

S1-10-rešitev

1. Prvi sklop vprašanj se nanaša na odgovore, ki jih lahko najdeš na [PDB](#):

- Organizma: *Aedes aegypti*, *Apocrypta bakeri*
- Insektni vohalni receptorji do od liganda odvisni ionski kanalčki. Ko se nanje veže ligand se konformacija kompleksa spremeni tako, da v celico vdrejo ioni, kar sproži signal po nevronih. Do odgovora je najlažje priti tako, da na PDB najdeš **April molecule of the month** in si prebereš kaj piše v opisu.
- Da receptor je oligomeren, sestavlja ga 4 polipeptidne verige, ki pripadajo 2 proteinoma. Trije proteini iz družine Orco, in en protein iz družine OR (OR10). Kompleks je asimetričen.

Slike na katerih so označene rešitve:

 8V00 | pdb_00008v00 

AegOR10 apo structure

PDB DOI: <https://doi.org/10.2210/pdb8V00/pdb>

EM Map EMD-42848: [EMDB EMDataResource](#)

Classification: MEMBRANE PROTEIN

Organism(s): ***Aedes aegypti*, *Apocrypta bakeri***

Expression System: Homo sapiens

Mutation(s): No 

Membrane Protein: Yes   





How insects smell

Insects also rely heavily on smell to navigate their environment. It turns out, however, that insects use a very different mechanism to sense airborne molecules. The primary olfactory organ of many insects are their antennae, which are covered in tiny hair-like structures called sensilla. Odor molecules can enter olfactory sensilla through pores, where they can then interact with odorant receptors on olfactory neurons.

Unlike mammalian odorant receptors, insect receptors are not GPCRs. Rather, insect odorant receptors are ligand-gated ion channels made up of four subunits. Odorant binding opens the channel and allows for the entry of ions into the cell, leading to neuronal activation. In most insects, odorant receptors are tetrameric complexes composed of two kinds of subunits: a variable odorant-binding subunit (called OR) and a conserved co-receptor known as Orco. ORs have rapidly expanded and diversified across different insect species to represent one of the largest and most divergent family of ion channels in nature, with potentially millions of different variants. This rapid evolution is thought to contribute to the ability of insects to adapt to very different ecologies.

Z rdečim pravokotnikom je označen paragraf, v katerem se nahaja odgovor na drugo vprašanje. [Dostop do odgovora.](#)

Macromolecule Content

- Total Structure Weight: 203.11 kDa 
- Atom Count: 12,479 
- Modeled Residue Count: 1,557 
- Deposited Residue Count: 1,797 
- **Unique protein chains: 2**

Z rdečim pravokotnikom je označena rešitev tretjega vprašanja.

Odgovor kateri proteini kompleks sestavljajo najdemo pod razdelkom Macromolecules, kjer je vsaka polipeptidna veriga/protein opisana posebej. Na spodnjih slikah so označene informacije o imenu proteinov in globalni simetriji.

Entity ID: 1					
Molecule	Chains	Sequence Length	Organism	Details	Image
Odorant receptor OR10	A [auth D]	375	Aedes aegypti	Mutation(s): 0 Gene Names: GPRor10 Membrane Entity: Yes	

Entity ID: 2					
Molecule	Chains	Sequence Length	Organism	Details	Image
Odorant receptor Orco	B [auth A], C [auth B], D [auth C]	474	Apocrypta bakeri	Mutation(s): 0 Gene Names: Or2 Membrane Entity: Yes	

Podatek o asimetričnosti:

Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report | Predict Membrane

Global Symmetry: Asymmetric - C1

Global Stoichiometry: Hetero 4-mer - A3B1

Pseudo Symmetry: Cyclic - C4 (Explore in 3D)

Pseudo Stoichiometry: Homo 4-mer - A4

Find Similar Assemblies

Biological assembly 1 assigned by authors.

