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In silico Analysis Of 5'-Nucleotidase from *Discopyge ommata*

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Abstract:

Bioinformatics and computational biology involve the use of techniques with the goal of analyzing the sequence of *Discopyge ommata* to identify the genes. A marine ray *Discopyge ommata* gene (5'-nucleotidase) is used in the first cycle to search for predicting the locations and exon-intron structures of genes in genomic sequences from Genscan. The protein predicted more number of Glycine with divergence value is 328.67. More number of adenine is predicted in the gene of *Discopyge ommata*. The predicted protein of marine ray *Discopyge ommata* shows more number of aliphatic compounds and is closely related to *Xenopus laevis* (African clawed frog), *Danio rerio* (zebrafish) and mouse with Calcineurin-like phosphoesterase.

Keywords: *Discopyge ommata*, Bioinformatics, Phylogeny

Introduction

Bioinformatics and computational biology involve the use of techniques to solve biological problems usually on the molecular level [1]. The human brain often compared to digital computer, where information processing and information flow occur in the course of an organism's development and throughout its lifespan, within a complex ecosystem [2]. The relationships between organisms from nanobes and microbes to macrobes are complex and multidimensional [1,3, 4].

Bioinformatics is not simply a set of tools but rather as a science increasingly essential to navigate and manage the host of information at all stages of genetics research: to improve study design, to assist [5] in candidate gene identification, to aid data interpretation and management and to shed light on the molecular pathology of disease-causing mutations [6]. As the increasing of data availability of genomics sequences, computational analysis of these data has become increasingly important [7,8] . In the present genomic age, a major research goal is to find the functions of genes and to define their interactions in a particular organism.

Gene finding is remarkably accurate in prokaryotic genomes such as bacteria, archaea and viruses, in contrast to be complicated problem of finding genes in higher eukaryotes. The absence of introns by computational analysis of the genome sequence removes one of the major barriers, with the result that the gene finder such as Genscan [9,10, 11].

Several disciplines have recently emerged within scientific discipline that enables biological systems to be studied on a scale commensurate with their inherent complexity[12].

According to shark taxonomists, the number of known shark species estimates to be approaching to 500. Combined with the 700 or more species of rays and skates there are above thousand valid species of elasmobranches, already described from other geographic areas [13,14].

Synaptotagmin (p65) is an integral membrane protein of synaptic vesicles and is considerating to be involved in calcium-dependent exocytosis of synaptic vesicles. Rat synaptotagmin III is a protein of 588 amino acids having 64.0% identity with synaptotagmin isoform of marine ray *Discopyge ommata*. These results suggest that rat synaptotagmin III is a mammalian homolog of o-p65-C and is involved in Ca(2+)-dependent exocytosis of secretory vesicles in endocrine cells, as well as in neurons [15]. The sequences of the p65 synaptic vesicle-specific protein from the marine ray *Discopyge ommata* have been involved in binding of PKC to RACKs (Receptors for activated protein kinase C). The p65 synaptic vesicle-specific protein contains two regions homologous to the C2 region of PKC. Since the only homologous region between PKC and the p65 fragments is the C2 region, suggest that the C2 region on PKC contains at least part of the RACK binding site [16].

Materials and Methods

Data extraction of cDNA sequence of *Discopyge ommata* (electric ray) gene

Discopyge ommata (common name: ocellated electric ray) belongs to family Narcinidae. The mRNA sequence for 5'-nucleotidase (GenBank Accession Number X62278) of *Discopyge ommata* gene is used in the first cycle to search for predicting the locations and exon-intron structures of genes in genomic sequences from Genscan. DNA and protein analysis is done by using EMBOSS package.

