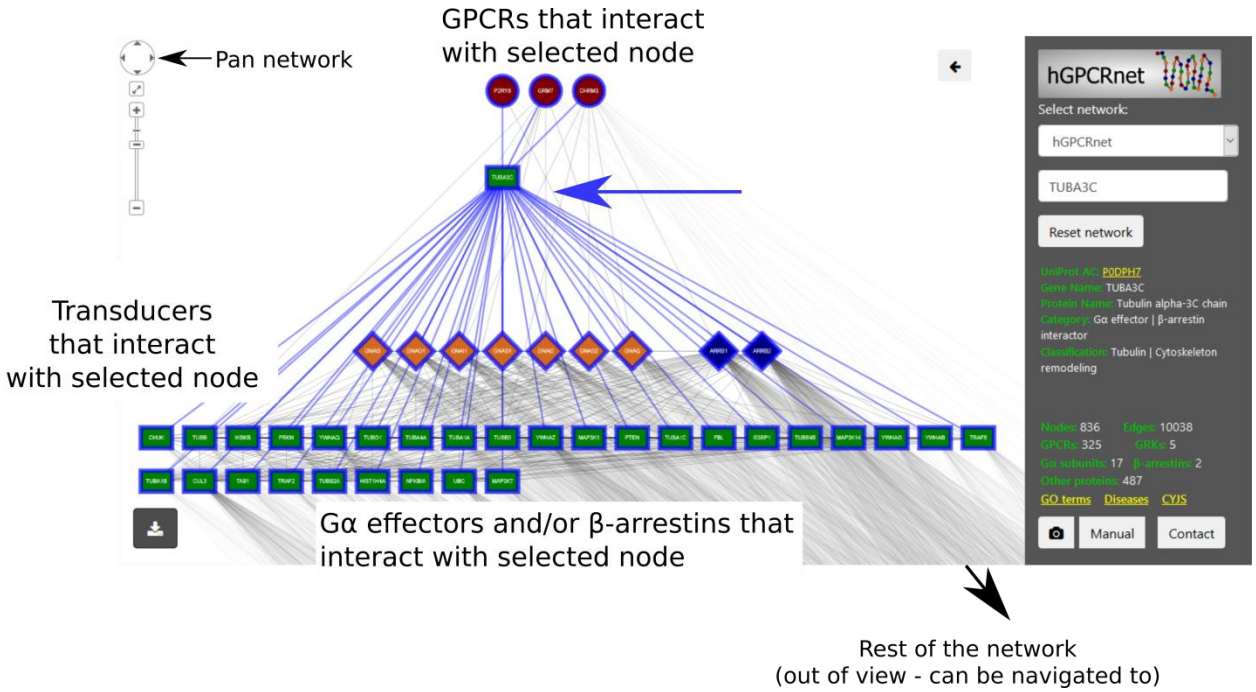
The *Reset Network button* will return all nodes to their original position and state.

View signaling pathway



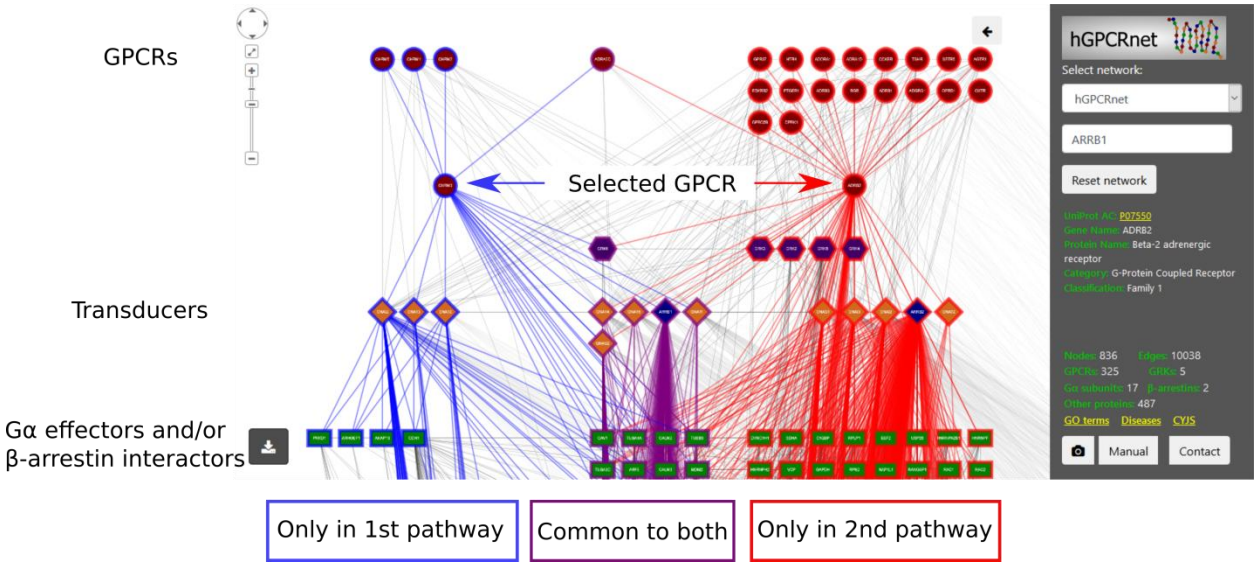
To display a GPCR signaling pathway, *Click* on the GPCR of interest using the *Left mouse button*. The nodes participating in said pathway will be moved and the view will zoom IN on them. Above the selected receptor are GPCRs, right below it are the GRKs and then are Gα subunits and β-arrestins that the selected receptor interacts with. Below the Gα subunits and β-arrestins are effectors that are regulated by them, or that directly interact with the receptor. Nodes in this pathway have a *blue border*, while edges are also colored blue indicating the flow of information from the selected GPCR to the rest of the signaling pathway.