Biological Assembly Evidence: electron microscopy

2. Drugo vprašanje je zastavljeno tako, da uporabiš BLAST in UniProt. Protein najdemo z [blastp](#), tako da v iskalno okno vstavimo podano zaporedje. Če upoštevamo namig v nalogi pridemo do dveh zadetkov, od katerih eden pripada iskanemu proteinu OR10, oz. variabilnemu proteinu insektih receptorskih kompleksov. Uporabimo obkroženo zaporedje, ki ima E vrednost enako 0, procent identičnosti pa 100%.

BLAST® » blastp suite » results for RID-ZSYWSXGU014

Home Recent Results Saved Strategies Help

[< Edit Search](#) [Save Search](#) [Search Summary](#)

How to read this report? BLAST Help Videos Back to Traditional Results Page

Job Title	Protein Sequence
RID	ZSYWSXGU014 Search expires on 05-08 19:46 pm Download All
Program	BLASTP Citation
Database	pdb See details
Query ID	lcl Query_11564936
Description	unnamed protein product
Molecule type	amino acid
Query Length	375
Other reports	Distance tree of results Multiple alignment MSA viewer

Filter Results

Organism only top 20 will appear exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to E value to Query Coverage to

[Filter](#) [Reset](#)

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments [Download](#) Select columns Show 100

select all 2 sequences selected [GenPept](#) [Graphics](#) [Distance tree of results](#) [Multiple alignment](#) [MSA Viewer](#)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> Chain D: Odorant receptor OR10 [Aedes aegypti]	Aedes aegypti	773	773	100%	0.0	100.00%	375	8V00_D
<input checked="" type="checkbox"/> Chain D: OR28 [Anopheles gambiae]	Anopheles gambiae	88.2	88.2	50%	1e-18	25.79%	398	8V3C_D

Iskanje proteina iz podatkov iz BLASTA – podatki ki jih imamo:

- Gre za vohalni receptor (odorant receptor), natančneje, gre za OR10
- Gre za organizem *Aedes aegypti*

[Download](#) [GenPept](#) [Graphics](#)

Chain D, Odorant receptor OR10 [*Aedes aegypti*]

Sequence ID: [8V00_D](#) Length: 375 Number of Matches: 1

[See 1 more title\(s\)](#) [See all Identical Proteins\(IPG\)](#)

Chain D, Odorant receptor OR10 [*Aedes aegypti*]

Sequence ID: [8V02_D](#)

Range 1: 1 to 375 [GenPept](#) [Graphics](#)

[Next Match](#) [Previous](#)

Score	Expect	Method	Identities	Positives	Gaps
773 bits(1996)	0.0	Compositional matrix adjust.	375/375(100%)	375/375(100%)	0/375(0%)

To so podatki, ki jih uporabimo za iskanje po UniProt. Pomaga če filtriramo po taksonomiji, ter da zapis ni pregledan – nahaja se v TrEMBL. Na sliki je prikazan ustrezen zapis:

Entry	Entry Name	Protein Names	Gene Names	Organism	Length
A0A6I8U2J8	A0A6I8U2J8_AEDAE	Odorant receptor	5569825	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	384 AA
A0A6I8U2M9	A0A6I8U2M9_AEDAE	Odorant receptor	110675627	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	387 AA
A0A6I8U6F1	A0A6I8U6F1_AEDAE	Odorant receptor	110678539	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	396 AA
Q177X3	Q177X3_AEDAE	Odorant receptor	GPRor10	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	375 AA
Q177X4	Q177X4_AEDAE	Odorant receptor	GPRor2, 5567334	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	376 AA
A0A1S4FBJ8	A0A1S4FBJ8_AEDAE	Odorant receptor	AaeL_AAEL005680	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	372 AA
A0A1S4G5R6	A0A1S4G5R6_AEDAE	Odorant receptor	AaeL_AAEL017294	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	384 AA
J9HTA3	J9HTA3_AEDAE	Odorant receptor	23687499, GPRor47	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	384 AA
Q16T07	Q16T07_AEDAE	Odorant receptor	5573335, GPRor30	<i>Aedes aegypti</i> (Yellowfever mosquito) (Culex aegypti)	384 AA

