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FUNCTIONAL ANNOTATION AND STRUCTURAL CHARACTERISATION OF OPSIN PROTEIN IN *LEPTUCA PUGILATOR*

S. Harshini[1] and Dr. K. Shoba*[2]

[1]M.Sc., Biochemistry, P.G Research Department of Biochemistry, D.K.M College for Women (Autonomous), Vellore – 632001.

[2]Assistant Professor, P.G. Research Department of Biochemistry, D.K.M College for Women (Autonomous), Vellore – 632001.

***Corresponding Author: Dr. K. Shoba**

Assistant Professor, P.G. Research Department of Biochemistry, D.K.M College for Women (Autonomous), Vellore – 632001.

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ABSTRACT

Fiddler crabs, which belong to the Ocypodidae family and subfamily Ucinae, are prevalent across the world's tropics and subtropics. Decapod crustaceans in the Uca family include *Leptuca pugilator*(Fiddler crabs), often known as the Atlantic sand fiddler crab or calling crab. They are brachyuran crabs from the Ocypodidae family, which are among the most recent aquatic organisms to reach the shore. Fiddler crabs are easily recognised by their distinctly asymmetrical claws, and they can be found in the Algarve regions of Portugal, West Africa, the Western Atlantic, the Eastern Pacific, and the Indo-Pacific. These species are present in seacoasts and brackish intertidal mud flats, lagoons and swamps, mangroves, salt marshes, and sandy or muddy coasts. Opsin, a photoreceptor protein found in fiddler crab species, functions as a fundamental piece of molecular machinery for colour perception and maybe trichromatic vision. The Protein and Nucleotide sequence of opsin protein from *Leptuca pugilator* were retrieved using NCBI database in fasta format. The Functional analysis were done through using AmiGO and T-COFFEE followed by Gene Expression analysis using JCAT Server. The Structural and Biophysical Characterization done through using SWISS-MODEL and Dipole Moment Server. Furthermore, our study investigates which shows the different insilico analysis of the Opsin protein in *Leptuca pugilator* which will increase the Opsin protein characteristics for the range of various medicinal applications.

KEYWORDS: Bioinformatics; Leptuca pugilator; Opsin; Photoreceptor protein; Functional Analysis; Structural Analysis; Medicinal applications.

INTRODUCTION

Crustacea (phylum Arthropoda), a group of invertebrate animals consisting of some 45,000 species distributed worldwide. Crabs, lobsters, shrimps, and wood lice are among the best-known crustaceans, but the group also includes an enormous variety of other forms without popular names.

Fiddler crabs, which are widespread throughout the world's tropics and subtropics, are members of the Ocypodidae family and subfamily Ucinae. The males' feeding behaviour, which comprised a tiny claw that travelled from the ground to its mouth in a manner like how a bow would be moved across a fiddle, gave rise to the name "Fiddler Crab" (the large claw). They are most closely related to the ghost crabs of the "sister genus", Ocypode. There are currently 97 recognised species and subspecies of fiddler crabs. Using a data matrix made up of 236 discrete morphological features, a phylogenetic analysis of 88 species was conducted after researching the systematic history of the genus and its members. Fiddler crabs have five pairs of legs, like other crabs do. The first pair was known as chelipeds due to the presence of claws. The remaining four pairs are called legs or ambulatories and are utilised for walking. Fiddler crabs typically inhabit intertidal areas with muddy or sandy open flats of protected shoreline, river banks, salt marshes, and mangrove muck. While certain species can be found upstream to the highest intertidal limits of mangroves, others can be found in close contact to the sea.

Antimicrobial peptides and proteins form an important means of host defense in eukaryotes. Antimicrobial peptides serve as endogenous antibiotics as well as being involved in inflammation, wound healing, and adaptive immune system modulation. Many antimicrobial peptides have been identified and discovered in crabs over the past few years.

