GPCRdb workshop

Introduction

First-time users will benefit from the easy introduction, whereas also experienced users will find new features and all have the chance to test on their own target and to ask questions. GPCRdb and this workshop is intended for the wide community of GPCR crystallographers, modellers, pharmacologists, medical chemists etc.

Preparation

1. Read the article GPCRdb: the G protein-coupled receptor database – an introduction
2. Test access to www.gpcrdb.org and www.test.gpcrdb.org
3. Test a help video (to the right of each GPCRdb menu item)

Table of Contents

1. Receptors

1.1. Receptor search and snake diagrams

1. Go to menu: Receptors – Receptor search
2. Search for your receptor
3. Scroll down to the snake diagram
4. Click "Properties" (grey button under the snake diagram and colour panel)
5. Hover your computer mouse over an amino acid to see its residue number
6. Inside the snake diagram, click on N-term, ECL2, ICL3 and C-term

Question: Which segments (transmembrane helices, loops or termini) have most hydrophobic (yellow) residues and why?

Answer:

1.2. Structure-based alignments and residue conservation

1. Go to menu: Receptors – Sequences – Structure-based alignments
2. Select the crystallized receptors (blue button under "Predefined sets)
3. Click Continue to next step (green button in upper right corner)
4. Select TM3 and ECL2 (blue buttons under "Helix" and "Loop", respectively)
5. Click Continue to next step (green button in upper right corner)
6. If needed, scroll down or right (drag the vertical blue bar at the top) to see more

Question: Most GPCRs have two conserved cysteine residues in TM3 (position 3x25) and ECL2 (position 45x50) that form a disulphide bond. Which receptors DO NOT have these cysteine residues and therefor lack the disulphide bridge?

Answer:

1.3. Sequence similarity

1. Go to menu: Receptors – Sequences – Similarity – Similarity search-GPCRdb
2. Select your own target receptor
3. Select "All TMs" (blue button under "Predefined selections")
4. Select a GPCR "Class" under Browse (at the bottom)
5. Click "Show similarity" (green button in upper right corner)
6. Wait 1-2 minutes (for class A it can take 10 minutes, so use a subset or another class)

Question: To the right of the receptor names, two columns and show the sequence identity (%I) and similarity (%S). What is the sequence similarity of your target to the most similar receptor?

Answers: Identity   %, similarity   %

1.4. Structure statistics

1. Go to menu: Receptors – Structures – Structure statistics
2. Scroll down to the (circular) receptor tree

Question 1: A red filled circle by a receptor name (see outer ring) means that it has been crystallised. Two GPCR Classes (see tree centre) have no structures, which?

Answer:

Question 2: One receptor family (outer groups) have structures for all its (four) receptors, which?

Answer:

1.5. Template selection

1. Go to menu: Receptors – Structures – Template selection
2. Select your receptor (either in Search box or Browse)
3. Click "Find template" (green button in upper right corner)

Question: A red filled circle by a receptor name (see outer ring) means that it has been crystallised. Which is the most homologous crystal structure template?

Answer:

2. Ligands

2.1. GPCR-ligand interactions

1. Go to menu: Ligands – Structure Sites – GPCR-ligand interactions
2. Select a pdb code (droplist "Select from list" in top middle)

Question 1: Which interaction type is most frequent? (see Ligand Interaction Browser)

Answer:

Question 2: In the two residue diagrams, are the interacting residues where you expect (between the upper halves of the TM helices)?

Answer:

2.2. Mutation browser

1. Go to menu: Ligands – Mutations – Mutation Browser
2. Write mglur5 in the search box
3. Click Continue to next step (green button in upper right corner)
4. Select All TMs (blue buttons under "Predefined selectins")
5. Click Show mutants (green button in upper right corner)
6. Scrool down to the residue diagrams

Question: Red mutations have decreased ligand affinity/potency. Are these mutations located inside the binding cavity (upper half of the 7 helices, and facing inwards the center)?

Answer:

2.3. Mutation suggestions for ligand binding sites

1. Go to menu: Ligands – Mutations – Mutation design tool – By receptor name
2. Select your receptor (either in Search box or Browse)
3. Assess the strength of the mutation suggestions.
    a. Checking the homology level (mouse over the green circle) of the receptors from which the data was collected.
    b. Also check the number of supporting receptors, ligands, interactions and mutagenesis experiments (4 rightmost columns).

Question: For the top/first suggested mutation, what is the homology level (mouse over green button) and number of supporting receptors?

Answer:

3. Crystal constructs

3.1. Schematic alignments

1. Go to menu: Crystal constructs – Schematic alignments
2. Filter your receptor(s) of interest in the 2nd top row (Class, Family, Receptor or Species)
3. Click "Apply filter"
4. Select all your constructs (by ticking the box in the leftmost column header)
5. Click "Construct Schematic" (2nd blue bottom in the top row)
6. Scroll right (using the blue bar at the bottom)
7. Tick TM5, ICL3 and TM6 (in the browser header)
8. Click Sequence and Align selected (rightmost blue button at the top)
9. Scroll right (using the blue bar at the bottom)

Question 1: Write the position ranges of left and right side deletion (e.g. 5x69-5x70 & 6x24-26).
Answer:

3.2. Experiment browser

1. Go to menu: Crystal constructs – Experiment browser
2. Scroll down and then right (blue bars on right side and bottom)
3. Sort by "Host cell type" and then crystallization "Method"

Question: What is the most commonly used host cell type and crystallisation method?
Answer:

3.3. Construct design tool

1. Go to menu: Crystal constructs – Construct design tool
2. Do not change the selection "Inactive" and "ICL3" (this has most data)
3. Choose the topmost/first suggestion for N-term, ICL3 and C-term
4. Choose all "Glycosylation site removal" selected Thermostabilising mutations

Question: Repeat the above selection for N-term, ICL3 and C-term, but use the first/top suggestion for the Class instead of the topmost (Receptor, Receptor Family or Ligand Type). This gives you the common solutions from the class instead of the most homologous/closest template. Do the N-term, ICL3 and C-term deletions differ much (red residues in snake diagram)?
Answer:

4. Workshop evaluation form

Hand in this form to the workshop organiser!

Use pen and paper, or email the form to david.gloriam@sund.ku.dk

Question: Which part(s) was most relevant for you?
Answer:

_____

Question: Should any part(s) be removed for next time?
Answer:

_____

Question: Do you have wishes for improvement of any of the GPCRdb tools you have tested?
Answer:

_____

Question: Which new features would you like to see in GPCRdb?
Answer:

_____

Question: Do you have any other comment?
Answer:

_____

May we contact you to discuss your feedback? If yes, please provide
Name:

_____

Email address:

_____

Institute/University:

_____