DNA ANALYSIS

1. Genscan

This server provides access to the program Genscan for predicting the locations and exon-intron structures of genes in genomic sequences from *Discopyge ommata*. The program was designed primarily to predict genes in *Discopyge ommata*

Submission method:

1. Select the vertebrate parameter file from the drop down menu
2. Paste your sequence in FASTA format into the sequence box
3. Click buttons to add additional options

2. Restriction Summary
Restriction Summary accepts a DNA sequence and returns the number and positions of restriction endonuclease cut sites. Use this program if you wish to quickly determine whether or not an enzyme cuts a particular segment of DNA, and to produce a table to complement the output of Rest and Trans Map.

3. Codon Usage
Codon Usage accepts a DNA sequence and returns the number and frequency of each codon type. The program also compares the frequencies of codons that code for the same amino acid.

4. **Positional Base Frequencies**
Positional Base Frequencies calculates the divergence of the positional base frequencies from random for the DNA sequence. The magnitude of the divergence value is dependent on the length of the sequence. A useful way to interpret the divergence value is to redo the analysis using a shuffled version of the DNA sequence. A coding sequence will often give a much higher divergence value than its shuffled counterpart.

5. DNA Stats
DNA Stats returns the number of occurrences of each residue in the sequence of *Discopyge ommata*. Percentage totals are also given for each residue, and for certain groups of residues, allowing you to quickly compare the results obtained for different sequences.

PROTEIN PREDICTION AND ANALYSIS
1.nnpredict
nnpredict is a program that predicts the secondary structure type for each residue in an amino acid sequence. The basis of the prediction is a two-layer, feed-forward neural network.

2. Protein Stats
Protein Stats returns the number of occurrences of each residue in the *Discopyge ommata* sequence.

3. Protein Molecular Weight
Protein Molecular Weight accepts a protein sequence and calculates the molecular weight.

PHYLOGENETIC ANALYSIS

1. BLASTp

BLASTP takes protein (AA) sequences and compares them against the NCBI protein databases. This search is similar to the standard protein-protein BLAST with the parameters set to optimize for searching with short sequences.

Submission method:
Step 1. Choose the program to use and the database to search.
Step 2. Input the data.
Step 3. Set the program options or choose defaults.
Step 4. Set the output formatting options
Step 5. Perform the search

2. ClustalW

ClustalW is an online tool to construct multiple sequence alignment. The selection of multiple sequences from BLASTP were retrieved and submitted to ClustalW for construction of phylogenetic tree/cladogram.

PROTEIN MODELING

Swiss-Model

SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server. The purpose of this server is to make Protein Modelling accessible to all biochemists and molecular biologists World Wide.

Results and Discussions

Most biologists were interested in "doing bioinformatics" due to use of computers to store, compare, retrieve, analyze or predict the composition or the structure of biomolecules. Computers in this generation become more powerful tool which could probably add simulate to this list of bioinformatics verbs [17.] "Biomolecules" include your genetic material---nucleic acids---and the products of your genes: proteins. These are the concerns of "classical" bioinformatics, dealing primarily with sequence analysis. According to this scheme, the monomers in a given macromolecule of DNA or protein can be treated computationally as letters of an alphabet, put together in pre-programmed arrangements to carry messages or do work in a cell.

Bioinformatics is an emerging and rapidly growing research area which has attracted great attention from medical, biological, and information scientists [17]. After the

Discopyge ommata (electric ray) gene has been completed and stored in GenBank, it provides important information for the study of *Discopyge ommata* genomic structures and functions by *in silico* models [18]. Meanwhile, a great number of tools for data mining are available online, and these tools constitute the foundation for *in silico* cloning.

Discopyge ommata found in Atlantic, Indian and Pacific Oceans, Head equipped with powerful electric organs, developed from branchial muscles. Eyes are small. Well developed caudal fin. Dorsal fins 0-2. Skin is soft and loose. Disc rounded anteriorly; jaws stout; strong labial cartilages; rostrum present. Numbfishes; A nocturnal and solitary species found on sand and rock bottoms, in bays and on coral reefs. Feeds on small crustaceans, worms, and fishes

The family Narcinidae belongs to the Class Elasmobranchii (sharks and rays) and the Order Torpediniformes [13,14]. It contains 9 genera and 24 species. It may be found in Marine environments. Members of this family are not used in the aquarium trade. Reproductively, most members of this family are bearers. The main mode of swimming of adult fish in this family is rajiform. Etymology of this family name: Greek, narke = paralysis.