Node neighborhood



In a similar manner, left click on a node that is not a GPCR will cause the display of its neighborhood, i.e. all nodes it interacts with. These nodes will be positioned the same way as when displaying a pathway, from top to bottom layers of: GPCRs, selected node, GRKs, transducers and Gα effectors and/or β-arrestin interactors.

Compare signaling pathways/node neighborhoods



The signaling pathway of a GPCR likely overlaps with other GPCR's pathways. Two pathways overlap because the GPCRs interact with the same GPCR, transducer or exert control, directly or indirectly, on the same Gα effectors and/or β-arrestin interactors or are phosphorylated by the same GRK. The application permits the user to visualize the overlap between two pathways. First one of the GPCRs of interest must be located (e.g. by search) and selected with a ***Left click***. Once that GPCR's signaling pathway is displayed (nodes colored blue), the other GPCR of interest, whose pathways the user wants to compare, must be located. Once located, the second GPCR may be selected with a ***Right click*** to examine the two receptor's signaling pathways overlap. The firstly selected GPCR and all proteins exclusive to its pathway will retain their *blue border* and be placed on the *left side*. The secondly selected GPCR and all proteins exclusive to its pathway will have a *red border* and be placed on the *right side*. Lastly, all proteins common to the two pathways proteins will have a *purple border* and be placed in the *middle*.

Links to files

Upon clicking on one of these links a new tab will open containing the information/results for the current network in plain text format. This file can be saved by right clicking and selecting Save as.

GO terms – results of functional analysis using the BiNGO application and GO terms for biological processes.

Diseases – results of disease enrichment using disease associations from DisGeNET.

CYJS – the network in cyjs format that can be imported in Cytoscape and is available for further analysis of the network. It contains the protein interactions and several node attributes. For a network style similar to the one in the application please download and use the style file for Cytoscape found here:
http://thalis.biol.uoa.gr/hGPCRnet/cyjs/hGPCRnet_style.xml

Download selection nodes – Additionally, when a signaling pathway, node neighborhood or comparison is displayed, a file containing all nodes (proteins) in the selection becomes available for download.

UniProt/Accession	GeneName/Label	Neighborhood
P08172	CHRM2	A
O75899	GABBR2	B
P08912	CHRM5	A
P63096	GNAI1	AB
P49407	ARRB1	A
P09471	GNAO1	AB
P11229	CHRM1	A
P10523	SAG	A
…	…	…

Example

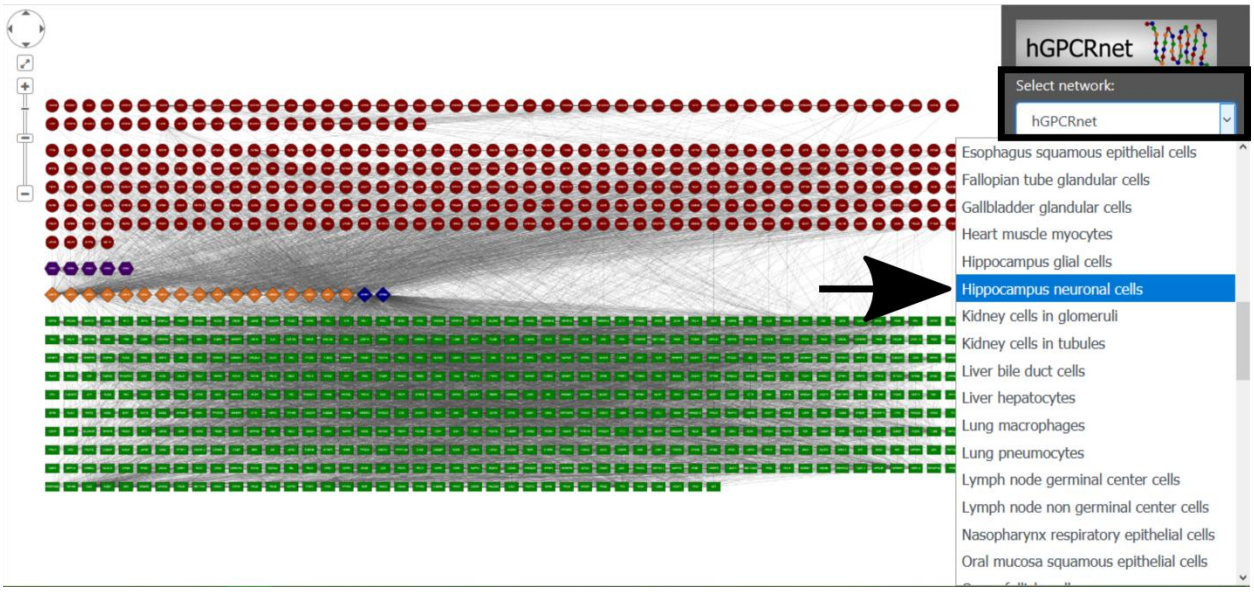
According to previous studies that muscarinic acetylcholine receptor action can affect the progression of Alzheimer's disease. More specifically activation of the M1 subtype (CHRM1) has been shown to slow the progression of the disease, while activation of the M2 subtype (CHRM2) has the reverse effect.