Leptuca pugilator (Fiddler crabs) also called Atlantic sand fiddler crab or calling crab are decapod crustaceans belong to the Ucafamily**.** They belong to the brachyuran crab family Ocypodidae and are the most recent species

of aquatic animal to reach the shore. In this Ocypodidae crab family, there are more than 100 species of fiddler crabs, which are divided among 11 of the 13 genera. Fiddler crabs are easily recognised by their distinctly asymmetrical claws, and they can be found in the Algarve regions of Portugal, West Africa, the Western Atlantic, the Eastern Pacific, and the Indo-Pacific. These species are present in seacoasts and brackish intertidal mud flats, lagoons and swamps, mangroves, salt marshes, and sandy or muddy coasts.

Rhodopsin and its relatives are photosensitive molecules composed of a protein component called an opsin and a non-protein moiety termed the chromophore retinal. GPCRs, also referred to as opsins, are found in animals. More than a thousand opsins have been found so far.

Opsins are membrane proteins that primarily function as light sensors in animals. They have molecular weights of 30–50 kDa and are related to the protein moiety of the photoreceptive molecule rhodopsin. Opsins are the common photoreceptor molecules found in all animal visual systems. During light absorption, they have the ability to switch from a resting state to a signalling state, activating the G protein and triggering a signalling cascade that results in physiological reactions. Opsins are proteins that bind to substances that react to light and are the basis for circadian rhythms, phototaxis, and other light-mediated responses in living things. Type I opsins, common in bacteria, evolved separately from type II opsins that are common in animals. This protein which is essential for normal colour vision. Photoreceptor cells are responsible for transduction of visual signals, and in arthropod compound eyes are clustered together ommatidia. Opsin protein present in *Leptuca pugilator* contains 380 amino acids with the molecular weight of 42.13926 KDA or 42139.26 g/mol. **Formula:** $C_{1950}H_{2962}N_{480}O_{517}S_{23}$

A collection of uncommon eye conditions known as retinitis pigmentosa (RP) affect the retina (the light-sensitive layer of tissue in the back of the eye). RP causes the retinal cells to gradually deteriorate over time, resulting in visual loss. RP is a hereditary condition that affects people from birth.

Cancer is any of a wide range of illnesses characterised by the growth of aberrant cells with the capacity to invade and damage healthy bodily tissue while dividing uncontrollably. It is frequently possible for cancer to spread throughout your body. Cancer is the second-leading cause of death in the world.

Lung adenocarcinoma is the most common primary lung cancer seen in the United States.Adenocarcinoma of the lung usually evolves from the mucosal glands and represents about 40% of all lung cancers. Lung adenocarcinoma usually occurs in the lung periphery, and in many cases, may be found in scars or areas of chronic inflammation.

In this research, functional analysis and structural analysis of opsin protein in *Leptuca pugilator* were done using various bioinformatics tools. So it will be very useful for further research process in drug designing for life threatening diseases.

METHODOLOGY

- Target protein sequence of Opsin in *Leptuca pugilator* were retrieved from NCBI database.
- Functional Annotation of Opsin protein in *Leptuca pugilator* were analysed using AmiGO.
- Phylogenetic relationships of annotated sequence were identified usingT-COFFEE.
- The Gene Expression Analysis of Opsin protein were examined using JCAT Server.
- Structural Characterization like primary, secondary and tertiary structure of Opsin protein were done through using SAPS, PSIPRE and SWISS – MODEL.
- Biophysical characterization of Opsin protein in *Leptuca pugilator* were done using.

Dipole movement server

Results and Discussion

1. NCBI

PROTEIN SEQUENCE

>ADQ01811.1 opsin protein [Leptucapugilator]

MMAAMKVLNATGPQAMAYGSGGYSFGFPEGVSV
TDFVPDHIKHMIHPHWEKFPPVNPMWHYLLGVVY
LFL
GAISLFGNGMVLLLFMKNKNLRSPANYLVANLAIF
DFIMMLKTPVFIVNSFNEGPVWGKLGCDVFALMG
S
YAGIGGAVTNAAIAYDRYKTIAKPFEAKMSRSTAF
LMVVGIWAYASPWSLLPLFGIWGRFVPEGFLTTCS
FDYLSEDLNTRSFVGAIFVFSYILPGMLIVYFYSQIF
SHVKSHEKAMHEQAKKMNVTNLRSNAEANAQSA
EVRIAKVAMTNVALWLVCWTPYAAVVVQGLFFN
QEDITPIVSMLPALLAKSASVYNPIIYAINHTKFRLA
LTKQMPGFCIHEEEEKASGADSKSTDTQKA