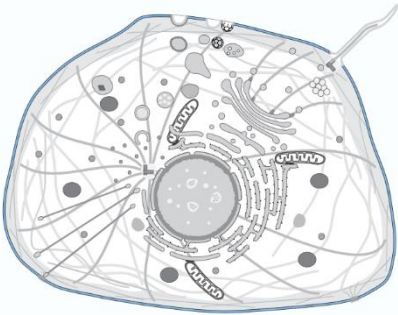
Možna alternativna rešitev: če bi vpisali 'or10' in upoštevali taksonomske filtre, nas UniProt usmeri na UniParc. Dobimo 3 zapise, ki so med sabo identični (zbirka UniParc je redundantna). Pri A0A1S7UEA4 bi do enakih zaključkov, kot pri Q177X3, pri A0A1S4FCA1 pa kot ime gena dobimo 5567346, ostalo pa se ne razlikuje.

Entry	Common taxonomies	Length	UniProtKB	First seen	Last seen
UPI00017FB632	<i>Aedes aegypti</i> (cellular organisms)	375	B5M8U3.1 (obsolete) Q177X3 A0A1S7UEA4	2008-09-14	2026-01-28
UPI000B793C65	<i>Aedes aegypti</i> (cellular organisms)	375	A0A1S4FCA1	2017-07-21	2026-01-28

- Identificirali smo kodo UniProt: Q177X3, ime gena: GPRor10 in ime organizma: Aedes aegypti. Z rdečim pravokotnikom so označene rešitve.
- Protein se nahaja v celični membrani in ima 6 transmembranskih heliksov

Subcellular Locationⁱ

UniProt Annotation GO Annotation



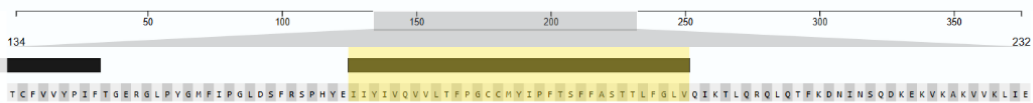
Q177X3 · Q177X3_AEDAE

Proteinⁱ | Odorant receptor
 Geneⁱ | **GPRor10**
 Statusⁱ | UniProtKB unreviewed (TrEMBL)
 Organismⁱ | **Aedes aegypti (Yellowfever mosquito) (Culex aegypti)**

Amino acids | 375 (go to sequence)
 Protein existenceⁱ | Evidence at protein level
 Annotation scoreⁱ | 2/9

Entry Variant viewer Feature viewer Genomic coordinates Publications External links History

Download



±	TYPE	ID	POSITION(S)	DESCRIPTION	Tools	Add
+	Transmembrane		28-46	Helical Automatic Annotation	Tools	Add
+	Transmembrane		58-79	Helical Automatic Annotation	Tools	Add
+	Transmembrane		120-142	Helical Automatic Annotation	Tools	Add
-	Transmembrane		167-199	Helical Automatic Annotation	Tools	Add
Sequence: IYIVQVVLTFPGCCMYIPFTSFFASTTLFGLV						
+	Transmembrane		247-270	Helical Automatic Annotation	Tools	Add
+	Transmembrane		276-298	Helical Automatic Annotation	Tools	Add

- Aminokislinsko zaporedje četrtega transmembranskega heliksa:

IYIVQVVLTFPGCCMYIPFTSFFASTTLFGLV

3. Tretja naloga je zastavljena tako, da moraš uporabiti [ProtParam](#).

For purification, cell pellets were thawed in room-temperature water, and resuspended in 100 mL of solubilization buffer per liter of cell culture. The solubilization buffer was composed of 20 mM HEPES/NaOH (pH 7.5), 150 mM NaCl, 0.5% (w/v) Lauryl Maltose Neopentyl Glycol (LMNG; Anatrace), 0.1% (w/v) cholesterol hemisuccinate (CHS; Sigma-Aldrich), 2.5 µg/mL leupeptin, and 160 µg/mL benzamidine. The cell pellets in the solubilization buffer were homogenized with a Dounce homogenizer in an ice-water bath and stirred with a magnetic stir bar in the cold room for an hour. The mixture was clarified by centrifugation at 90,000 g for 25 minutes, and the supernatant was added to 1 mL of anti-GFP nanobody-coupled Sepharose resin (56) per liter of cell culture. After washes with 20 mM HEPES pH 7.5, 150 mM NaCl, 0.01% LMNG, and 0.002% CHS, the OR/Orco complex was eluted by mixing 50 µg of 3C protease (Sigma-Aldrich) with every 1 mL of resin and gentle rotation at 4 °C for an hour. The sample was then concentrated and injected into a Superose 6 Increase column (Cytiva) pre-equilibrated with 20 mM HEPES pH 7.5, 150 mM NaCl, 0.002% LMNG, 0.0004% CHS. Peak fractions containing OR/Orco complex were pooled and concentrated to A280 = 4.7 (the OR10/Orco sample) or 1.8 (the OR28/Orco sample). The proteins were used immediately for cryo-EM grid preparation, or flash-frozen and kept at -80 °C until use. A single freeze-thaw cycle does not significantly affect the protein behavior on grids. To prepare ligand-bound samples, *o*-cresol was added to the OR10/Orco sample to a final concentration of 1 mM, and 2,4,5-trimethylthiazole was added to the OR28/Orco sample to a final concentration of 5 mM. The ligands were incubated with samples at room temperature for 1 hour before making grids.

Prilagajam sliko, ki je vir moje trditve, da je protein stabilen pri pH=7,5 (vzeta je iz članka, v katerem so mu določili strukturo - DOI: 10.1126/science.adn6384). Če bi želeli izolirati celoten kompleks, bi bila najboljša izbira afinitetna kromatografija, pa tudi gelska izključitvena kromatografija. Kompleks je velik (203,11 kDa), zato bi se dobro ločil od ostalih proteinov. Ta način so uporabili, tudi v izbranem članku (moder pravokotnik označuje izbrano kolono, če uporabimo brskalnik ugotovimo, da gre za kolono za gelsko izključitveno kromatografijo). Možna alternativna rešitev je tudi HIC – hidrofobna interakcijska kromatografija (celoten kompleks je močno hidrofoben, nisem pa našla članka, pri katerem bi to tehniko uporabili – gre bolj za špekulacijo).

Number of amino acids: 375
Theoretical pI: 8.69
Molecular weight: 43451.36

Amino acid composition:

Ala (A)	22	5.9%
Arg (R)	16	4.3%
Asn (N)	17	4.5%
Asp (D)	11	2.9%
Cys (C)	7	1.9%
Gln (Q)	14	3.7%
Glu (E)	17	4.5%
Gly (G)	12	3.2%
His (H)	7	1.9%
Ile (I)	39	10.4%
Leu (L)	42	11.2%
Lys (K)	17	4.5%
Met (M)	14	3.7%
Phe (F)	28	7.5%
Pro (P)	11	2.9%
Ser (S)	27	7.2%
Thr (T)	18	4.8%
Trp (W)	5	1.3%
Tyr (Y)	20	5.3%
Val (V)	31	8.3%
Py1 (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

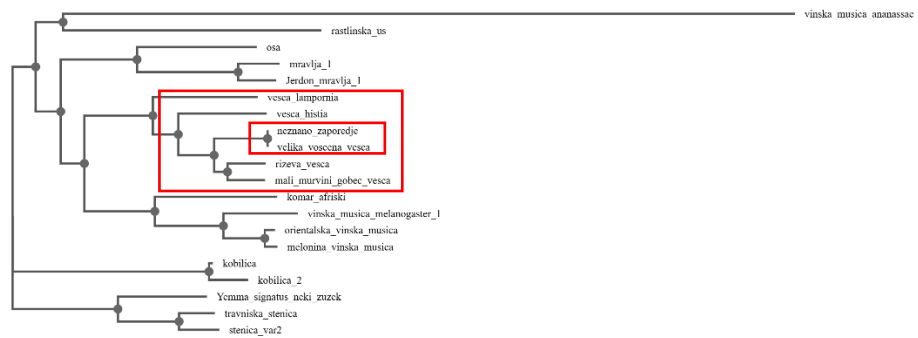
Total number of negatively charged residues (Asp + Glu): 28
Total number of positively charged residues (Arg + Lys): 33