An mRNA with 2986bp has been taken from NCBI site with Accession Number X62278 belongs to *Discopyge ommata*, an electric ray from Pacific coast of Colombia.

The nucleic acid of this organism was separated from the flat file and has submitted to Genscan for prediction of peptide sequence. The result shows 577 amino acids from the submitted query sequence of *Discopyge ommata*, a vertebrate (Figure 1).

Protein and Nucleic acid analysis has been analyzed using EMBOSS software (Figure 2 to 4 and Table 1, 2).

Positional Base Frequencies predicted a divergence value is 328.67. The Protein weighs 63.62 kilodaltons

The translated CDS (protein sequence) predicted from Genscan has been submitted to BLASTP for protein characterization. The protein sequence has the close characters as mentioned (Figure 5, 6)

1.Hydrolyzes extracellular nucleotides into membrane permeable nucleosides.
[CATALYTIC ACTIVITY] A 5'-ribonucleotide + H(2)O = a ribonucleoside + phosphate.
[COFACTOR] Zinc.
[SUBUNIT] Homodimer; disulfide-linked (By similarity).

[SUBCELLULAR LOCATION] Cell membrane; lipid-anchor; GPI-anchor.

2.*Xenopus laevis* (African clawed frog) with Calcineurin-like phosphoesterase

3.*Danio rerio* (zebrafish) with Calcineurin-like phosphoesterase

4.*Danio rerio* (zebrafish) Exposure to Hg2+ and Pb2+ changes NTPDase and ecto-5'-nucleotidase activities in central nervous system of zebrafish (*Danio rerio*).The sequence has enzymatic properties of an ecto-5'-nucleotidase activity in brain membranes of zebrafish

5. Precursor – mouse with Calcineurin-like phosphoesterase

Swiss modeled image has been presented in Figure 7.

Figure 1: Genescan Output

GENSCANW output

Sequence kk : 2986 bp : 45.41% C+G : Isochore 2 (43 - 51 C+G%)

Gn.Ex	Type	S	.Begin	...End	.Len	Fr	Ph	I/Ac	Do/T	CodRg	P....	Tscr..
1.01	Sngl	+	71	1804	1734	1	0	74	34	2039	0.999	189.96
1.02	PlyA	+	1861	1866	6							-0.45

Predicted coding sequence(s):

>OM|GENSCAN_predicted_peptide_1|577_aa
MPRVPSASATGSSALLSLLCAFSLGRAAPFQLTILHTNDVHARVEETNQDSGKCF
TQSFAGVARRWTKIEELRARDKNVLLLDAGDQYQGTIWFNYYKGAEAAHFIEA
VGYNAMALGNHEFDNGAEGLLDPFLLNVSFPVLSANLEQGEDQVPSLIGYYKPS
TVLDVNGEKIGVVGYTSKETPTLSSPGPHLIFKDEIQAVQHEVDILVSQGIDKIIAL
GHSGFETDKLIAQKVRGVDVVVGGHSNTFLYTGKAPSNDVPVGPYPFLVNSDDQ
RTIPVVQAYAYGKYLGYLKLTFDKGEVIKREGNPILLNSSIIQDPVLLAEVNKWK
ESLANFGKEVIGRTVVYLNGTTEECRNRECNMGNLICDAMIQQNIRNPDEKFWN
HVSICIFQGGGIRAPINEQNNGTIQVDSLLAVLPFGSTIDLLEVYGSTLRAAFDHSV
RRYGQNTGEFLQVSGIQVQFNLKRPPGSRVVKIDVLCADCRVPHYQPLLDNKIY
KIVTNSYIAEGGDGFTMLKNERLRYDTGSTDISVVSSYIKQMKVVYPAVEGRILF
VENSATLPIINLKIGLSLFAFLTWFLHCS

