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BIOINFORMATICS PROFILING OF SALIVARY AMELOBLASTIN

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ABSTRACT

Amelogenesis imperfecta represents a heterogeneous group of inherited disorders characterized by very thin dental enamel. Defects in mineralization or matrix formation during tooth development lead to enamel hypoplasia and/or hypo mineralization. Mutations in several tooth-specific genes are associated with the disease. In this study we desired to study the ameloblastin protein at different levels of protein structure. The functional and structural role of ameloblastin is not clearly understood. We found a possible kinase phosphorylation site based on signal at

position 27. Therefore, this study would help us to gain deeper insights into the same using in-silico approaches.

Keywords: Bioinformatics Profiling, Salivary Ameloblastin, abnormal enamel

INTRODUCTION

Ameloblast cells secrete tooth specific enamel matrix protein called Ameloblastin (AMBN, is also known as Amelin or Sheathlin) **[1–3]**. Recent researches have reported that AMBN is also expressed during the development of mesenchymal dental hard tissue **[4]**, during the formation of reparative dentin formation **[5]**, and formation of early stages of craniofacial bone **[6]**. The function of AMBN protein is not very clear, however it has been implicated in enamel biomineralization **[7–9]** and also in interactions between the enamel extracellular matrix and the ameloblasts **[2, 10, 11]**. Furthermore, it has been proven that AMBN also acts as a signal molecule in epithelial–mesenchymal interactions **[4, 12, 13]**. Recent in vivo studies demonstrated that AMBN could induce hard-tissue regeneration, by promoting the differentiation and growth of mesenchymal cells at the healing site **[14, 15]**.

Ameloblastin knocked out mice showed severe enamel hypoplasia that resulted from a dramatically reduced expression of amelogenin from the ameloblast cells which form multiple cell layers of poorly differentiated epithelial cells. These findings supported that AMBN gene product is involved in cell attachment and signalling, cell differentiation, and maturation processes **[11, 16]**. Ameloblastin overexpression in transgenic mice demonstrated abnormal enamel crystal structure formation and enamel rod morphology **[10]**. All of the experiments suggest that either loss-of-function or gain-of-function for AMBN in the enamel matrix disrupts normal enamel formationas a consequence of the uncontrolled differentiation of ameloblast cells.

The primary structure of ameloblastin is well conserved over all the species. In most of the species, the AMBN molecule existed as two variants which were generated by alternative splicing of a 15 amino acid segment in the N-terminus region. The rat and mouse with 422 amino acid residues of AMBN contained the DGEA integrin-binding domain as well as the VTXG thrombospondin celladhesion domain, which suggested that AMBN might be involved in cell–matrix interactions. The human homologue had a unique 26 amino acid insert in the center of the molecule that

appeared to be a duplication of short exon 7 **[17, 18]**. Extensive researches on porcine AMBN, called sheathlin, identified 4 different calcium binding peptides of molecular weight 13, 15, 27 and 29 kD, all of which were derived from the C-terminus part of the molecule, starting with Leu3271 **[19, 20]**. Several post-translational modifications have been identified and described, particularly in sheathlin, including sulphated O-linked glycosylation at Thr387 **[20]** and Ser112 **[21]**, hydroxylatedprolines, and phosphorylations at Ser43 and Thr277 **[22]**.

A new generation of prediction method was developed recently by building hybrid models from fragments that was obtained from the experimentally determined protein structures or from the fold-recognition models **[25]**. This was based on the assumption that the global protein conformation could be well approximated by the presence of shorter fragments whose local structures are identified from similar, but not necessarily peptide homologs **[25]**. This method is powerful, as the resulting hybrid models arecomplete and accurate than the input models. This can also predict the three-dimensional (3D) structure of a protein with a completely new fold that is inaccessible by homology modeling **[25]**. This model does not consider the influence of post-translational modifications on protein conformation. Here we report and discuss the ameloblastin protein at different levels of protein structure.