NUCLEOTIDE SEQUENCE

>HM765427.1:103-1245 Celucapugilatoropsin protein (Rh3) mRNA, complete cds

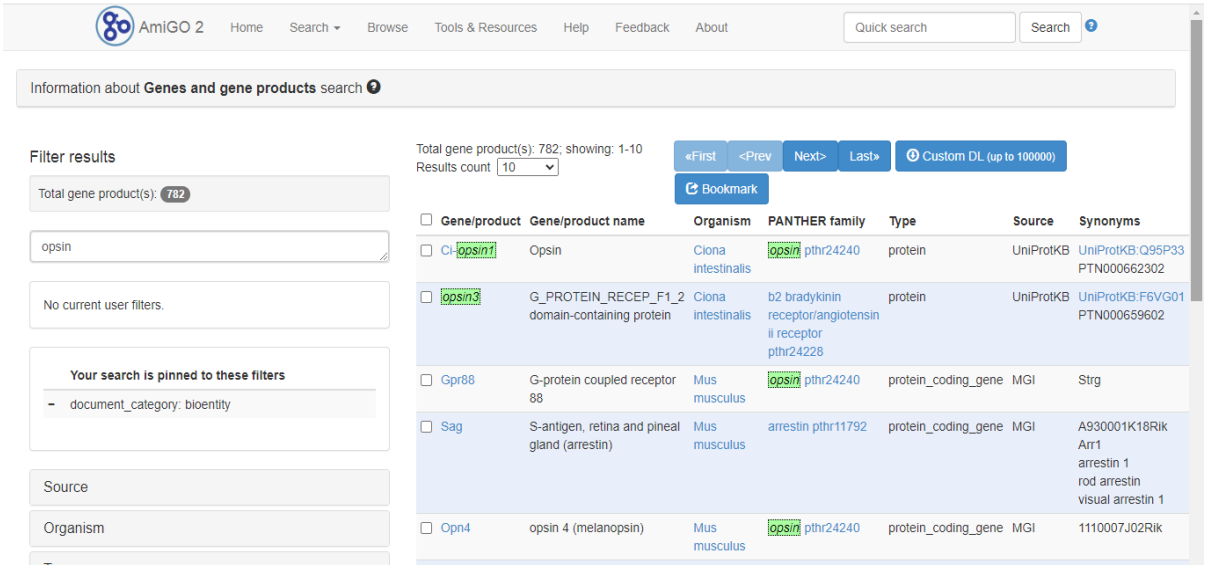
ATGATGGCTGCTATGAAGGTGCTTAACGCGACT
GGCCCACAGGCCATGGCCTACGGCTCAGGAGGA
TATT
CTTTCGGTTTTCCCGAAGGTGTATCTGTCACTGA
CTTTGTTCCTGACCACATTAAGCATATGATTCAC
CC
TCACTGGGAAAAATTTCCACCCGTTAACCCCAT
GTGGCACTACCTTCTGGGTGTTGTGTATCTCTTC
CTC
GGCGCCATTTCCTTGTTCGGCAACGGAATGGTG
CTGTTGTTGTTCATGAAGAATAAGAATCTACGG
AGTC
CAGCCAACTACCTTGTAGCCAACCTGGCGATAT
TCGACTTCATAATGATGCTGAAAACGCCTGTGTT
CAT

TGTTAACTCCTTCAACGAGGGGCCGGTGTGGGG
CAAGCTAGGATGTGACGTGTTTGCGCTCATGGG
CTCC
TACGCTGGTATTGGCGGCGCTGTGACCAACGCT
GCCATCGCCTATGATAGATATAAAACCATCGCC
AAGC
CGTTTGAAGCTAAGATGTCCCGCAGCACAGCCT
TCCTCATGGTGGTGGGAATCTGGGCCTACGCCTC
ACC
ATGGTCCCTCCTTCCACTCTTTGGGATATGGGGA
AGATTCGTGCCAGAGGGCTTCCTGACAACCTGC
AGC
TTCGACTACCTATCAGAGGACCTCAACACTCGCT
CCTTCGTCGGTGCCATCTTCGTGTTCTCATACAT
CC
TGCCGGGGATGCTAATTGTCTACTTCTACAGCCA
GATTTTCAGTCACGTCAAGAGCCACGAGAAGGC
GAT