Izračun iz ProtParam:

- Izoelektrična točka tega proteina je 8,69; torej bo pri pH=7,5 pozitivno nabit → potrebujemo kationski izmenjevalec.
- Aminokislinska ostanka, ki sta najbolj zastopana sta levcin in izolevcin (11,2% ter 10,4%). Sledi jima valin z 8,3%. To so hidrofobni aminokislinski ostanki, ki so za protein pomembni, saj protein prehaja membrano → mora imeti hidrofobne regije.

Primer, kaj dobimo v Phylio (zaporedja iz datoteke S1-10-zaporedja-primer):

phylio.io



0.0753

Če so bila vstavljena zaporedja vseh organizmov ugotovimo, da je neznano zaporedje identično zaporedju organizma *Galleria mellonella* (velika voščena večča). Prilagam sliko, na kateri je razvidno, da je za ta organizem znan le en zapis za Orco.

UniProt BLAST Align Peptide search ID mapping SPARQL UniProtKB Orco AND (taxonomy_name:"Galleria mellonella") Advanced | List Search

Please read our help page, view affected entries and proteomes, or contact us with any questions.

UniProtKB 4 results

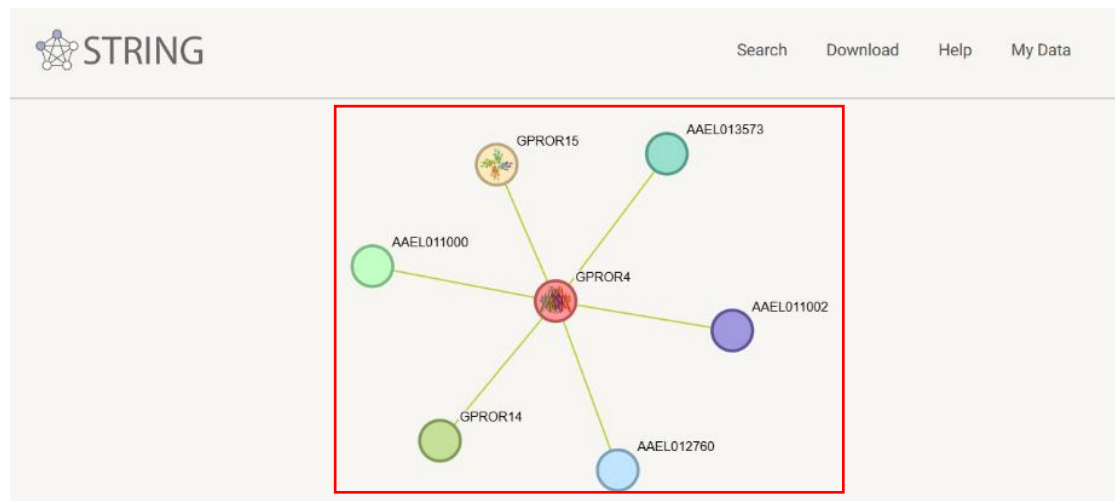
Tools | Download (4) | Add | View: Cards | Table | Customize columns | Share

