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IN SILICO STRUCTURAL AND FUNCTIONAL ANALYSIS OF HUMAN CALCIUM BINDING PROTEIN-5 (CABP5)

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ABSTRACT

Studies showing the role of Ca^{2+}-binding proteins (CaBPs) in mammalian retinal neurons has yielded new insights into the function of these proteins in normal and abnormal states. CaBP5 is a neuronal calmodulin-like Ca^{2+}-binding protein that is expressed in the cochlea and retina. Although CaBP5 knockout mice displayed reduced sensitivity of retinal ganglion cell light responses, the exact 3D structure and function of CaBP5 is still unknown. To gain further insight into CaBP5 structure and function, we tried *in silico* structural and functional analysis of CaBP5. In this study, human Ca^{2+}-binding protein (CaBP-5) having 173 amino acid residues was retrieved from NCBI (Accession no AAF25793.1) and analysed for the structural and functional characteristics using various bioinformatic tools and databases. The analysis revealed structurally and functionally important domains and families and protein-protein interacting partners as PPP3R1, GRK5, MYO5A, MYO5B, MYO5C, OBSCN, MYO1G, RAB30, POTEF and POTEE which might have a role in disease. The structural and binding site prediction of this protein has been done with an aim that it would be useful in docking studies for aiding in the drug discovery.

KEY WORDS: **Ca^{2+}-binding protein5 (CaBP5), Structure analysis, Functional analysis, Bioinformatics tools**

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INTRODUCTION

Ca^{2+} ions play a significant role in many biological systems.[1-4] Among organisms as diverse as yeast and human, an array of signalling pathways are initiated with the changes in the intracellular Ca^{2+} ion concentration. Ca^{2+} ions exert its function in a number of ways. They can act as a diffusible signal that can effect directly or through Ca^{2+}-binding proteins on plasma membrane and intracellular channels, intracellular proteins involved in membrane trafficking, and a wide spectrum of enzymes, including kinases and adenylyl cyclases.[5] Another important research area involves controlling of retinal processes by Ca^{2+} and Ca^{2+}-binding proteins (CaBPs).[6] Much has been learned from studies on Ca^{2+} effects on retinal physiology, and in particular, on regulation of photo transduction in photoreceptor cells.[7-11] The largest group of Ca^{2+}-binding proteins belongs to the calmodulin (CaM) superfamily. They are structurally related and comprise four EF-hand motifs.[11] Several novel subfamilies of CaBPs related to calmodulin (CaM) were discovered including guanylate cyclase (GC)-activating proteins (GCAPs)[12-17], recoverin[18] and hippocalcin-like CaBPs[19,20], or CaM-like CaBPs.[12] Some of these proteins have been found to play important role in the physiology of neurons in other tissues.[19-29] Most of the work done on GCAPs over the last few years has established rapid progress in understanding their structural organization, physiological function and potential implication in human pathologies and in better interpretation of the mechanism involved.[11,14] Information on CaM-like CaBPs in the retina has come out only recently, and it is the beginning, to understand the role of these proteins in various pathways. Both groups of proteins, CaBPs and GCAPs, appear to be related to an CaM like CaBP having four EF hand loops for Ca^{2+}-binding. It has been found that some point mutations in the genes encoding these proteins lead to altered functions causing retina disease, an interesting example being GCAP1 (Y99C) linked to autosomal cone dystrophy.[30] CaBP4 function has been well characterized and has been found to be localized at the photoreceptor synaptic terminals which is essential for photoreceptor synaptic function through enhanced activation of Cav1.4 L-type voltage-gated $Ca2^{+}$ channels and transmitter release.[31,32] It has been documented that cone-rod synaptic disorder and autosomal recessive incomplete congenital stationary night blindness patients have mutations in the CaBP4.[33-35] On other hand, the specific function of CaBP5 *in vivo* has not yet been clearly established. In mice, CaBP5 is expressed in rod bipolar cells.[36-38.] In this communication, we present bioinformatics (*In silico* analysis) data related to structural and functional analysis of human CaBP5 protein, which may be helpful in understanding the role of this protein in the retinal neurons and in other human pathologies. It may also be used for the designing of various drugs.