GCACGAACAGGCCAAGAAGATGAACGTCACCA
ACCTCAGGTCCAATGCCGAGGCTAACGCTCAGT
CTGCT
GAGGTTCGCATCGCCAAGGTGGCCATGACCAAC
GTGGCTCTGTGGCTCGTCTGCTGGACGCCATACG
CCG
CCGTTGTTGTTCAGGGTTTGTTCTTCAACCAGGA
GGACATCACTCCCATCGTTTCCATGTTGCCTGCC
CT
GCTGGCCAAAAGTGCCTCTGTCTACAATCCAAT
AATTTACGCCATCAACCATACCAAATTCCGTCTG
GCC
CTCACGAAGCAGATGCCCGGCTTCTGCATTCAT
GAGGAAGAGGAGAAGGCGTCCGGTGCTGACAG
CAAGT
CCACGGATACCCAAAAAGCGTAA

The above results shows the protein and Nucleotide sequence of opsin protein in *Leptuca pugilator.*

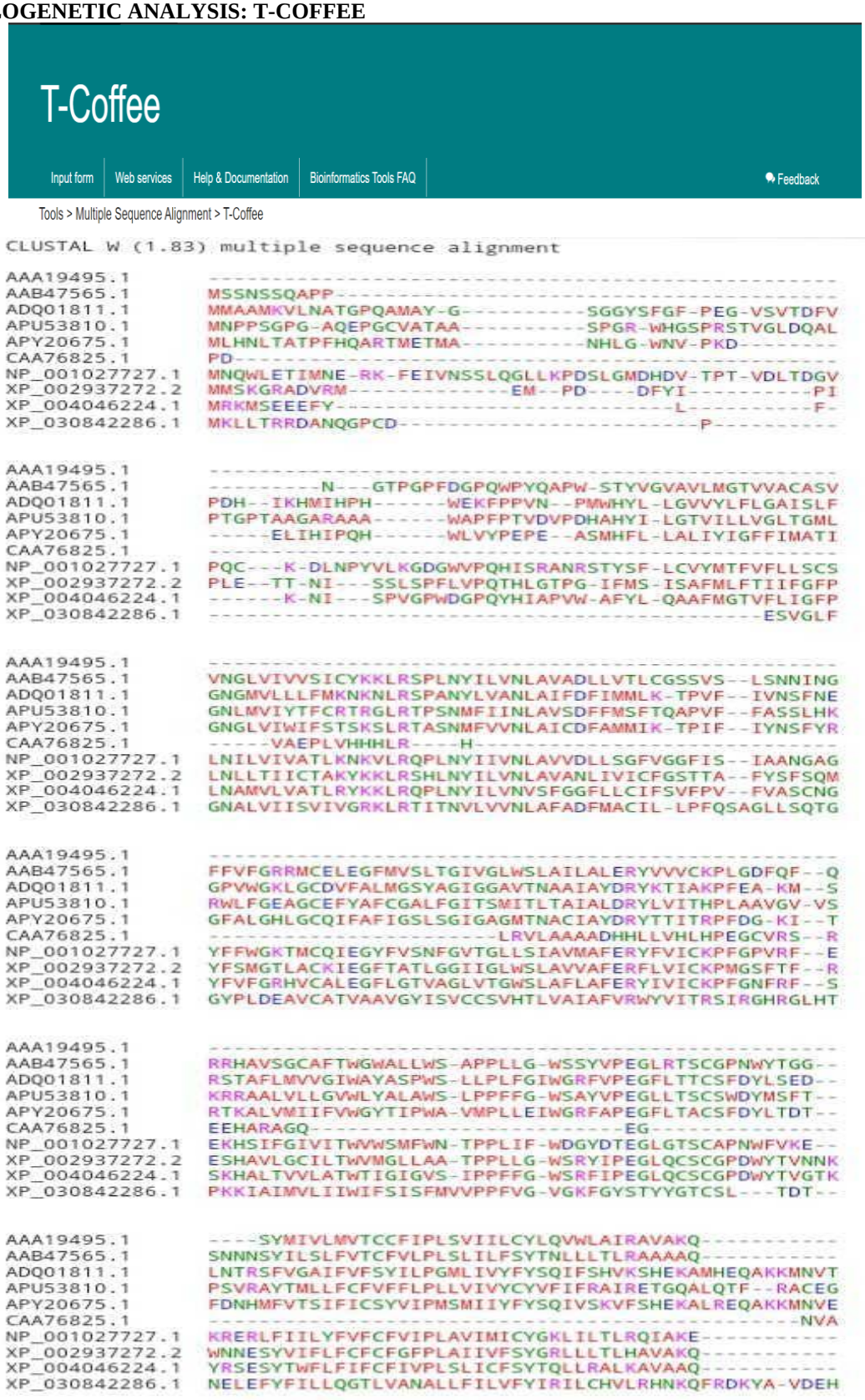
2. AMIGO TOOL

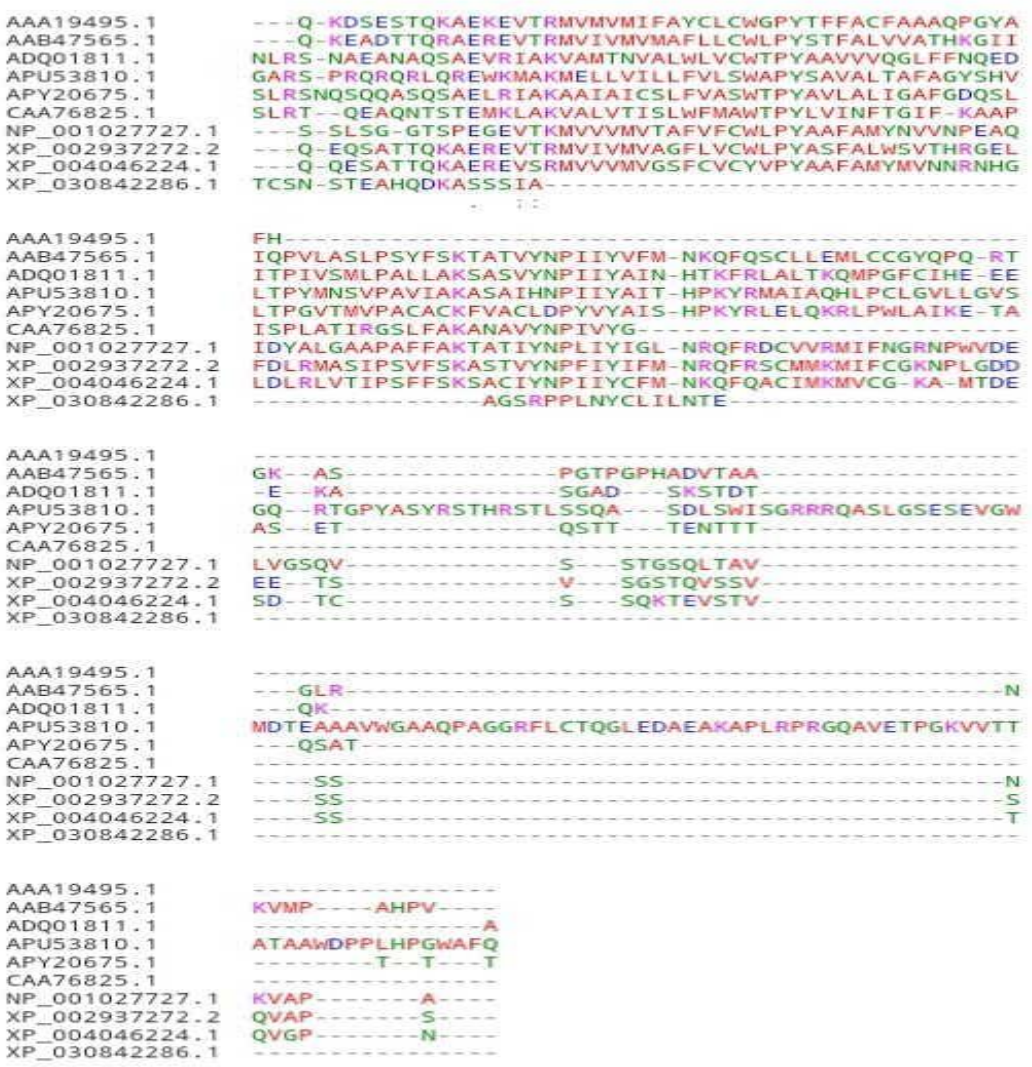


S. No.	SPECIES	PROTEIN NAME
1.	*Leptucapugilator*	Opsin
2.	*Cionaintestinalis*	Opsin
3.	*Bostaurus*	Opsin, partial
4.	*Canis lupus familiaris*	Opsin 4
5.	*Gallus gallus*	Opsin
6.	*Strongylocentrotuspurpuratus*	Opsin, ultraviolet-sensitive-like