Goal: Find where the receptors' pathways differ in order to explain their opposite effect on the progression of Alzheimer's.

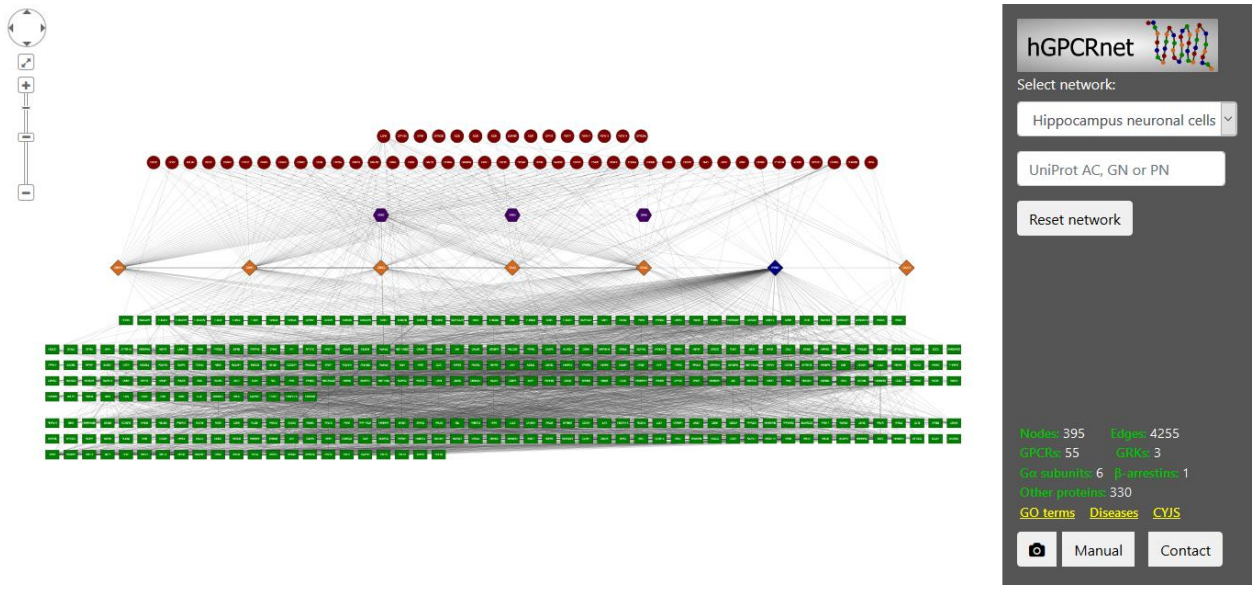
Selecting a network

The hGPCRnet contains all possible signaling pathways and is the by default selected network. We can use this network to check if our protein of interest is in the network (i.e. interaction data were available) and to view its entire signaling pathway. Since in this example we are concerned with the Alzheimer's disease we will select the cell-type-specific network for hippocampus neuronal cells because these cells are affected in diseased individuals.