MATERIALS AND METHODS

Data Retrieval and Analyses

The aminoacid sequence of ameloblastin was retrieved from the Uniprotdatabase (reference) which was stored under the accession number Q9NP70 and it was 447 amino acids long. The sequence was uploaded to AAcomp (reference) application to identify similar sequences in the Swissprot database based on similar aminoacid composition both from humans and other species. Isoelectric point and molecular weight of the protein was predicted using AAcompsim (reference). KEGG database portal was used to understand further about the role of ameloblastin in different possible diseases and the hierarchy of the disease was retrieved from the KEGG Brite database **(Figure 1)**.

Interproscan search was also performed to know more about ameloblastin and its family of proteins **(Figure 2)**.

Amino acid sequence of human ameloblastin was searched against nr protein database using Blastp program (reference). This resulted in several hits of intra and inter species ameloblastin homologs. Default

algorithm parameters of Blastp was used and only the orthologous sequences were chosen to understand the evolutionary pattern followed by ameloblastin over the years. A distance based neighbor joining method tree was constructed using the extended options available at the Blastptool at NCBI **(Figure 3)**.

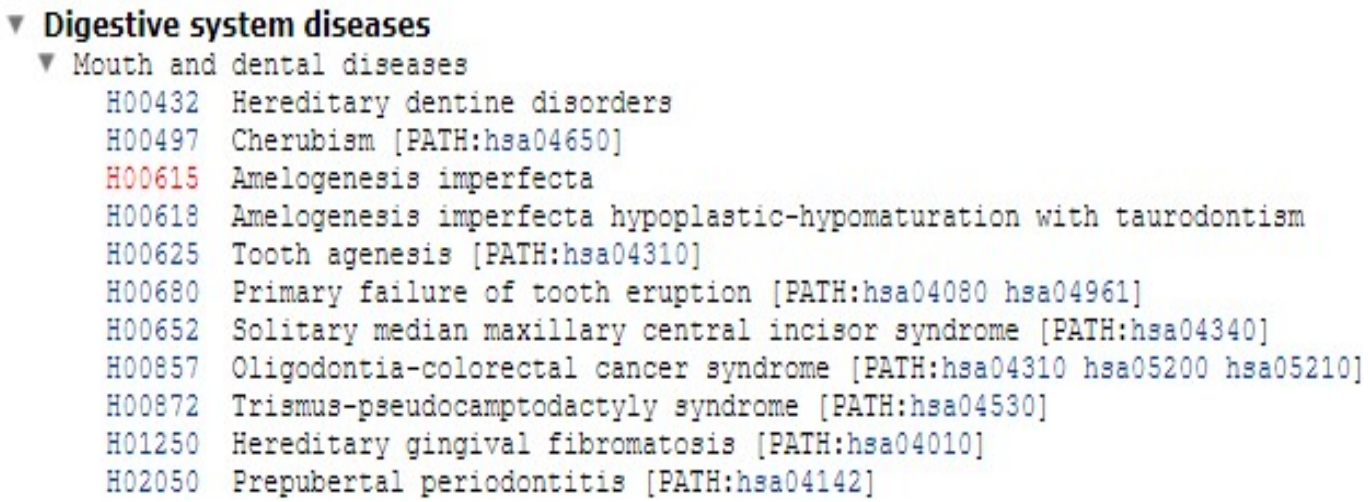

Figure 1: Ameloblastin related disease from KEGG Disease Database

Figure 2: Interproscan search details

Protein AMBN_HUMAN: Ameloblastin. /FTId=PRO_0000001192.

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pI: 4.72 Range: (4.47, 4.97)

Mw: 45344 Range: (36275, 54413)

The closest SWISS-PROT entries (in terms of AA composition for the species HUMAN:

Rank	Score	Protein	(pI	Mw)	Description
1	0	AMBN_HUMAN	4.72	45344	Ameloblastin. /FTId=PRO_0000001192.
2	40	NOBOX_HUMAN	5.79	73906	Homeobox protein NOBOX.
3	43	KMT2D_HUMAN	5.40	593389	Histone-lysine N-methyltransferase 2D.
4	44	FOXN1_HUMAN	5.93	68925	Forkhead box protein N1.
5	46	ZFHX2_HUMAN	5.59	274176	Zinc finger homeobox protein 2.
6	46	ZMIZ2_HUMAN	6.68	96537	Zinc finger MIZ domain-containing
7	47	MYRF_HUMAN	6.39	63758	Myelin regulatory factor, N-terminal.
8	48	UBQL3_HUMAN	5.01	70841	Ubiquilin-3. /FTId=PRO_0000211013.
9	49	FOXN4_HUMAN	5.93	55215	Forkhead box protein N4.