7.	*Gorilla gorillagorilla*	Short-wave-sensitive opsin 1
8.	*Anopheles gambiae*	Opsin, partial
9.	*Triboliumcastaneum*	Ultraviolet sensitive opsin
10.	*Xenopustropicalis*	Blue-sensitive opsin

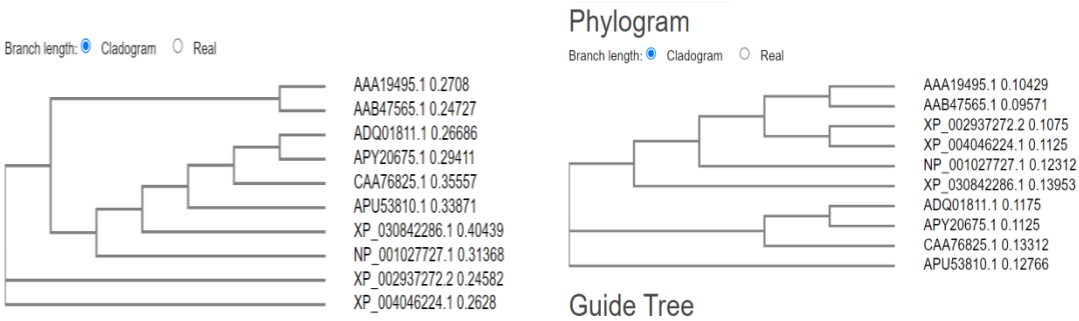
The above results shows the Functional Annotation of Opsin protein in *Leptuca pugilator*.

3. PHYLOGENETIC ANALYSIS: T-COFFEE





Phylogenetic Tree



The above resultsshows the evolutionary relationship of opsin protein with annotated sequence.

4. GENE EXPRESSION ANALYSIS - JCAT

S.No.	Protein Name	Species	CAI Value
1.	Opsin	*Leptucapugilator*	**0.955261450769427**

The above results shows the CAI Value of opsin protein (*Leptuca pugilator*) where the value indicates that gene expression is highly expressed.

5. PRIMARY STRUCTURE ANALYSIS - SAPS

SAPS

Input form | Web services | Help & Documentation | Bioinformatics Tools FAQ | Feedback

