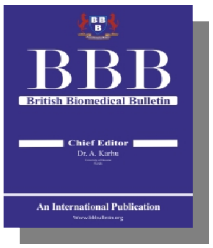
Journal Home Page www.bbbulletin.org

BRITISH BIOMEDICAL BULLETIN




Original

# Bioinformatics Analysis of α-Amylase Three-Dimensional Structure in *Aspergillus oryzae*

**Javad Sharifi-Rad[1,2], Fatemeh Taktaz*[3] and Seyedeh Mahsan Hoseini-Alfatemi[4]**

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran*
[2]*Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran*
[3]*Department of Biology, Faculty of Basic Sciences, Hakim Sabzevari University, Sabzevar, Iran*
[4]*Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran*

A R T I C L E  I N F O

Received 18 Sep. 2014
Received in revised form 27 Sep. 2014
Accepted 28 Sep. 2014

***Keywords:***
Three-dimensional structure,
α-Amylases,
Secondary structures.

**Corresponding author:** Department of Biology, Faculty of Basic Sciences, Hakim Sabzevari University, Sabzevar, Iran.
**E-mail address:** f.taktaz@gmail.com

A B S T R A C T

The major aim of this study is to introduce bimolecular structures from a bioinformatic perspective and informs about the bioinformatic softwares in this field. Each protein has a specific chemical or structural function, strongly suggesting that each has a unique three-dimensional structure. In this study, we investigated α-amylases in *Aspergillus oryzae*. Among starch converting enzymes, the α-amylases (endo-1, 4-α-D-glucan glucanohydrolase [E.C.3.2.1.1]) are of special importance and extensively studied. PDB and NCBI databases and Chimera, Predict Protein and Multalign softwares used for this research. By using these softwares, multiple analyses, including determination of residue composition, secondary structures, conserved regions, ligand binding site done. Starch-hydrolyzing enzymes such as amylases, pullulanases and glucoamylases play an important role in food, chemical, and pharmaceutical industries. In the certain applications like detergent and bakery industries the properties of α-amylase needed is quite challenging Using of bioinformatic softwares improves our understanding about proteins 3D structures and their functions.

## Introduction

The most known amylolytic enzymes are α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3) that are, however, quite different from each other. They don't only differ in their primary and tertiary structures, but also in their catalytic machineries and reaction mechanisms employed. They have therefore been classified into different GH families: GH13-α-amylases, GH14-β-amylases, and GH15–glucoamylases[1]. Among starch converting enzymes, the α-amylases (endo-1, 4-α-D-glucan glucanohydrolase [E.C.3.2.1.1] are of special importance[2] and extensively studied starch-hydrolyzing enzymes such as amylases[3], pullulanases and glucoamylases play an important role in food, chemical and pharmaceutical industries[4]. In the certain applications like detergent and bakery industries the properties of α-amylase needed is quite challenging[5]. Fungal amylases have been used to manufacture starches, starch derivatives and starch saccharification products. The most important fungi used for α-amylase production is *Aspergillus oryzae*, *A. niger*, and *Rhizopus oryzae*[6,7]. Three-dimensional structures of amylases shown in Figure 1.

## Materials and Methods

In this study the three-dimensional structure of the alpha-amylase enzymes in *Aspergillus oryzae* was studied in various aspects. At the first step, amino acid sequences of *Aspergillus oryzae* α-amylase or Taka-Amylase A (PDB ID: 2TAA) was taken from the Protein Data Bank website. The amino acid sequence of this enzyme in several strains from NCBI databases were obtained and aligned by Multalign software. Three-dimensional structure of the enzyme was carried out with the help of Chimera UCSF software. Predict protein software was used for prediction of amino acid content of the enzyme and its secondary structure.

## Results and Discussion

### Protein structure analysis

The Taka-Amylase A (EC=3.2.1.1) has three chains A, B and C that each of them has 479 amino acids in monomer structure with signal domain, and its molecular weight is 54,810 (Da). Amino acid sequence analysis of Taka-Amylase A for each chain (www.uniprot.org) shown in Table 1.

The application forms that conserved regions in the sequence of the enzyme determined by Multalign software that shown in Figure 2.