10	50	NTM2D_HUMAN	6.98	86276	NUT family member 2D.
11	51	BAG6_HUMAN	5.40	119409	Large proline-rich protein BAG6.
12	52	NTM2B_HUMAN	8.12	93984	NUT family member 2B.
13	53	PO2F2_HUMAN	8.60	51209	POU domain, class 2, transcription
14	53	TCAM1_HUMAN	5.23	76422	TIR domain-containing adapter molecule 1
15	54	NTM2E_HUMAN	7.97	93979	NUT family member 2E.
16	54	IASPP_HUMAN	6.37	89091	RelA-associated inhibitor.
17	54	ZMIZ1_HUMAN	7.09	115483	Zinc finger MIZ domain-containing
18	55	NTM2A_HUMAN	8.60	93890	NUT family member 2A.
19	56	FMN2_HUMAN	5.32	180106	Formin-2. /FTId=PRO_0000194888.
20	57	ALX3_HUMAN	8.81	36935	Homeobox protein aristaless-like 3.

The closest SWISS-PROT entries (in terms of AA composition) for any species:

Rank	Score	Protein	(pI	Mw)	Description
1	0	AMBN_HUMAN	4.72	45344	Ameloblastin. /FTId=PRO_0000001192.
2	15	AMBN_RAT	5.24	42357	Ameloblastin. /FTId=PRO_0000001195.
3	16	AMBN_MOUSE	5.58	40814	Ameloblastin. /FTId=PRO_0000001193.
4	17	AMBN_BOVIN	5.10	39307	Ameloblastin. /FTId=PRO_0000001191.
5	20	AMBN_PIG	5.15	42009	Ameloblastin. /FTId=PRO_0000001194.
6	34	FOXN1_MOUSE	5.67	69245	Forkhead box protein N1.
7	40	NOBOX_HUMAN	5.79	73906	Homeobox protein NOBOX.
8	42	ZMIZ2_MOUSE	6.70	96719	Zinc finger MIZ domain-containing
9	43	KMT2D_HUMAN	5.40	593389	Histone-lysine N-methyltransferase 2D.
10	44	FOXN1_HUMAN	5.93	68925	Forkhead box protein N1.
11	44	BOP_PONAB	4.81	39061	Protein Bop. /FTId=PRO_0000295911.
12	46	MYRF_MOUSE	6.37	63644	Myelin regulatory factor, N-terminal.
13	46	ZFHX2_HUMAN	5.59	274176	Zinc finger homeobox protein 2.
14	46	ZMIZ2_HUMAN	6.68	96537	Zinc finger MIZ domain-containing
15	47	MYRF_HUMAN	6.39	63758	Myelin regulatory factor, N-terminal.
16	48	UBQL3_HUMAN	5.01	70841	Ubiquilin-3. /FTId=PRO_0000211013.
17	48	NOBOX_MOUSE	5.09	57565	Homeobox protein NOBOX.
18	49	KMT2D_MOUSE	5.54	600245	Histone-lysine N-methyltransferase 2D.
19	49	RX2_CHICK	8.68	34057	Retinal homeobox protein Rx2.
20	49	FOXN4_HUMAN	5.93	55215	Forkhead box protein N4.