MATERIALS AND METHODS

Sequence retrieval

Retrieved the sequence of CaBP5 Human from NCBI (http://www.ncbi.nlm.nih.gov/) with sequence ID/ Accession number AAF25793.1 and was used in this study. Various physiochemical, structural and functional properties were analysed by using various bioinformatics tools/software and databases.

Physiochemical properties study

For physicochemical study, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY) were computed by using the Expasy's Protparam Server (http://us.expasy.org/tools/protparam.html).

PFAM

Pfam (http://pfam.sanger.ac.uk/) is a collection of multiple protein-sequence alignments and HMMs (Hidden Markov Model), and provides a good repository of models for identifying protein families, domains and repeats. There are two parts to the Pfam database: Pfam A, a set of manually curated and annotated models; Pfam B, which has higher coverage but is fully automated (with no manual curation). Pfam B HMMs are created from alignments generated by ProDom in the automatic clustering of the protein sequences in SWISS-PROT and TrEMBL. So by using PFAM tool we have identified the protein superfamily and domain regions.

CDD-BLAST

CD-Search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/.) is NCBI's interface for searching the Conserved Domain Database with protein query sequences. It uses RPSBLAST, a variant of PSI-BLAST, to quickly scan a set of precalculated position-specific scoring matrices (PSSMs) with a protein query.[39]

Protein-Protein interactions prediction

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database of known and predicted protein interactions (http://string.embl.de/.). The interactions include direct (physical) and indirect (functional) associations; they are derived from four sources: Genomic Context, High-throughput Experiments, (Conserved) Co-expression and Previous Knowledge. STRING quantitatively integrates interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable. We have employed this STRING tool and analyze the result.

Protein secondary structure prediction

Online server GOR4 (https://npsa-prabi.ibcp.fr) was used for the prediction of secondary structure of CaBP5 protein which accepts the protein FASTA sequence as a query to predict the alpha helix, beta sheet, turn or random coils secondary structure at each position based on 17-amino acid sequence windows.

The original description of the method included four scoring matrices of size 17×20.

Protein tertiary structure prediction

Online server PS2 (PS Square) Protein Structure Prediction Server (http://www.ps2.life.nctu.edu.tw/.) was used which accepts the protein query sequences in FASTA format and uses the strategies of Pair-wise and multiple alignment by combining powers of the programs PSI-BLAST, IMPALA and T-COFFEE in both target – template selection and target–template alignment and finally it constructs the protein 3D structures using integrated modeling package of PS2 using best scored orthologous template. The best model was selected on the basis of Ramachandran plot and protein stability analysis by SAVES on line software (https://services.mbi.ucla.edu).

Metapocket 2.0

Metapocket 2.0 is a Meta approach for protein ligand-binding sites prediction. Active sites of the receptor were analysed by metapocket software (http://metapocket.eml.org). Binding sites are the sites where active site is surrounded by residues.

RESULT AND DISCUSSION

After taking the primary sequence of CaBP5 protein we analyzed its physiochemical properties using Protparam tool. The physiochemical property of this CaBP5 protein is tabulated in Table-1. Among various properties studied, the calculated isoelectric point (pI) is useful because at pI, solubility is least and mobility in an electro-focusing system is zero. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge on protein is zero. At pI, proteins are stable and compact. The computed isoelectric point (pI) could be used for developing buffer system for purification by

Table 1

Physicochemical properties of CaBP5 protein by Protparam tool

Protein name	CaBP5
Accession number	AAF25793.1
No. Of amino acid	173
Molecular weight	19825.7
pI	4.45
+ Residues	21
- Residues	36
Atomic formula	$C_{880}H_{1377}N_{235}O_{271}S_{15}$
Total no. Of atoms	2758
Ext. coefficient	1615
Instability index	39.35
Aliphatic index	78.32
GRAVY	-0.401