### Secondary structure and solvent accessibility prediction for chain

PROFsec predicts secondary structure elements and solvent accessibility using evolutionary information from multiple sequence alignments and a multi-level system. Three states of secondary structure predicted: helix (H; includes alpha-, pi- and 3_10-helix), (beta-) strand (E = extended strand in beta-sheet conformation of at least two residues in length) and loop. Secondary structure predicted by a system of neural networks with an expected average accuracy of more than 72%. Evaluation of enzyme molecules indicated that the active form of the enzyme is composed of three chains in the same sequence and secondary structure of the enzyme is related to the structure shown in the following diagram. The secondary structure of the enzyme has 59% loop, 20.9 % helix and 20.09 % strand. Based on the sequence diagram of the bulk structure of the enzyme is made of loops and the amount of helix and strand is approximately equal. Amino acid and secondary structure, composition shown in Figures 3 and 4, respectively. The structure of the enzyme also

examined in terms of solvent accessibility shown in Figure 5.

The NetPhos 2.0 server produces neural network predictions for serine, threonine and tyrosine phosphorylation sites in eukaryotic proteins. By this software phosphorylation site on enzymes identified. Figure 6 that shown phosphorylation site on enzyme structure.

This enzyme has three calcium ions in each of the three chains at position 479 that shown in Figure 7. The position of the essential calcium ion may be assumed to be near both site C and the maltose residual. The calcium ion may play a role in tightening the catalytic sites suitable for binding amylase and/or for supplying water molecules to the reaction.

## Conclusions

The illustration of the whole chain folding indicates some characteristic features of enzyme structure which have so far been recognized in general. The molecule is composed of two domains: the main and the C-terminal domains which are connected to each other by a single polypeptide chain. In fact, a molecular model of Taka-amylase A (α-amylase) from *A. oryzae* by Matsuura et al suggests that both His and Asp in these homologous areas functions as active sites, while Asp and Lys in region 2 and His in region 4 may function as a substrate-binding sites. Since another active site of Glu and the neighboring substrate-binding sites of Val, Leu and Asp between regions 2 and 4 have been suggested[8], the sequences were examined for an alignment containing these four amino acid residues. Understanding the three-dimensional structure of proteins is essential for its function. Combining Bioinformatics and biochemistry helps in our understanding of the biochemical model. Today with the increasing volume of information, familiarity with Bioinformatics software is important to achieve desired results quickly and the accurately.

## References


1. Stefan J. Amylolytic Enzymes - Focus On The Alpha-Amylases From Archaea And Plants. 2009. *Nova Biotechnologica* 9-1.
2. Nielsen JE. Borchert TV. Protein engineering of bacterial α-amylases. 2000. *Biochim Biophys Acta*; 1543:253–74.
3. MacGregor EA. Janeček Š. Svensson B. Relationship of sequence and structure to specificity in the -amylase family of enzymes. 2001. *Biochim Biophys Acta*; 1546:1–20.
4. Burhan U. Nisa C. Gokhan C. Omer A. Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic Bacillus sp. isolate ANT-6. 2003. *Biochem*. 38; 1397-1403.
5. Gupta R. Gigras P. Mohapatra H. Microbial α-amylase: a biotechnological perspective. 2003. *Biochem*. Vol. 38 1599-1616.
6. Bogar B. Szakacs G. Tengerdy R. P. Linden C .Pandey A. Production of α amylase with Aspergillus oryzae on Spent Brewing Grain by Solid Substrate Fermentation 2002. *Applied Biochemistry and Biotechnology*. Vol. 102–103.
7. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Setzer WN. Chemical Composition, Antifungal and Antibacterial Activities of Essential Oil from *Lallemantia Royleana* (Benth. in Wall.) Benth. *Journal of Food Safety*. (2014); doi: 10.1111/jfs.12139. In Press.
8. Lesk MA. Introduction to Bioinformatics. 2002. Oxford University Press Inc., New York.
9. Matsuura Y. Kusunoki M. Harada W. Kakudo M. Structure and possible catalytic residues of Taka-amylase. A. 1984. *Biochem*. 95:697-702.

**Table 1.** Amino acid sequence analysis of Taka-amylase a for each chain (www.uniprot.org)

| No. | Feature key | Position (s) | Length | Description |
|---|---|---|---|---|
| 1 | Signal peptide | 1 – 21 | 21 | |
| 2 | Chain | 21 – 499 | 479 | Alpha-amylase Atype-1/2 |
| 3 | Active site | 227 | 1 | Nucleophile |
| 4 | Active site | 251 | 1 | Proton donor |
| 5 | Binding site | 56, 104, 143<br>225, 255, 317<br>365 | 1, 1, 1<br>1, 1, 1<br>1 | Substrate |
| 6 | Site | 318 | 1 | Transition state stabilizer |