The closest SWISS-PROT entries (in terms of AA composition) and having pI and Mw values in the specified range for the species HUMAN:

Rank Score Protein (pI Mw) Description

==========
1 0 AMBN_HUMAN 4.72 45344 Ameloblastin. /FTId=PRO_0000001192.
2 61 BOP_HUMAN 4.93 39299 Protein Bop. /FTId=PRO_0000295910.
3 66 CS067_HUMAN 4.78 39779 UPF0575 protein C19orf67.
4 80 E2F2_HUMAN 4.75 47506 Transcription factor E2F2.
5 81 SHD_HUMAN 4.88 38264 SH2 domain-containing adapter protein D.
6 84 PRR22_HUMAN 4.97 43980 Proline-rich protein 22.
7 85 ME3L2_HUMAN 4.89 39220 Putative homeobox protein Meis3-like 2.
8 95 NAF1_HUMAN 4.76 53717 H/ACA ribonucleoprotein complex non-core
9 97 SPNDC_HUMAN 4.92 41037 Spindlininteractor and repressor of
10 100 ADRM1_HUMAN 4.95 42022 Proteasomal ubiquitin receptor ADRM1.
11 101 HERP2_HUMAN 4.93 45147 Homocysteine-responsive endoplasmic
12 101 NFE2_HUMAN 4.88 41473 Transcription factor NF-E2 45 kDa
13 102 PAIP1_HUMAN 4.71 53525 Polyadenylate-binding protein
14 104 CAH9_HUMAN 4.61 45824 Carbonic anhydrase 9.
15 105 E2F1_HUMAN 4.79 46920 Transcription factor E2F1.
16 105 MINY1_HUMAN 4.75 51778 Ubiquitin carboxyl-terminal hydrolase
17 106 ANR40_HUMAN 4.88 41088 Ankyrin repeat domain-containing protein
18 114 PDD2L_HUMAN 4.71 39286 Programmed cell death protein 2-like.
19 115 SPT32_HUMAN 4.70 42325 Spermatogenesis-associated protein 32.
20 115 GORS2_HUMAN 4.73 47014 Golgi reassembly-stacking protein 2.

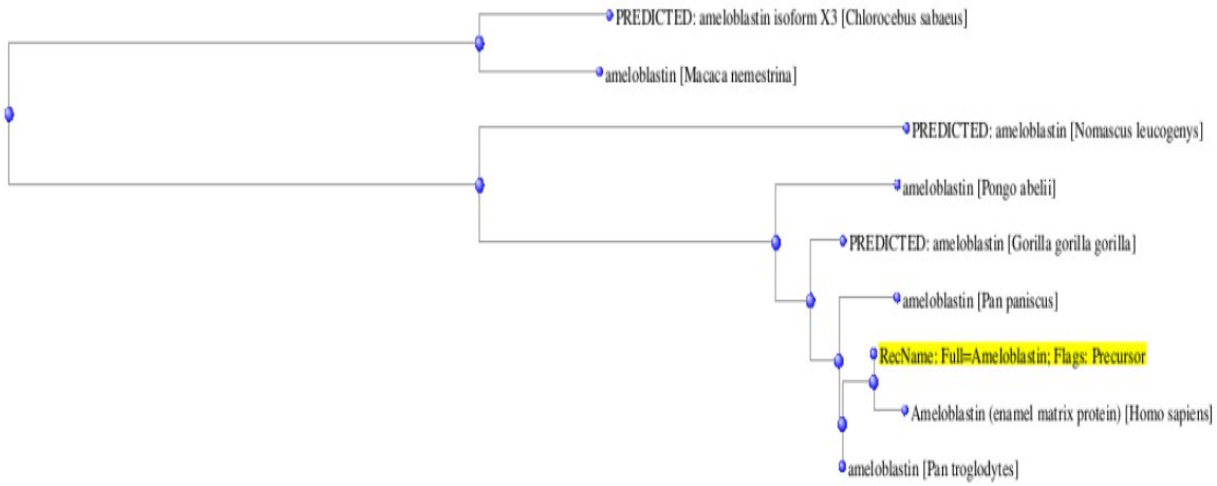


Figure 3: Orthologous Ameloblastins – Evolutionary tree

Further, other primary structure analyses such identification of phosphorylation sites or signal peptides were identified prior to secondary and tertiary structure prediction studies. Signal P server (reference) available at the Expasy server at the Swiss Institute of Bioinformatics was used for this purpose.