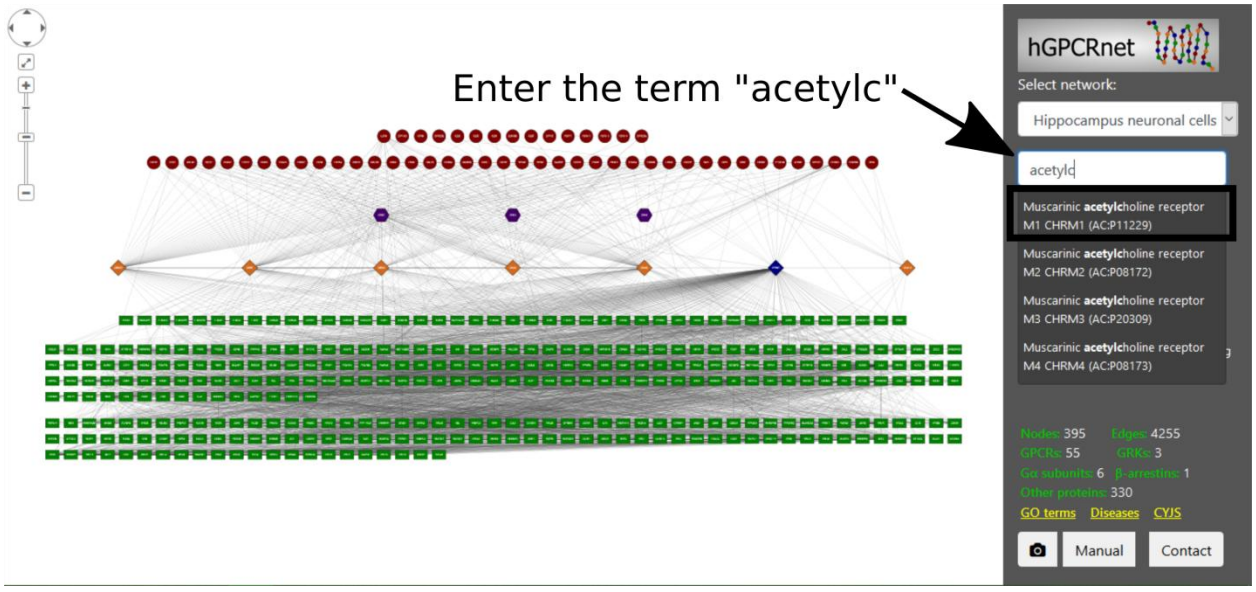
In order to select the network we choose to study we click on the upper right where the name of the current network (in this case hGPCRnet) is displayed, right under the Select network. This will reveal a drop-down list with all available networks, the hGPCRnet is on the top and the cell-type-specific networks are in alphabetical order. We scroll down until we find Hippocampus neuronal cells and then click on it to select it.



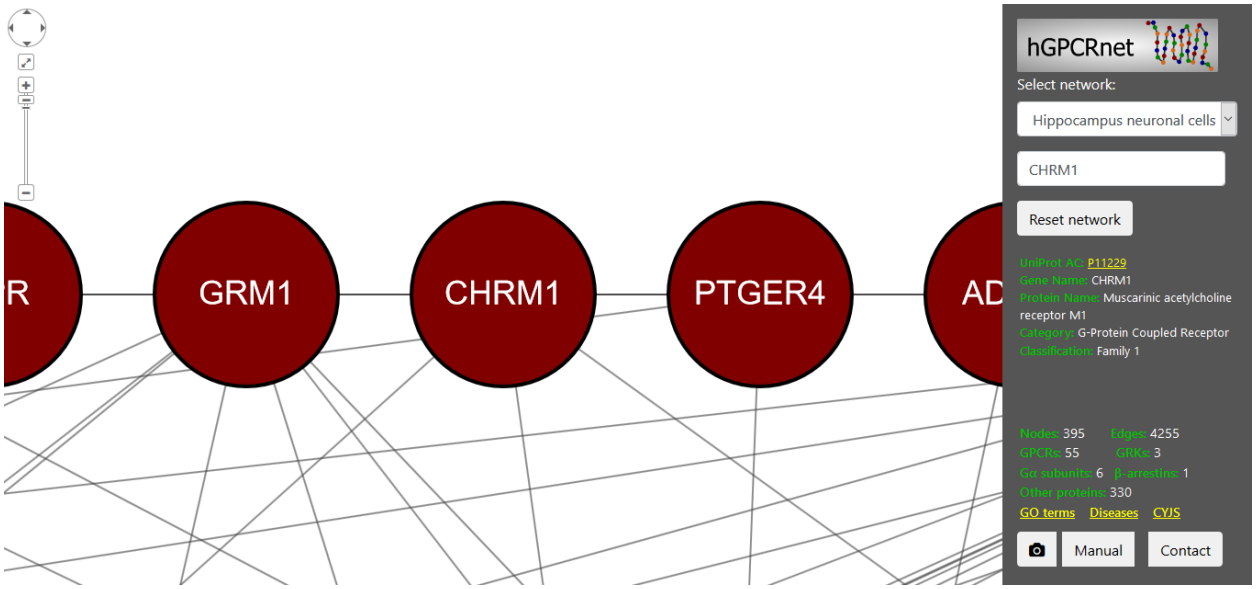
Finding the proteins of interest



We are now viewing the network we chose and can see from the network statistics on the lower right side that it contains 395 nodes, 55 GPCRs, 3 GRKs, 6 Gα subunits, 1 β-arrestin and 330 other proteins. To explore these proteins we may zoom IN, move around the network and hover over any node to view its basic information. However, in this particular case we know which two proteins we are interested in studying, so we can just use the Search box located underneath the "Select network" menu. As the text in the box displays suggestions, we can use UniProt Accession number, Gene Name or Protein Name. If we start typing part of the protein name (acetylcholine receptor) we will see our two proteins of interest, CHRM1 and CHRM2, included in the network.