Figure 2: Summary of Restriction enzymes

Item: Positions:
AatII gacgt|c none
AccIII t|ccgga none
AluI ag|ct 15, 164, 393, 669, 947, 1371, 1660, 2083, 2105, 2258, 2507
ApaI gggcc|c none
AvaI c|ycgrg 141, 196, 221, 734
BamHI g|gatcc 458, 1019
BclI t|gatca 970, 1376, 2879
BglII a|gatct 1543, 2922
BssHII g|cgcgc 146
ClaI at|cgat 1486
DraI ttt|aaa 1861, 1923
EcoRI g|aattc none
EcoRV gat|atc 939
HincII gty|rac 686, 782, 875, 1295, 2668
HindIII a|agctt none
HinfI g|antc 219, 629, 662, 896, 986, 1056, 1133, 1249, 1952, 2155, 2382, 2423, 2524, 2694, 2703, 2740
HpaI gtt|aac none
HpaII c|cgg 56, 100, 250
KpnI ggtac|c none
MboI |gatc 458, 646, 715, 970, 1019, 1174, 1334, 1376, 1483, 1543, 1705, 1753, 1939, 2499, 2570, 2879, 2922
MboII gaagannnnnnnnnn| 291, 528, 593, 1402, 1466, 1493, 1763, 2467
MboII n|nnnnnnnntc ttc 641, 1947, 2510
MluI a|cgcgt 916
MspI c|cgg 56, 100, 250
NaeI gcc|ggc none
NarI gg|cgcc 367
NcoI c|catgg none
NdeI ca|tatg none
NdeII |gatc 458, 646, 715, 970, 1019, 1174, 1334, 1376, 1483, 1543, 1705, 1753, 1939, 2499, 2570, 2879, 2922
NheI g|ctagc 90
NotI gc|ggccgc none
NruI tcg|cga none
PstI ctgca|g 1423, 1694
PvuI cgat|cg none
PvuII cag|ctg 164, 2258
RsaI gt|ac 333, 526, 861, 921, 930, 2300
SacI gagct|c none
SacII ccgc|gg 32, 78
SalI g|tcgac 1293
ScaI agt|act none
SmaI ccc|ggg none
SpeI a|ctagt none

Figure 3: Output of codon usage

AmAcid	Codon	Number	/1000	Fract:
Gly	GGG	12	20.76	0.24
Gly	GGA	15	25.95	0.31
Gly	GGT	8	13.84	0.16
Gly	GGC	14	24.22	0.29
Glu	GAG	16	27.68	0.52
Glu	GAA	15	25.95	0.48
Asp	GAT	16	27.68	0.53
Asp	GAC	14	24.22	0.47
Val	GTG	25	43.25	0.5
Val	GTA	4	6.92	0.08
Val	GTT	12	20.76	0.24
Val	GTC	9	15.57	0.18
Ala	GCG	5	8.65	0.14
Ala	GCA	14	24.22	0.38
Ala	GCT	11	19.03	0.3
Ala	GCC	7	12.11	0.19
Arg	AGG	4	6.92	0.17
Arg	AGA	7	12.11	0.29
Ser	AGT	2	3.46	0.05
Ser	AGC	11	19.03	0.29
Lys	AAG	18	31.14	0.64
Lys	AAA	10	17.3	0.36
Asn	AAT	14	24.22	0.41
Asn	AAC	20	34.6	0.59
Met	ATG	6	10.38	1
Ile	ATA	2	3.46	0.05
Ile	ATT	18	31.14	0.46
Ile	ATC	19	32.87	0.49
Thr	ACG	3	5.19	0.1
Thr	ACA	8	13.84	0.28
Thr	ACT	5	8.65	0.17
Thr	ACC	13	22.49	0.45
Trp	TGG	5	8.65	1
End	TGA	1	1.73	1
Cys	TGT	7	12.11	0.78
Cys	TGC	2	3.46	0.22
End	TAG	0	0	0
End	TAA	0	0	0
Tyr	TAT	6	10.38	0.27
Tyr	TAC	16	27.68	0.73
Leu	TTG	9	15.57	0.16
Leu	TTA	6	10.38	0.11
Phe	TTT	13	22.49	0.5
Phe	TTC	13	22.49	0.5
Ser	TCG	8	13.84	0.21
Ser	TCA	3	5.19	0.08
Ser	TCT	3	5.19	0.08
Ser	TCC	11	19.03	0.29
Arg	CGG	7	12.11	0.29
Arg	CGA	4	6.92	0.17
Arg	CGT	1	1.73	0.04
Arg	CGC	1	1.73	0.04
Gln	CAG	13	22.49	0.52
Gln	CAA	12	20.76	0.48
His	CAT	4	6.92	0.33
His	CAC	8	13.84	0.67
Leu	CTG	24	41.52	0.42
Leu	CTA	1	1.73	0.02
Leu	CTT	10	17.3	0.18
Leu	CTC	7	12.11	0.12
Pro	CCG	3	5.19	0.12
Pro	CCA	11	19.03	0.42
Pro	CCT	6	10.38	0.23
Pro	CCC	6	10.38	0.23