Secondary and tertiary structure prediction

Secondary structure gives us better insights into the localised pattern followed in the tertiary structure. NNvPDB server (reference) of SRMBIT portal was used to predict the secondary structure of the ameloblastin. NNvPDB follows neural networks based prediction with very vital advantage of getting the results validated as well. This was followed by subjecting the protein to Modbase (reference) server for modeling the tertiary structure of this protein.

RESULTS AND DISCUSSIONS

This family consists of mammalian Ameloblastin precursor (Amelin) proteins. Matrix proteins of tooth enamel consist mainly of amelogenin but also of non-amelogenin proteins, which, although their volumetric percentage is low, have an important role in enamel mineralization. One of the non-amelogenin proteins is ameloblastin, also known as amelin and sheathlin. Ameloblastin (AMBN) is one of the enamel sheath proteins which is thought to have a role in determining the prismatic structure of growing enamel crystals.

The gene ontology for ameloblastin has been predicted to be involved in odontogenesis of dentin-containing tooth under the biological performing and behaving as the structural constituent of tooth enamel. However, the gene ontology for ameloblastin is not yet clear at the cellular component level.

Protein structure models are computational predictions which may contain errors. The suitability of a specific model for a particular application is determined by its accuracy. The sequence identity between target protein and template structure is commonly seen as a first indicator for the expected accuracy of a model, as confirmed by various studies. This model is based on target-template sequence alignment of 30% sequence identity (A), where substantial alignment errors and suboptimal template selection are frequently observed.

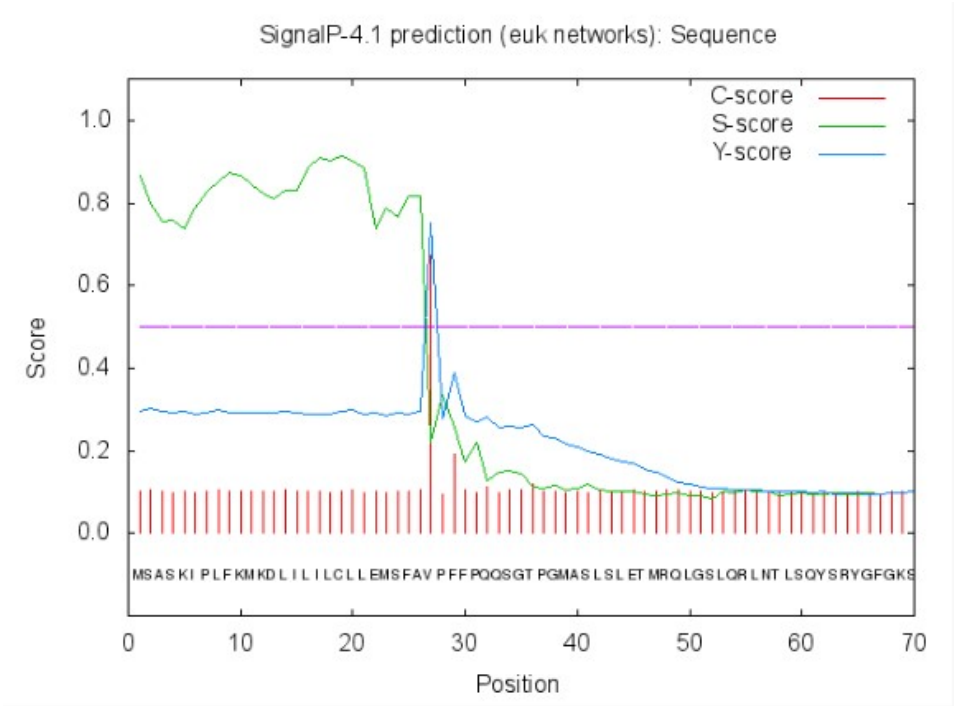
The idea of this study was understand the structural properties of ameloblastin. From the primary sequence analysis performed using AAcompsim it was predicted that this protein has various orthologs. Based on the aminoacid composition similar sequences from the human species were searched and predicted. Also the isoelectric point predicted

for this sequence was 4.72 and the molecular weight was 45322 Kj/mol. The closest entries to ameloblastin in humans were found to be having homeobox domain, Histone-lysine N-methyltransferase, Zinc finger box, etc,. Other matches in terms of pI and MW are shown in table both in humans and other species.