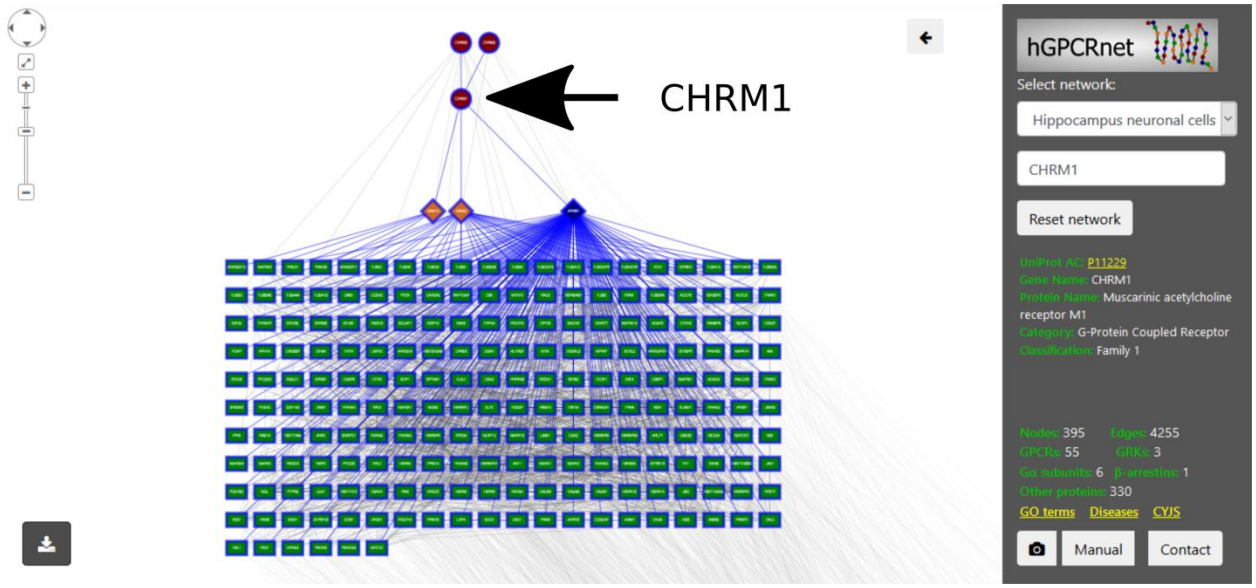


We click on one of the suggestions, CHRM1, and the network is zoomed IN with the chosen node appearing in the center of the view.

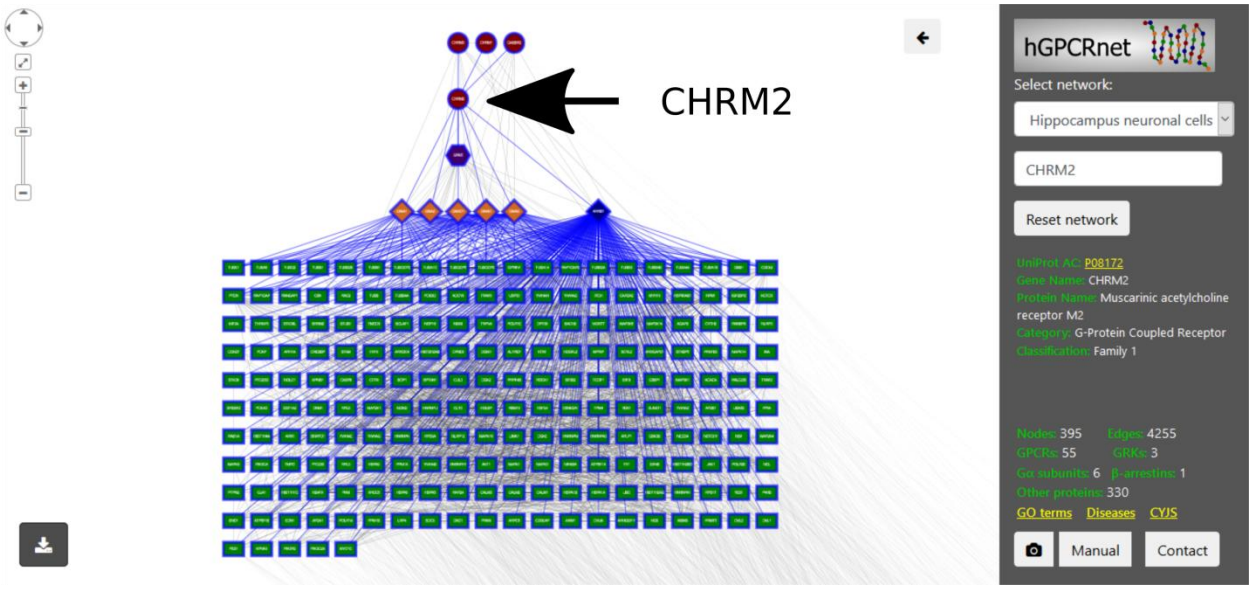


View GPCR signaling pathway

To view the CHRM1 signaling pathway we simply left click on the node. CHRM1 and all nodes participating in its signaling pathway are moved to the upper left side of the view, zoomed IN on and given a blue border. It is apparent that this signaling pathway is rather large with several proteins involved in it.