Table 1: The Sequence Manipulation Suite by DNA Stats

Pattern	Times found	Percent
G	721	24
A	849	28
T	781	26
C	635	21
GG	157	11
GA	216	14
GT	162	11
GC	152	10
AG	193	13
AA	210	14
AT	212	14
AC	169	11
TG	258	17
TA	118	8
TT	182	12
TC	171	11
CG	79	5
CA	241	16
CT	173	12
CC	119	8
G or C	1356	45
A or T	1630	55

Figure 4: Secondary structure prediction using nnpredict

Sequence *electric ray*:

```
MPRVPSASATGSSALLSLLCAFSLGRAAPFQLTILHTNDVHARVEETNQDSGKCFTQSFA
GVARRWTKIEELRARDKNVLLLDAGDQYQGTIWFNYYKGAEAAHFIEAVGYNAMALGNHE
FDNGAEGLLDPFLLNVSFPVLSANLEQGEDQVPSLIGYYKPSTVLDVNGEKIGVVGYTSK
ETPTLSSPGPHLIFKDEIQAVQHEVDILVSQGIDKIIALGHSGFETDKLIAQKVRGVDVV
VGGHSNTFLYTGKAPSNDVPVGPYPFLVNSDDQRTIPVVQAYAYGKYLGYLKLTFDKGEV
IKREGNPILLNSSIIQDPVLLAEVNKWKESLANFGKEVIGRTVVYLNGTTEECRNRECNM
GNLICDAMIQQNIRNPDEKFWNHVSICIFQGGGIRAPINEQNNGTIQVDSLLAVLPFGST
IDLLEVYGSTLRAAFDHSVRRYGQNTGEFLQVSGIQVQFNLKRPPGSRVVKIDVLCADCR
VPHYQPLLDNKIYKIVTNSYIAEGGDGFTMLKNERLRYDTGSTDISVVSSYIKQMKVVYP
AVEGRILFVENSATLPIINLKIGLSLFAFLTWFLHCS
```

Secondary structure prediction ***(H = helix, E = strand, - = no prediction)***:

```
------------HHHHHHHHHHH------HHEEEE--------H---------EEHHHH
HHHHHH-HHHHHHH----HHEH--------EEEEEHH---HHHHHHHHHHHHHHHH----
-----------HEE--------------------EE------EE------EEEEE----
-----------EE---HHHHHHHHHEEEH----HHEEEH------HHHHHHHHH---EEE
E------EEE---------------EEE--------EEEEE-HH--HE-EHEE------E
E-------EE---------HHHHHHHHHHHHH---HEE-EEEEEE--------------
----H--HH----------H----EEEEE---------------EEEH--HEE------
-EEEEHH-HHHHHHHH--H---------EEEE---EEEE---------EEEEEE------
-----------HHEEE---EEE------EEHH----EE-----EEEEE--HHH--EEE--
-----EEEE-------EEEEEE-HHHHHHHHHH---
```