Entry	Entry Name	Protein Names	Gene Names	Organism	Length
AOA051U2I3	AOA051U2I3_GALME	Odorant receptor	Orco	Galleria mellonella (Greater wax moth)	474 AA
AOA6J1WAC4	AOA6J1WAC4_GALME	Odorant receptor	LOC113510749	Galleria mellonella (Greater wax moth)	401 AA
AOA5C0E4N8	AOA5C0E4N8_GALME	Odorant receptor	LOC113509914	Galleria mellonella (Greater wax moth)	394 AA
AOA5C0E5M3	AOA5C0E5M3_GALME	Odorant receptor	LOC113519132	Galleria mellonella (Greater wax moth)	474 AA

```
# Aligned_sequences: 2
# 1: neznano_zaporedje
# 2: Galleria mellonella
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 474
# Identity: 474/474 (100.0%)
# Similarity: 474/474 (100.0%)
# Gaps: 0/474 (0.0%)
# Score: 2441.0
#
#
#=====
neznano_zapor 1 MHTKVKAGQLVTDLPNPKLQQAAGHFLFNHYSNHSIGNSMLLRKVVSSVH 50
Galleria_mell 1 MHTKVKAGQLVTDLPNPKLQQAAGHFLFNHYSNHSIGNSMLLRKVVSSVH 50
neznano_zapor 51 AVLIVNYYVCHAINAQYSDVEINELTANTITVLFFAHSHVIKLLFFALNSK 100
Galleria_mell 51 AVLIVNYYVCHAINAQYSDVEINELTANTITVLFFAHSHVIKLLFFALNSK 100
neznano_zapor 101 SFYRTLAIINQNSHPLFTESDARYHQLATKMRLLYICIGVTVLSVAS 150
Galleria_mell 101 SFYRTLAIINQNSHPLFTESDARYHQLATKMRLLYICIGVTVLSVAS 150
neznano_zapor 151 WTLTFFGESVRFIAKNETNETMTEPAPRLPKAHPFNTHSGTMYIAAF 200
Galleria_mell 151 WTLTFFGESVRFIAKNETNETMTEPAPRLPKAHPFNTHSGTMYIAAF 200
neznano_zapor 201 ALQIYNLWLFMAIANLQVDFCSWLIFFACEQLHLKAIKPLMELSASLD 250
Galleria_mell 201 ALQIYNLWLFMAIANLQVDFCSWLIFFACEQLHLKAIKPLMELSASLD 250
neznano_zapor 251 TYRPNTELFRASSTSEKSEKVPDPVLDIRGIYSTQDFGTLRGAGGKL 300
Galleria_mell 251 TYRPNTELFRASSTSEKSEKVPDPVLDIRGIYSTQDFGTLRGAGGKL 300
neznano_zapor 301 QTFGQPTPNPNGLTQKQELARSAIKYVVERHKHVRLVASIGDYGTA 350
Galleria_mell 301 QTFGQPTPNPNGLTQKQELARSAIKYVVERHKHVRLVASIGDYGTA 350
neznano_zapor 351 LLFHMILISTITLTLAYQATKIDGVNVPFSTLGLVYTLGQVPHFCIFG 400
Galleria_mell 351 LLFHMILISTITLTLAYQATKIDGVNVPFSTLGLVYTLGQVPHFCIFG 400
neznano_zapor 401 NRIIESSVMEAAVSCQVYDGSSEAKTFVQIVCQCQKAMISIGAKFFT 450
Galleria_mell 401 NRIIESSVMEAAVSCQVYDGSSEAKTFVQIVCQCQKAMISIGAKFFT 450
neznano_zapor 451 VSLDLFAVSLGAVVTFYFVLIQLK 474
Galleria_mell 451 VSLDLFAVSLGAVVTFYFVLIQLK 474
```

Gotovo lahko zaključimo, da je organizem, ki je 'lastnik' proteina s podanim zaporedjem gotovo iz družine večč. Ker pa je z drevesa razvidno tudi, da je neznano zaporedje identično zaporedju velike voščene večče lahko predvidimo tudi specifično vrsto organizma. Če nismo prepričani, lahko uporabimo tudi orodje EMBOSS NEEDLE. Ugotovimo, da sta zaporedji 100% identični

5. Zadnja naloga zahteva uporabo spletne zbirke [STRING](#). V iskalno vrstico vstavimo ime gena GPROR4 in zaženemo iskanje. Pridemo do naslednjega grafa:



S klikanjem na proteine, ki se v grafu pojavijo dobimo informacije o tem kaj počno: iščemo takšne, ki bi sodelovali pri prenosu signala

Da ugotovimo s katerimi proteini interagirajo najdeni proteini kliknemo na **»re-center network on this node«**.