It was decided to check if there are any sites of specific importance inferring in terms of signals. Using Signal P tool we found that the residues 1- 26 to have a cleave site at a cutoff score of 0.450 **(Figure 4)**. Further a multiple sequence alignment based distance tree constructed using neighbor joining method from the selected blast hits resulted in the Pan trogloyditus to be highly similar with our human ameloblastin sequence.

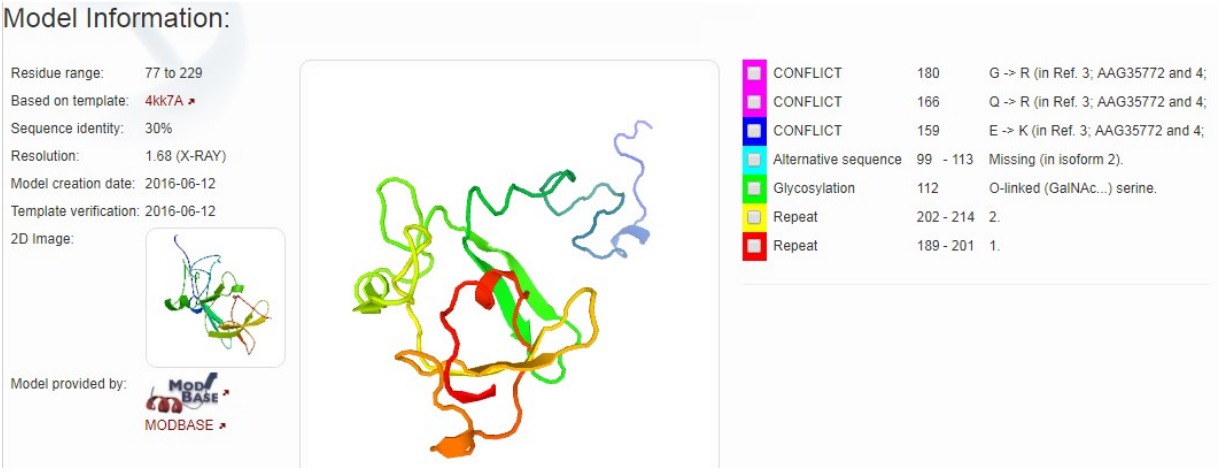
Secondary structure prediction was performed using NNvPDB tool resulted in only partial prediction. As shown in **Figure 5**, most of the regions were predicted as coiled region and few sheet regions. This is because there were no proper similar sequences with sounding homology.

A tertiary structure prediction exercise resulted in not so promising structures. This was due to fact as was earlier informed that this protein is scantily studied. We tried both homology modeling and fold recognition method for tertiary structure prediction. Both were unable to give satisfying results. However, results of the model generated are shown in **Figure 6** which was based on thirty percent sequence identity and less query coverage. From the model it can be seen only residues from 77 to 229 was modeled and most of the model structure consisted of coils as is expected if there are so many gap regions in an alignment.




```
# Measure  Position  Value    Cutoff   signal peptide?
  max. C    27       0.672
  max. Y    27       0.750
  max. S    19       0.912
  mean S     1-26    0.830
       D     1-26    0.793   0.450   YES
Name=Sequence   SP='YES' Cleavage site between pos. 26 and 27: SFA-VP D=0.793 D-cutoff=0.450 Networks=SignalP-noTM
```

Figure 4: Prediction of kinase phosphorylation sites using SignalP server



Input Summary
Description : sp|Q9NP70|AMBN_HUMAN Ameloblastin OS=Homo sapiens OX=9606 GN=AMBN PE=1 SV=1
Length : 447 bp