Table 2: The Sequence Manipulation Suite by Protein Stats

Pattern	Times Found	Percentage
A	37	6
C	9	2
D	30	5
E	31	5
F	26	5
G	49	8
H	12	2
I	39	7
K	28	5
L	57	10
M	6	1
N	34	6
P	26	5
Q	25	4
R	24	4
S	38	7
T	29	5
V	50	9
W	5	1
Y	22	4
Aliphatic I, L, V	146	25
Aromatic F, W, Y	53	9
Positive K, R, H	64	11
Negative D, E	61	11
Tiny G, A, S	124	21

Figure 5: BLASTP result

Sequences producing significant alignments:	Score (Bits)	E Value
gi|112824|sp|P29240|5NTD_DISOM 5'-nucleotidase precursor (Ect...	1184	0.0
gi|66910731|gb|AAH97618.1| MGC114869 protein [Xenopus laevis]	741	0.0
gi|68393614|ref|XP_702406.1| PREDICTED: hypothetical protein ...	724	0.0
gi|41055552|ref|NP_957226.1| 5' nucleotidase ecto [Danio reri...	723	0.0
gi|539794|pir||JC2001 5'-nucleotidase (EC 3.1.3.5) precursor - m	702	0.0
gi|6754900|ref|NP_035981.1| 5' nucleotidase, ecto [Mus muscul...	696	0.0
gi|109071909|ref|XP_001086989.1| PREDICTED: 5' nucleotidase, ect	696	0.0