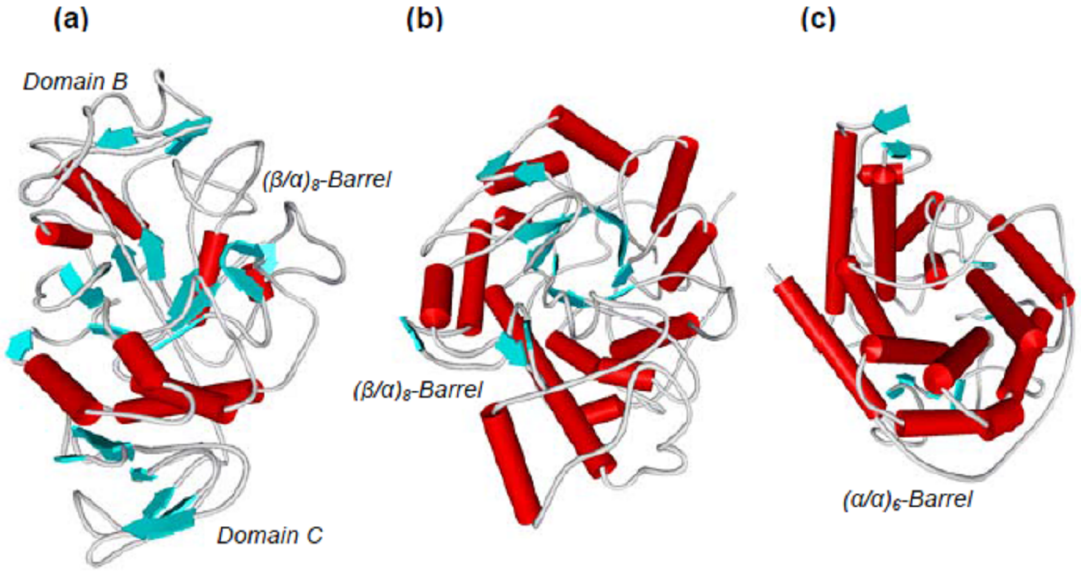



**Figure 1**. Three-dimensional structures of amylases. (a) GH13 α-amylase from *Aspergillus oryzae* (PDB code: 2TAA); (b) GH14 β-amylase from soybean (1BYA) and (c) GH15 glucoamylase from *Aspergillus awamori*