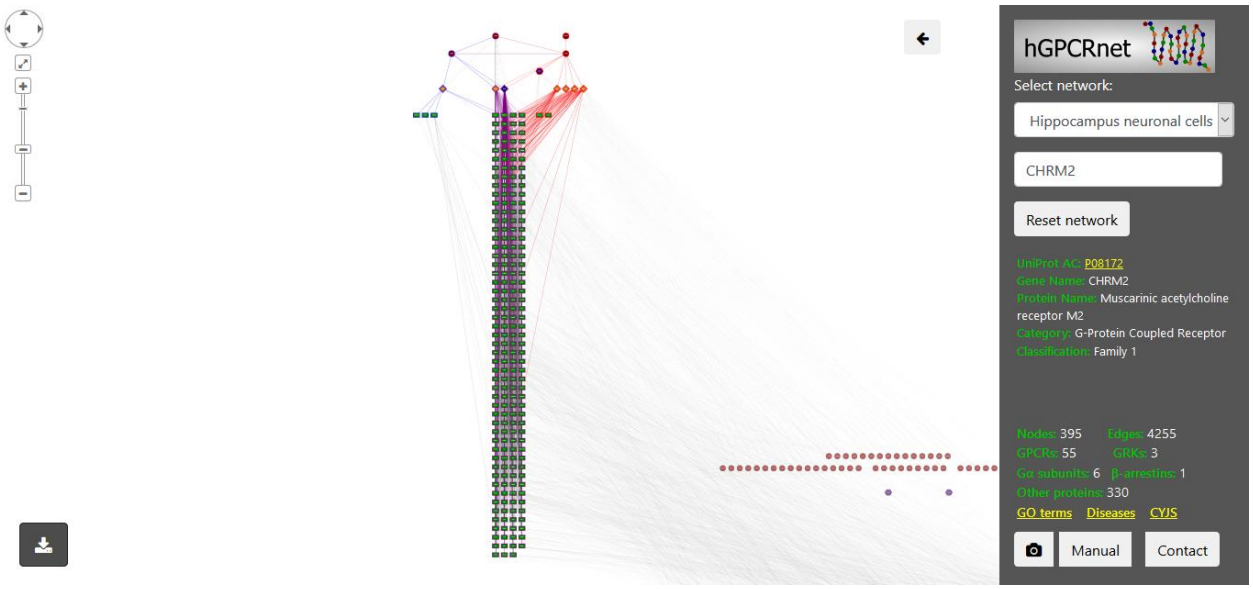


Similarly, we can view the signaling pathway of CHRM2 using the search functionality, or in this case, finding it amongst the GPCRs in the CHRM1 pathway, that it happens to participate in, using left click to select it. We see that this GPCR also has a large pathway and we can tell immediately it differs from the CHRM1 pathway due to the participation of more Gα subunits.

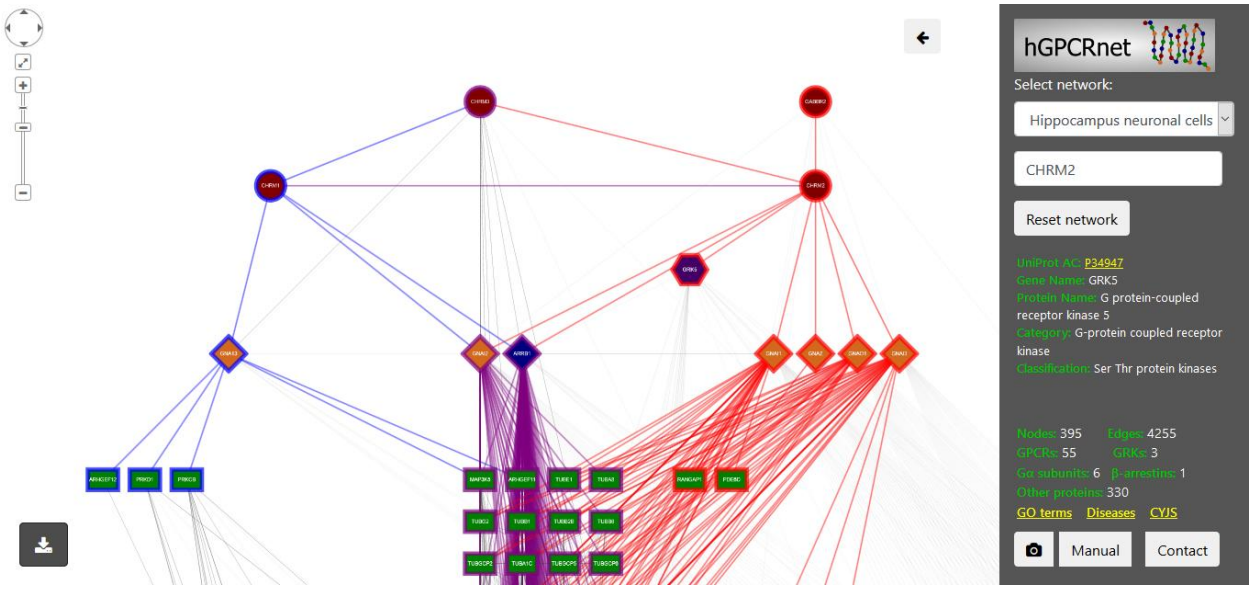


Comparison of the signaling pathways

In this example we are trying to determine possible explanations for the reverse effects that have the activation of CHRM1 and the activation of CHRM2 on the progression of Alzheimer's disease. We are, therefore, interested in finding where the two receptor's pathways diverge. Attempting to do so by visually comparing the two pathways would be time consuming and error prone due to their large size. That is not, however, necessary as the application provides us with a fast and automated way to compare two signaling pathways. First we select the first GPCR (e.g. CHRM1) and once the pathway is displayed we locate (e.g. by search) the second GPCR and initiate the comparison by Right Clicking on that node (e.g. CHRM2). Once the comparison is completed we can see that all nodes are separated and colored according to which pathway they participate in (both – middle & purple, first – left & blue, second – right & red).