Figure 6: Phylogenetic tree of Discopyge ommata

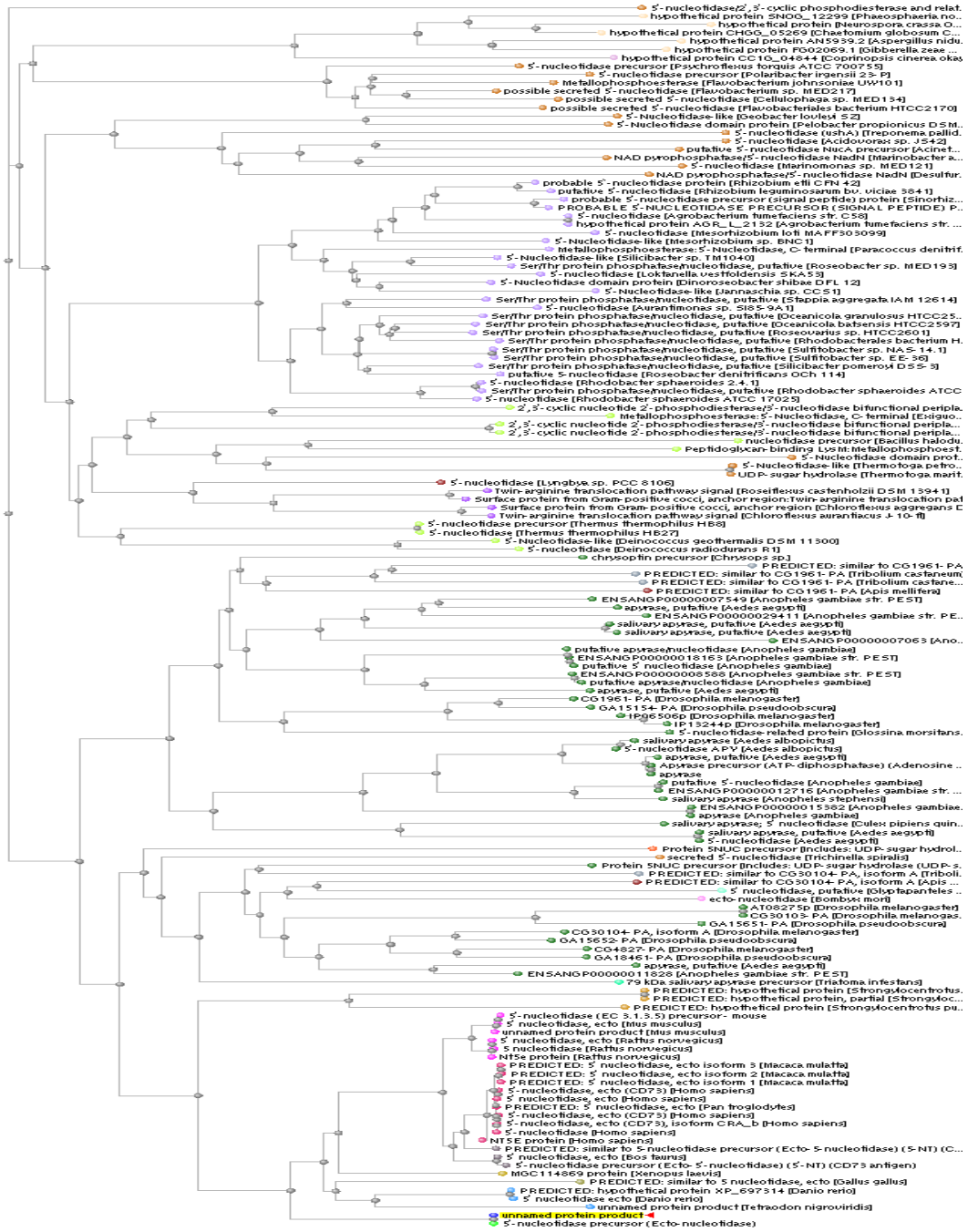
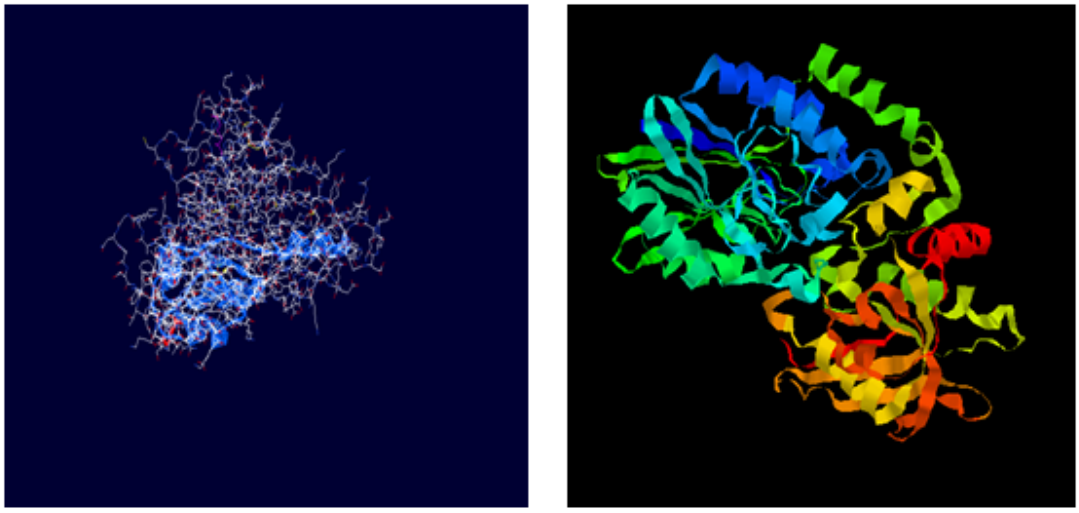


Figure 7: Modeled Image using Swiss Model



a. Image from ArgusLab v4.0.1

b. Image from RasMol v2.5.1

Conclusion

Analysis of gene and protein of *Discopyge ommata* (electric ray) sequence from NCBI with Accession number X62278 provides characterization by *in silico* models. The predicted protein is closely related to *Xenopus laevis* (African clawed frog), *Danio rerio* (zebrafish) and mouse with Calcineurin-like phosphoesterase.

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