British Biomedical Bulletin

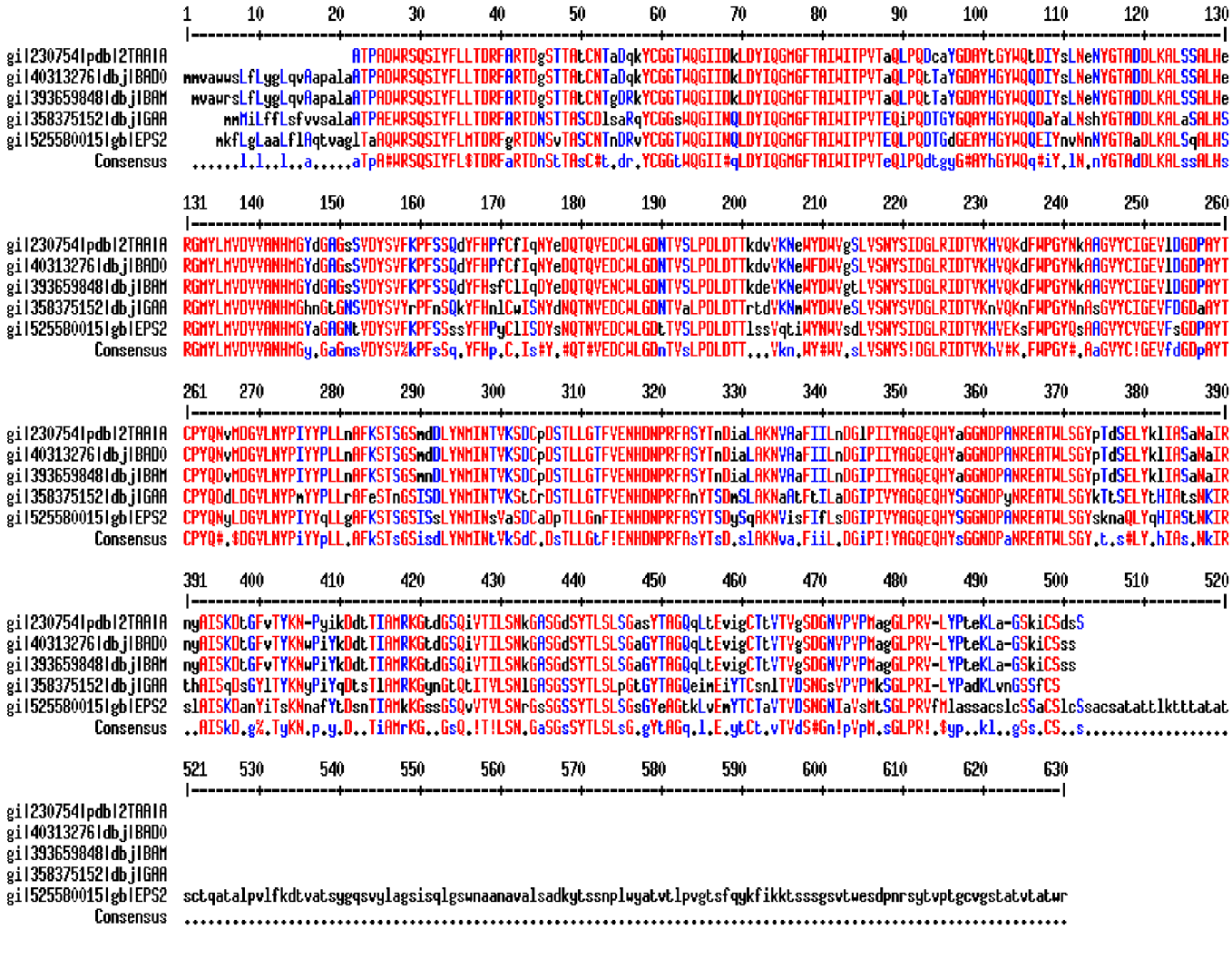


**Figure 2**. Multiple sequence alignment by Multalign software. Accession numbers: Taka-Amylase A (2TAA|A), alpha-amylase [*Aspergillus awamori*] (BAD06002.1), alpha-amylase [*Aspergillus sojae*] (BAM28635.1), alpha-amylase [*Aspergillus kawachii* IFO 4308] (GAA91738.1), alpha-amylase Amy13A [*Penicillium oxalicum* 114-2] (EPS26265.1)