Results for job saps-I20230311-080547-0142-45474164-p1m

Summary | Submission Details

View/download raw output file

```
SAPS.  Version of April 11, 1996.
Date run: Sat Mar 11 08:05:48 2023

File: saps-I20230311-080547-0142-45474164-p1m.sequence
ID   EMBOSS_001             Reviewed;         380 AA.

number of residues:  380;   molecular weight:  42.1 kdal

        1  MMAAMKVLNA TGPQAMAYGS GGYSFGFPEG VSVTDFVPDH IKHMIHPHWE KFPPVNPMWH
       61  YLLGVVYLFL GAISLFGNGM VLLLFMKNKN LRSPANYLVA NLAIFDFIMM LKTPVFIVNS
      121  FNEGPVWGKL GCDVFALMGS YAGIGGAVTN AAIAYDRYKT IAKPFEAKMS RSTAFLMVVG
      181  IWAYASPWSL LPLFGIWGRF VPEGFLTTCS FDYLSEDLNT RSFVGAIFVF SYILPGMLIV
      241  YFYSQIFSHV KSHEKAMHEQ AKKMNVTNLR SNAEANAQSA EVRIAKVAMT NVALWLVCWT
      301  PYAAVVVQGL FFNQEDITPI VSMLPALLAK SASVYNPIIY AINHTKFRLA LTKQMPGFCI
      361  HEEEEKASGA DSKSTDTQKA
```