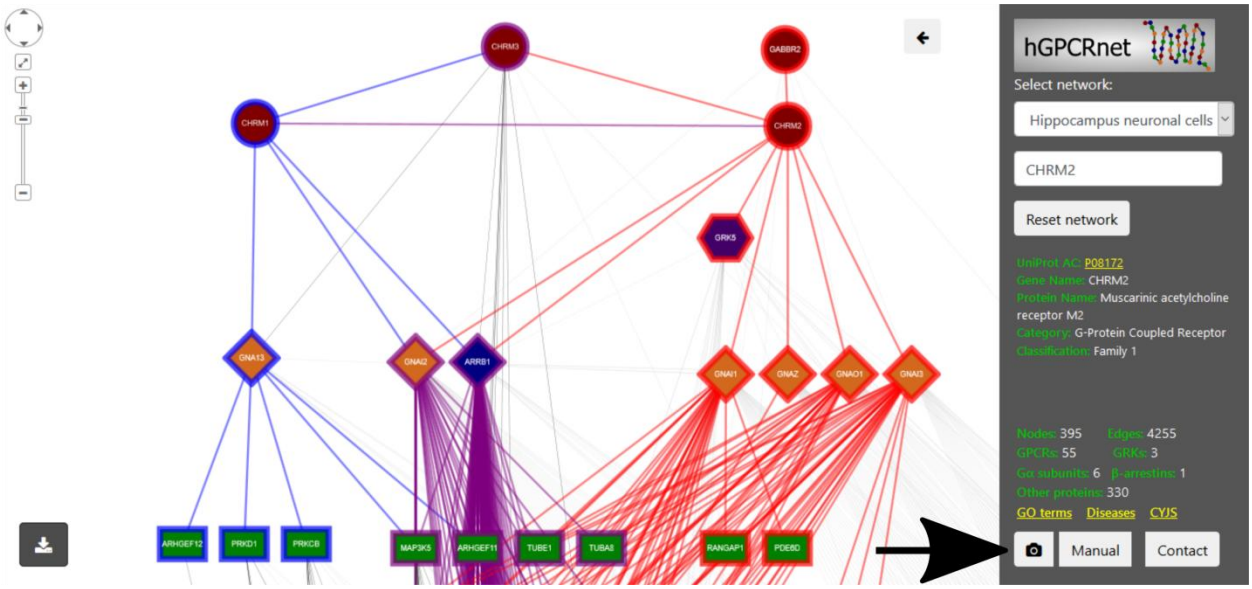
We can zoom IN using the + (upper left side) and move around the network by Click and Drag on an empty area (white background). This allows us to more carefully examine the proteins on display and makes their Label (gene name) visible.



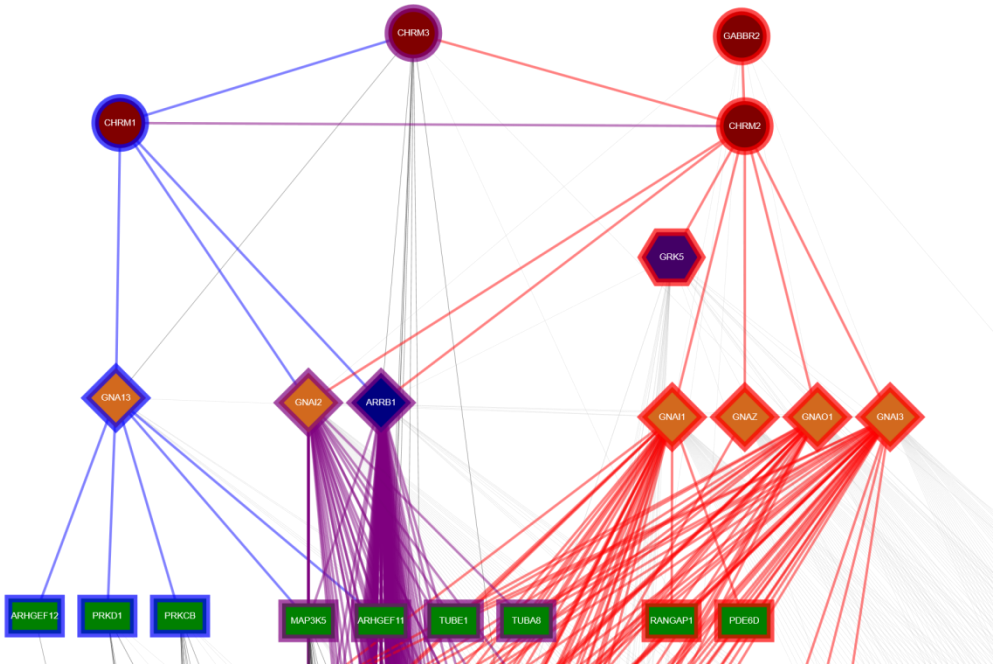
We can clearly see that CHRM1 and CHRM2 uniquely control three and two proteins respectively. These could very likely help explain their varying effect on Alzheimer's disease and can be targets of further investigation.