British Biomedical Bulletin

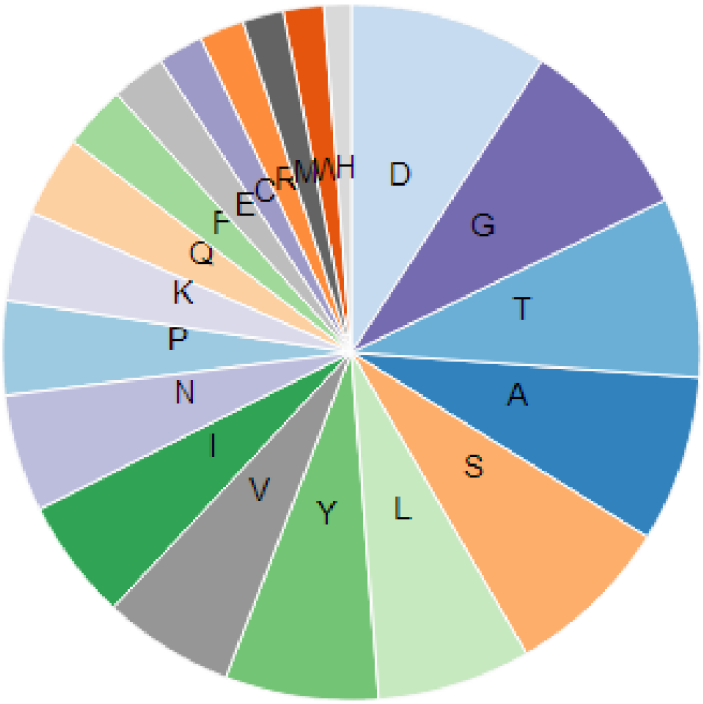



**Figure 3**. Amino acid composition

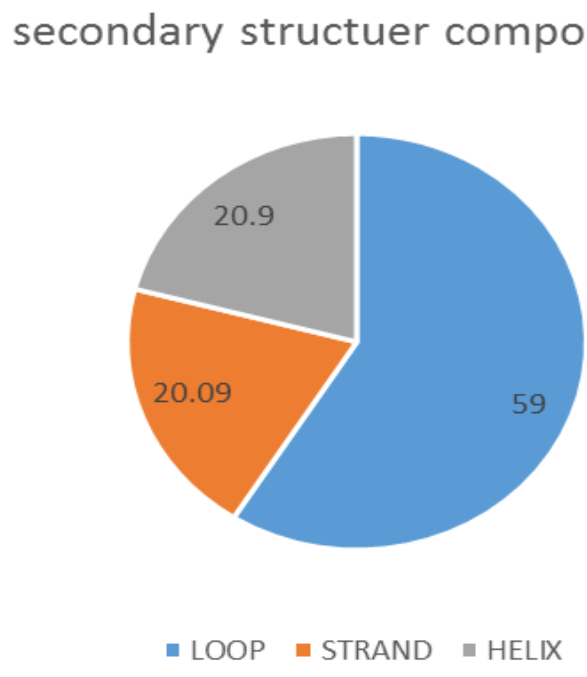



**Figure 4**. Secondary structure composition

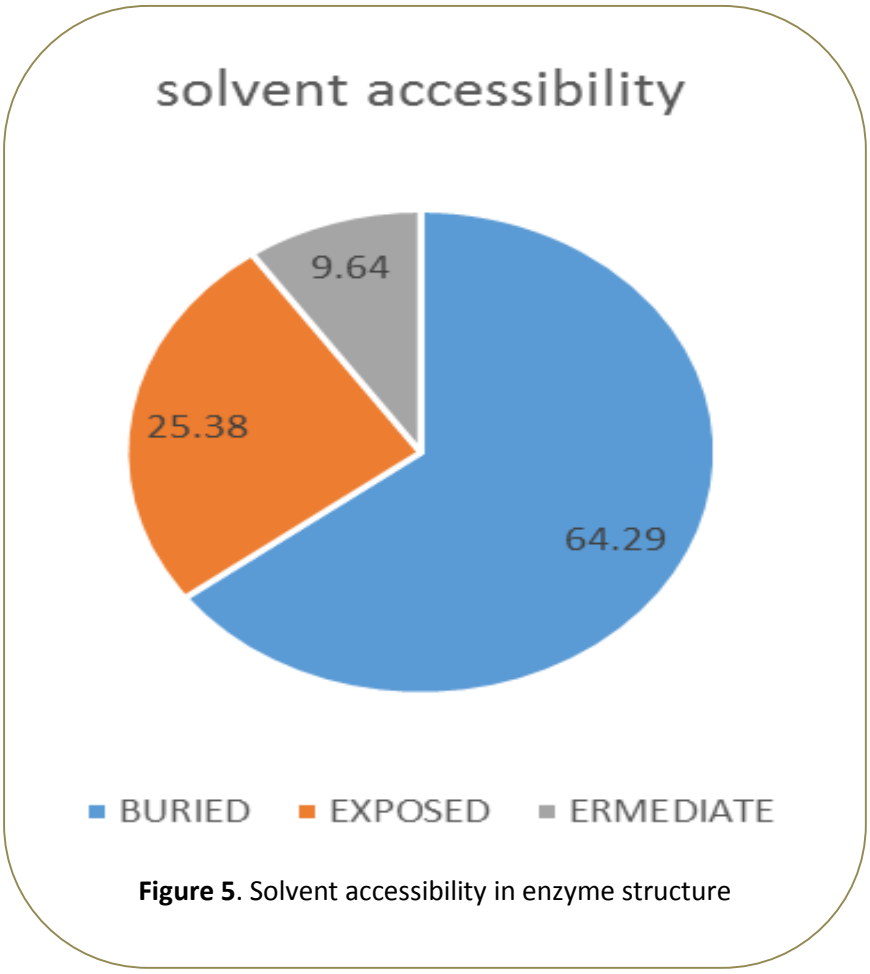