isoelectric focusing method. Although Expasy's Protparam computes the extinction coefficient for 276, 278, 279, 280 and 282 nm wavelengths, 280 nm is favored because proteins absorb light strongly there while other substances commonly in protein solutions do not .The extinction coefficient of CaBP5 protein at 280 nm is 1615 M cm with respect to the concentration of Cys, Trp and Tyr. The high extinction coefficient points towards the high concentration of Cys, Trp and Tyr. This extinction coefficient helps in the quantitative study of protein-protein interactions and ligand–protein interactions in the solution. The instability index provides an estimate of the stability of protein in a test tube. There are certain dipeptides, the occurrence of which is significantly different in the unstable proteins compared with those in the stable one. This method assigns a weight value of instability. Using these weight values it is possible to compute an instability index (II). A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable. The instability index value for the CaBP5 protein we found was to be 39.35, it means this protein is stable. The aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. Aliphatic index for this protein is 78.32.The Grand Average hydropathy (GRAVY) value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence. GRAVY indices of CaBP5 protein is -0.401. This low range of value indicates the possibility of better interaction with water (Table- 1). Functional analysis of this protein includes protein domains and family prediction and was done by Pfam and CDD Blast. Domains can be understood of as distinct functional and/or structural units of a protein. Domains are often identified as recurring (sequence or structure) units, which may exist in various contexts. In molecular evolution such domains may have been utilized as building blocks, and may have been recombined in different arrangements to modulate protein function. The protein was classified into particular family based on the presence of specific domain in the sequence. On analysis we found that CaBP5 protein, possessed specific family which was EF-hand 7 family pair with different e-value and bit score. The e-value was 2.9e-08 and 7.8e-15, whereas their bit score was 33.9 and 55 respectively (Figure 1A). Also it was found that CaBP5 protein have functionally three important domains which are EFh (Acc no. cd00051), EFh (Acc no. cd00051) and PTZ00184 (PTZ00184) (Figure 1B). The presence of these domains in the CaBP5 proteins reveals that

Figure 1
A: ***Predicted protein family by using Pfam;***
B: Domain analysed by using CDD BLAST tool

the proteins might be involved in performing the same function. The domains of the CaBP5 protein and their super-family descriptions are given in Figure 1A & Figure 1B. Protein-protein interactions (PPI) are essential for almost all cellular functions. Proteins often interact with one another to perform a common function. For example, the transcription factors interact among themselves to bring about transcription. It is therefore possible to know the functions of proteins based on their interaction partners. Proteins rarely carry out their function in isolation; rather, they operate through a number of interactions with other biomolecules. Experimental elucidation and computational analysis of these complex networks is one of the major challenges in the post-genomic era. Protein-protein interaction databases have become a major resource for investigating biological networks and pathways in cells.[40] On examination we found that CaBP5 protein was found to have interaction with G protein-coupled receptor kinase 5(GRK5), Calcineurin subunit B type 1(PPP3R1), Unconventional myosin-Vc (MYO5C), Unconventional myosin-Ig (MYO1G), Unconventional myosin-Va (MYO5A), POTE ankyrin domain family member E (POTEE), POTE ankyrin domain family member F (POTEF), Unconventional myosin-Vb (MYO5B) and Obscurin (OBSCN). GRK5 phosphorylates a variety of GPCRs, including adrenergic receptors, muscarinic acetylcholine receptors (more specifically Gi-coupled M2/M4 subtypes), dopamine receptors and opioid receptors. In addition to GPCRs, also phosphorylates various substrates: Hsc70-interacting protein/ST13, TP53/p53, HDAC5, and arrestin-1/ARRB1. Phosphorylation of ARRB1 by GRK5 inhibits G-protein independent MAPK1/MAPK3 signaling downstream of 5HT4-receptors. Phosphorylation of HDAC5, a repressor of myocyte enhancer factor 2 (MEF2) leading to nuclear export of HDAC5 and allowing MEF2-mediated transcription. Phosphorylation of TP53/p53, a crucial tumor suppressor, inhibits TP53/p53-mediated apoptosis. Phosphorylation of ST13 regulates internalization of the chemokine receptor. GRK5 phosphorylates rhodopsin (RHO) (*in vitro*) and a non G-protein-coupled receptor, LRP6 during Wnt signaling (*in vitro*).[41,42] PPP3R1 is regulatory subunit of calcineurin, a calcium-dependent, calmodulin stimulated protein phosphatase which confers calcium sensitivity.[43] Unconventional myosins are actin-based motor molecules with ATPase activity and serve in intracellular movements. MYO1G acts as a regulator of T-cell migration by generating membrane tension, enforcing cell-intrinsic meandering search, thereby enhancing detection of rare antigens during lymph-node surveillance, enabling pathogen eradication. Also required in B-cells, where it regulates different membrane/cytoskeleton-dependent processes. It is also involved in Fc-gamma receptor (Fc- gamma-R) phagocytosis.[44] MYO5A act as a processive actin-based motor that can move in large steps approximating the 36-nm pseudo-repeat of the actin filament. It has also a role in melanosome transport. It also mediates the transport of vesicles to the plasma membrane.[45] MYO5B may be involved in vesicular trafficking via its association with the CART complex. The CART complex is necessary for efficient transferrin receptor recycling but not for EGFR degradation. Required in a complex with RAB11A and RAB11FIP2 for the transport of NPC1L1 to the plasma membrane. Together with RAB11A participates in CFTR trafficking to the plasma membrane and TF (transferrin) recycling in nonpolarized cells. Together with RAB11A and RAB8A participates in epithelial cell polarization. Together with RAB25 regulates transcytosis.[46,47] OBSCN is involved in myofibrillogenesis. Seems to be involved in assembly of myosin into sarcomeric A bands in striated muscle. Isoform 3 together with ANK1 isoform Mu17/Ank1.5 may provide a molecular link between the sarcoplasmic reticulum and myofibrils.[48,49] The protein-protein interacting network of the CaBP5 protein is given in (Figure 2A and 2B). Thus this protein could have the function of their interacting proteins.