Taking a screenshot

We would like to "save" our results so that we may share them or refer back to them in the future. Clicking on the button with the camera on it, we can take a screenshot of the current view of the network. We want to save an image where we can see the differences between the two pathways, so not interested in including all purple nodes, and would like for the Labels to be legible. We zoom IN and move the network so all the proteins we are interested in are in view, but still we would like to be able to clearly see the labels. We can of course zoom IN even more, take two or more different screenshots with the application and join them using a Graphics program. Another option is to manually move the nodes, in this case to place them closer to one another. To move a single node we Click and Drag, while to move many at once, we select them using Ctrl and "Click and Drag" simultaneously, move them by Click and Drag on any one of them, and releasing them by clicking on an empty area.



Then we just press the screenshot button and the image is opened in a new tab. The image is in PNG format and can be saved by right clicking and selecting "Save image as".


CHRM3
GABBR2
CHRM1
CHRM2
GRK5
GNA13
GNAI2
ARRB1
GNAI1
GNAZ
GNAO1
GNAI3
ARHGEF12
PRKD1
PRKCB
MAP3K5
ARHGEF11
TUBE1
TUBA8
RANGAP1
PDE8D

Appendix

Data sources

- Protein interactions:
  - Human-gpDB
  - β-Arrestinome
  - GPCR-HetNet
  - IID (Integrated Interactions Database – Version 2018-05)
  - IntAct (Version 2018-10)
- Protein information:
  - UniProtKB (release 2018_08) – Protein name, Accession, Gene name
  - Classification of GPCRs – family from UniProtKB
  - Classification of Gα subunit – family from Human-gpDB
  - Classification of Gα effector – functional category from Human-gpDB
  - Classification of β-arrestin interactors – functional category from β-Arrestinome
- Cell type protein expression:
  - HPA (Human Protein Atlas – Version 18) immunohistochemistry data
- Disease associations:
  - DisGeNET (Version 5)
- Biological processes:
  - Gene Ontology (GO)
- P-values calculation:
  - Tools
    - GO terms: BiNGO
    - Diseases: R
  - Parameters for calculation:
    - Test: Hypergeometric Test
    - Multiple testing correction: Benjamini & Hochberg's FDR correction
    - Cut off: 0.05

Cell types available

Adrenal gland glandular cells
Appendix glandular cells
Appendix lymphoid tissue
Bone marrow hematopoietic cells
Breast adipocytes
Breast glandular cells
Breast myoepithelial cells
Bronchus respiratory epithelial cells
Caudate glial cells
Caudate neuronal cells

Cerebellum cells in granular layer
Cerebellum cells in molecular layer
Cerebellum Purkinje cells
Cerebral cortex endothelial cells
Cerebral cortex glial cells
Cerebral cortex neuronal cells
Cerebral cortex neuropil
Cervix uterine glandular cells
Cervix uterine squamous epithelial cells
Colon endothelial cells
Colon glandular cells
Colon peripheral nerve ganglion
Duodenum glandular cells
Endometrium cells in endometrial stroma
Endometrium glandular cells
Epididymis glandular cells
Esophagus squamous epithelial cells
Fallopian tube glandular cells
Gallbladder glandular cells
Heart muscle myocytes
Hippocampus glial cells
Hippocampus neuronal cells
Kidney cells in glomeruli
Kidney cells in tubules
Liver bile duct cells
Liver hepatocytes
Lung macrophages
Lung pneumocytes
Lymph node germinal center cells
Lymph node non germinal center cells
Nasopharynx respiratory epithelial cells
Oral mucosa squamous epithelial cells
Ovary follicle cells
Ovary ovarian stroma cells
Pancreas exocrine glandular cells
Pancreas islets of Langerhans
Parathyroid gland glandular cells
Placenta decidual cells
Placenta trophoblastic cells
Prostate glandular cells
Rectum glandular cells
Retina cells in photoreceptor layer
Salivary gland glandular cells
Seminal vesicle glandular cells
Skeletal muscle myocytes
Skin epidermal cells
Skin fibroblasts
Skin keratinocytes
Skin Langerhans
Skin melanocytes
Small intestine glandular cells
Smooth muscle smooth muscle cells
Soft tissue adipocytes
Soft tissue chondrocytes
Soft tissue fibroblasts
Soft tissue peripheral nerve
Spleen cells in red pulp
Spleen cells in white pulp
Stomach glandular cells
Testis cells in seminiferous ducts
Testis Leydig cells
Thyroid gland glandular cells
Tonsil germinal center cells
Tonsil non germinal center cells
Tonsil squamous epithelial cells
Urinary bladder urothelial cells
Vagina squamous epithelial cells