**Figure 5**. Solvent accessibility in enzyme structure

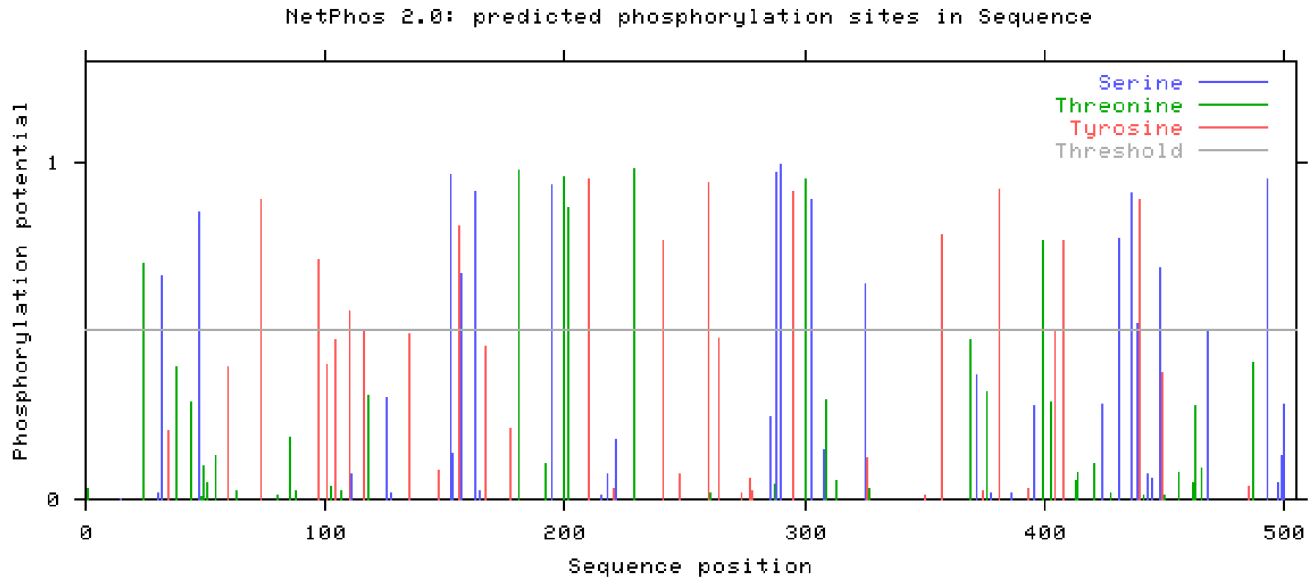



**Figure 6**. Phosphorylation sites on enzymes structure

 ISSN-2347-5447

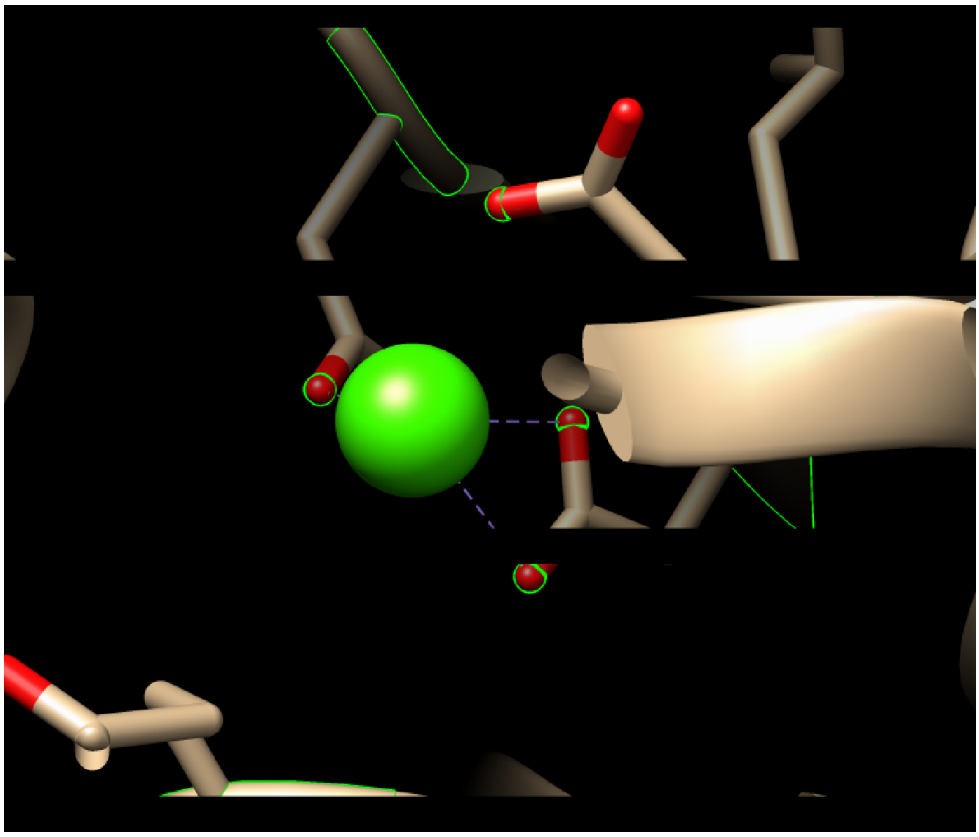


**Figure 7**. Calcium ions in enzyme structure