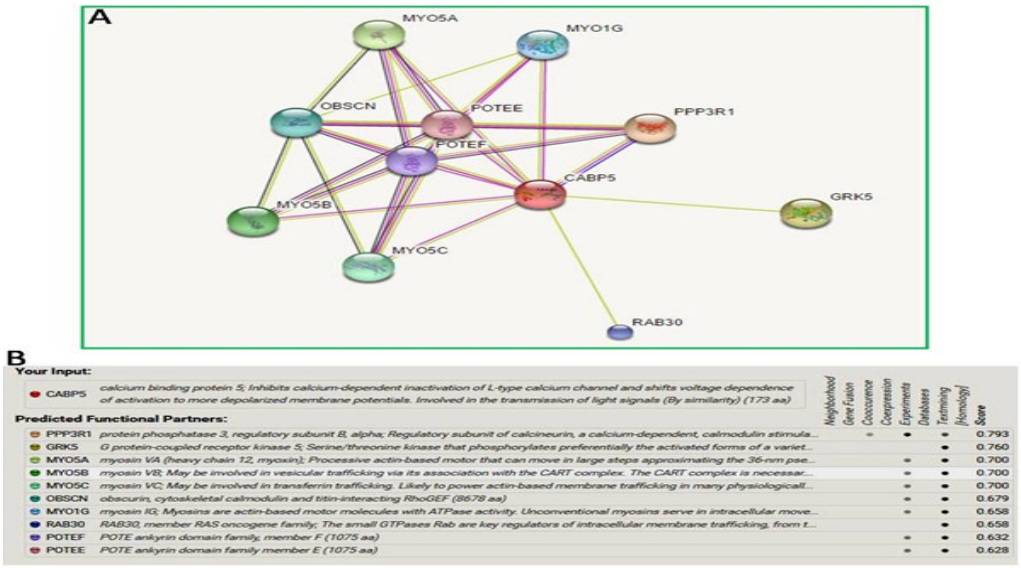

Figure 2A and B
Protein-protein interactions of CaBP5 protein predicted by STRING tool
The secondary structure of CaBP5 protein was predicted by GOR4 online server.
The properties we observed are shown in Figure 3.