The screenshot shows the STRING database interface with a network graph centered on GPROR4. A pop-up window for AAEL011002 is open, displaying information about the protein and a list of actions. The action 're-center network on this node' is highlighted in red.

Information
AAEL011002-PA; Belongs to the amiloride-sensitive sodium channel (TC 1.A.6) family.
Identifier: Q16RC2, AAEL011002
Organism: Aedes aegypti
UniProt e!

Plan
ASC

Actions

- re-center network on this node
- add this node to input nodes
- show protein sequence
- homologs among STRING organisms

Settings > Analysis

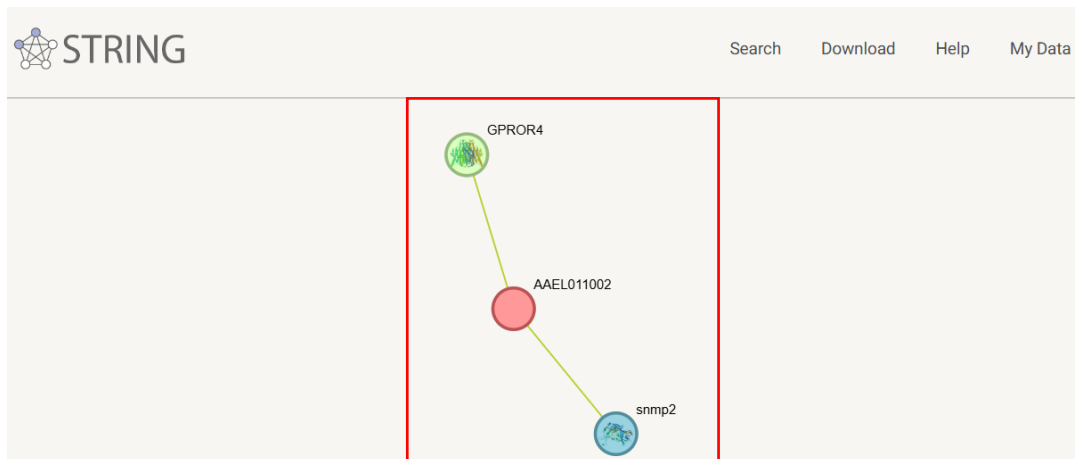
Node Color

- colored nodes: query proteins
- white nodes: second half

Enable node coloring mode

Show this node's terms in the analysis table

Pri proteinih AAEL01357, AAEL011000, AAEL011002 in AAL012760 pridemo do praktično identičnega grafa. Na sliki je primer za AAEL011002:



Na grafu se pojavita tudi proteinska kompleksa GPROR15 in GPROR14. Gre prav tako za receptorja, ki nista del signalne kaskade od GPROR4 do nevrona. Zaključek: proteini, ki sodelujejo pri prenosu signala so: AAEL01357, AAEL011000, AAEL011002 in AAL012760.

Nevronski protein, kateremu se signal prenese je snmp2/senzorni nevronski membranski protein 2. Spada v družino CD36 proteinov.

snmp2
Information
 Sensory neuron membrane protein 2; Plays an olfactory role that is not restricted to pheromone sensitivity; Belongs to the CD36 family.
 Identifier: C3U0S3, snmp2
 Organism: Aedes aegypti

Actions

- re-center network on this node
- add this node to input nodes
- show protein sequence
- homologs among STRING organisms

homology model (C3U0S3 / 4f7bA)
identity: 33.2%

Vredno je omeniti, da so povezave med proteini niso eksperimentalno dokazane, temveč predvidene. Točnega odgovora zato ne moremo podati.