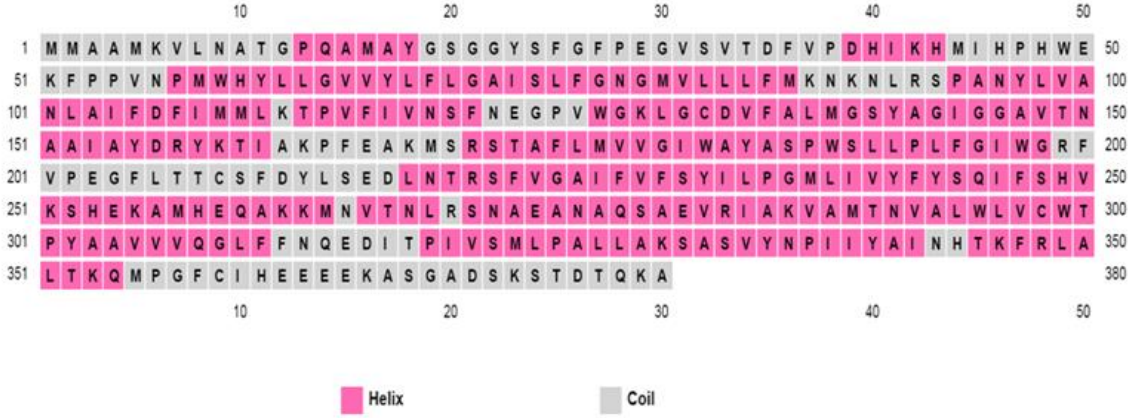
COMPOSITIONAL ANALYSIS

```
 (extremes relative to: swp23s.q)
A   : 40(10.5%); C   :  4( 1.1%); D   : 10( 2.6%); E   : 15( 3.9%); F+ : 27( 7.1%)
G   : 26( 6.8%); H   : 10( 2.6%); I   : 22( 5.8%); K   : 21( 5.5%); L   : 33( 8.7%)
M+ : 19( 5.0%); N   : 19( 5.0%); P   : 20( 5.3%); Q   :  8( 2.1%); R   :  8( 2.1%)
S   : 26( 6.8%); T   : 17( 4.5%); V   : 31( 8.2%); W   :  8( 2.1%); Y   : 16( 4.2%)

KR      :   29 (  7.6%);   ED      :   25 (  6.6%);   AGP     :   86 ( 22.6%);
KRED    :   54 ( 14.2%);   KR-ED   :    4 (  1.1%);   FIKMNY  :  124 ( 32.6%);
LVIFM   :  132 ( 34.7%);   ST      :   43 ( 11.3%).
```

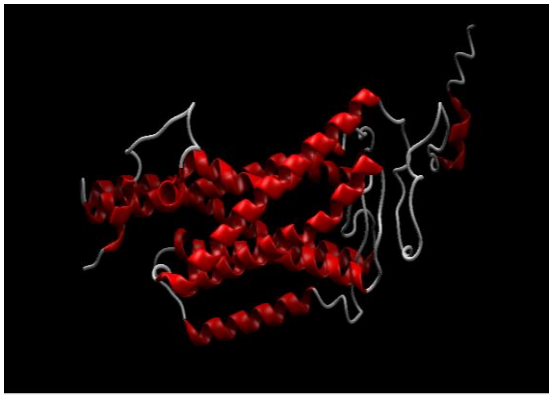
The above results shows the primary structure analysis like molecular weight, number of amino acids and compositional analysis of opsin protein in *Leptuca pugilator*.