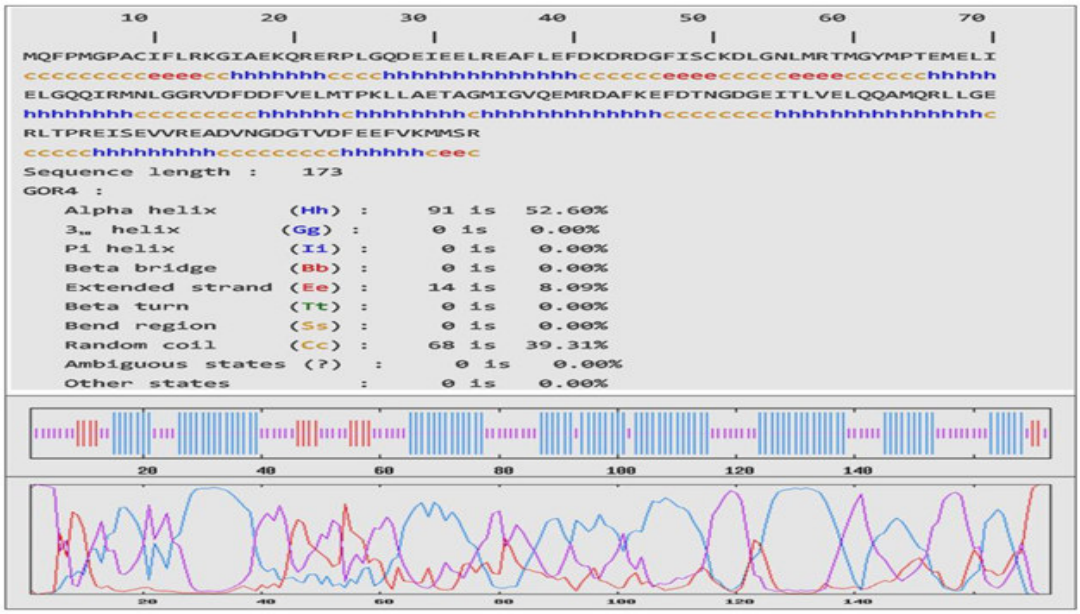

Figure 3
Prediction of secondary structure of CaBP5 by using GOR4 web server

The three dimensional structure of the CaBP5 protein were modeled by PS Square server (Figure 4). The template used by the server to model this protein is shown in Figure 5. After modelling the 3D structure, we validated the 3D structure of the target protein using SAVES online software tool https://services.mbi.ucla.edu (took help of PROCHECK and Ramachandran plot- Figure 6A & 6B) and we found 98.03% of the residues had an averaged 3D-1D score >= 0.2, (Pass) and at least 80% of the amino acids have scored >= 0. 2 in the 3D/ 1D profile, thus passing the criteria of verifying 3D predicted structure. Identifying the location of ligand binding sites on a protein is of fundamental importance for a range of applications including molecular docking, *de novo* drug design and structural identification and comparison of functional sites. Top three active site residues (metapockets) of the CaBP5 protein and its three dimensional structure predicted by Metapocket 2.0 server is shown in

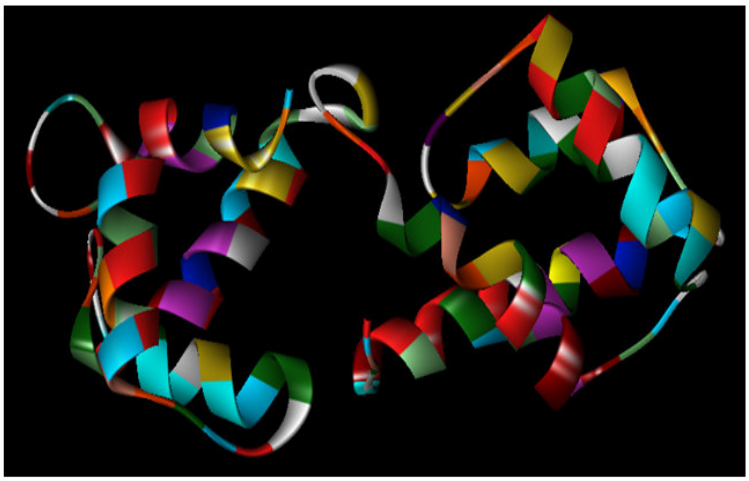

Figure 4
Structures of CaBP5 protein modeled by PS SQUARE server

Template	Seq. Identity	Description
3ox5.1.A	73.68%	Calcium binding protein-1

Model-Template Alignment

```
Model_01  MQFPMGPACIFLRKGIAEKQRERPLGQDEIEELREAFLEFDKDRDGFISCKDLGN  55
3ox5.1.A  ----------------------DRSLRPEEIEELREAFREFDKDKDGYINCRDLGN  35

Model_01  LMRTMGYMPTEMELIELGQQIRMNLGGRVDFDDFVELMTPKLLAETAGMIGVQEM 110
3ox5.1.A  CMRTMGYMPTEMELIELSQQINMNLGGHVDFDDFVELMGPKLLAETADMIGVKEL  90

Model_01  RDAFKEFDTNGDGEITLVELQQAMQRLLGERLTPREISEVVREADVNGDGTVDFE 165
3ox5.1.A  RDAFREFDTNGDGEISTSELREAMRKLLGHQVGHRDIEEIIRDVDLNGDGRVDFE 145

Model_01  EFVKMMSR                                                 173
3ox5.1.A  EFVRMMSR
```