Secondary Structure:

```
Query   :     MSASKIPLFKMKDLILILCLLEMSFAVPFFPQQSGTPGMASLSLETMRQLGSLQRLNTLSQYSRYGFGKSFN
SS      :     ********BBBBBBBBCBBCBBCBBBBBBCBCCCCBBBBBBBBBBBBBBBBBBBBCBBCBBBBBBBBBBBCC

Query   :     SLWMHGLLPPHSSLPWMRPREHETQQYEYSLPVHPPPLPSQPSLKPQQPGLKPFLQSAAATTNQATALKEAL
SS      :     CBBBCBCBBBBBCBBCBCCBCCBCBCCCCCCCCCCCCBCBCBCCCCBCBBCBBBBBBBBBBBBBBBBBBCB

Query   :     QPPIHLGHLPLQEGELPLVQQQVAPSDKPPKPELPGVDFADPQGPSLPGMDFPDPQGPSLPGLDFADPQGST
SS      :     CCCBBBCCCCBBBCBCBBCCCBBCBBCCCCCCCCCCCCCCCCCCCCBCCBCCCCCCCCCBCBBCCBCCCBBBB

Query   :     IFQIARLISHGPMPQNKQSPLYPGMLYVPFGANQLNAPARLGIMSSEEVAGGREDPMAYGAMFPGFGGMRPG
SS      :     BBBBBCBCBBBCCBBCCCCCBCCCCCCBCBBCCBCCBCBBBBBBCCCBBBCCCCCCCCCBBBBBBCCCCCCC

Query   :     FEGMPHNPAMGGDFTLEFDSPVAATKGPENEEGGAQGSPMPEANPDNLENPAFLTELEPAPHAGLLALPKDD
SS      :     BCCCCCCBBBBBCBCBCBBBCCBBBBCCCCCCCCCCCCCCCCCCCCCBCBBBCCCCCCCBBBBBBBBBCBCB

Query   :     IPGLPRSPSGKMKGLPSVTPAAADPLMTPELADVYRTYDADMTTSVDFQEEATMDTTMAPNSLQTSMPGNKA
SS      :     CCCCBBBCBBBBCBCCCBCCBBBBBBCCCBCBBBCCCBBBBBBBBBBBBBBCBBCCCBBBBBBBBBBCBBCCC

Query   :     QEPEMMHDAWHFQEP
SS      :     CCCCCCC********
```

Figure 5: Secondary structure prediction of ameloblastin using NNvPDB

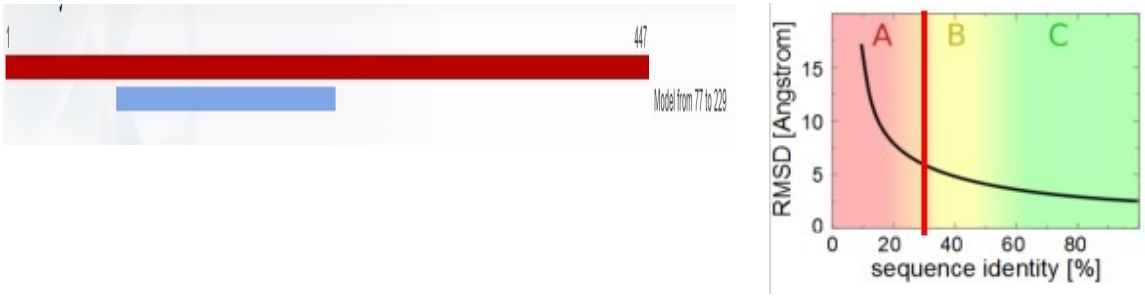


Figure 6: 3D model search results for ameloblastin from Modbase server

CONCLUSION

Therefore, we conclude, that the structural understanding of the ameloblastin protein is very necessary. Existing bioinformatics resources such as databases are unable to get us any lead into the structure and function when the approaches such as homology modeling, fold recognition methods, domain analysis, PSI Blast. The next higher approach that could be tried is tertiary structure prediction using ab initio predictions which involves rigorous calculation.

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