6. SECONDARY STRUCTURE ANALYSIS – PSIPRED

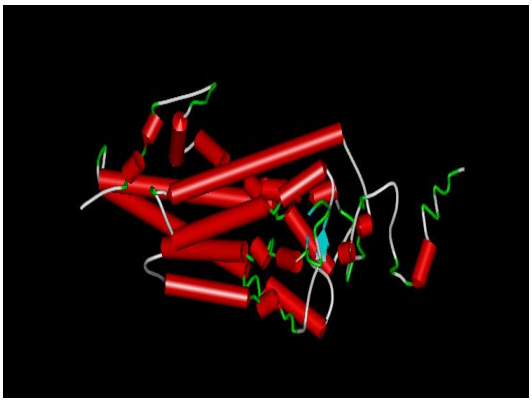


The above resultsshows the secondary structure analysis of opsin protein in *Leptuca pugilator*. i.e., pink colour indicates the Helix region and grey colour indicates the coil region identified in opsin protein.

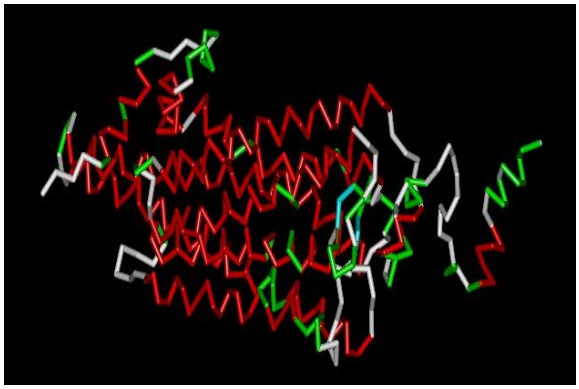
7. HOMOLOGY MODELLING – SWISS MODEL



Ribbon Structure



Pipeline Structure



Stick Structure

Swiss model - Discovery studio

The above results shows the 3 D Structure of Opsin Protein in *Leptuca pugilator*.

8. BIOPHYSICAL CHARACTERIZATION – DIPOLE MOMENT SERVER

Dipole moment for

	No. of Chains=1		Elongated							
	No.Atoms	No.Res.	R_M	Pos.Res.	Neg.Res.	Charge	Dipole	Quadrapole	Crg./Nat.	Dip./Nat.
Value	2483.	314.	376.77	22.	18.	4.	1180.	2235.	0.0016	0.4751
No.Dev.Units	0.61	0.60	0.76	-0.16	-0.50	0.95	1.52	-0.07	0.53	0.37

Dipole vector (in atomic units): -56.20 -239.10 2.93

Mass Moments vector: 415.67 917.53 512.83

Open a larger Jmol window.

Biophysical characterization of Opsin protein in *Leptuca pugilator* were done using dipole movement server. The dipole movement server shows the net charge, dipole moment and mean radius of 3D protein structure and its constituents peptide chains and display the dipole vector superimposed on a ribbon backbone of the protein.

CONCLUSION

In Humans, the overexpression of opsin protein (Melanopsin / OPN 4) in lung cancer cells and tissues which leads to **Lung Adenocarcinoma**.People with this condition suffers from breathing difficulties, pneumonia, pleaural effusion, metastatis and Horner's syndrome (eye muscle problems). Retinal degeneration is brought on by the genetic condition known as **retinitis pigmentosa**, or RP. Those who have this condition face a gradual loss of vision. It is brought on by the deterioration of photoreceptors. Over time, it is inevitable to experience central visual impairment, reduced rods and cones, colour perception, and restricted vision. Rhodopsin, a photopigment, will decline as a result of the damage in the rods.So there will be lacking of Opsin in the retina. Opsin protein is one of the most important antimicrobial protein. It is also responsible for many life threatening diseases. In this study opsin protein in *Leptuca pugilator* was selected. The Sequence of Opsin protein in *Leptuca pugilator* were retrieved using NCBI Database in fasta format and the protein were subjected to several insilico analysis like Functional Annotation, Phylogenetic relationship, Gene expression Analysis, Structural and Biophysical Characterization. Therefore, the Antimicrobial Protein Opsin in *Leptuca pugilator* may have potential application in the treatment of Lung Adenocarcinoma and Retinitis Pigmentosa.

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