Figure 5
Model template alignment for CaBP5 protein

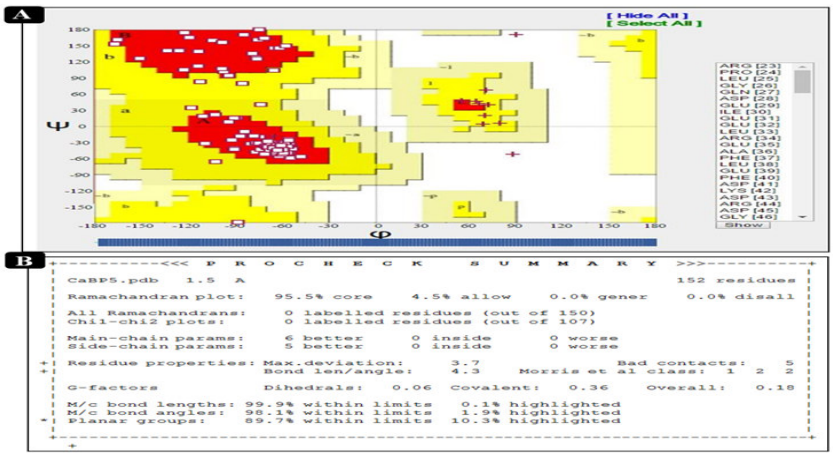

Figure 6A and B
Ramachandran plot & procheck summary of calcium binding protein-5

(CaBP5)

Figure 7A and 7B respectively. The active binding site residues would be helpful for docking with specific ligand to study the binding interactions between them.

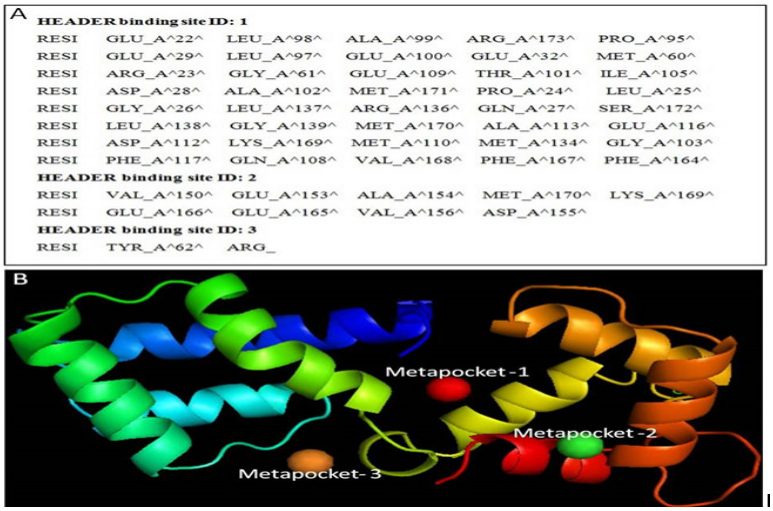

Figure 7A and B
Top three binding sites residues prediction by using metapocket 2.0 server

CONCLUSION

There is a need to annotate and find the functional and structural properties of CaBP5 protein which will be of some help in understanding the role of this protein in the retinal neurons and in other human pathologies. We retrieved CaBP5 protein from NCBI database and characterized its physicochemical properties and identified domains and families using various bioinformatics tools/software and various databases. The structure was modeled and their ligand binding sites were identified. The analysis showed functionally important domains and families. We also predicted the protein-protein interaction partners which may have some significant role in the development of various diseases and ultimately for the designing of therapeutics. The structural and binding sites prediction of this protein can thus be useful in docking studies for aiding in the drug discovery.

CONFLICT OF INTEREST

Conflict of interest declared none

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