

# Research Paper: Bioinformatics Analysis of Linear B-cell Viscumin Toxin Epitope With Potential Use in Molecularly Imprinted Polymer Biosensors



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**Citation:** Sasan Nasirahmadi, Jamil Zargan. Bioinformatics Analysis of Linear B-cell Viscumin Toxin Epitope With Potential Use in Molecularly Imprinted Polymer Biosensors. International Journal of Medical Toxicology and Forensic Medicine. 2020; 10(1):26172. <https://doi.org/10.32598/ijmtfm.v10i1.26172>

**doi** <https://doi.org/10.32598/ijmtfm.v10i1.26172>



## Article info:

**Received:** 10 Jul 2019

**First Revision:** 25 July 2019

**Accepted:** 23 Dec 2019

**Published:** 16 Feb 2020

## Keywords:

Bioinformatics, Biosensors,  
Viscumin, Mistletoe lectin 1

## ABSTRACT

**Background:** There are many diseases around the world that threaten human health and its related hygienic issues. Cancer is among the conditions mentioned above that cause many problems for health sectors worldwide.

**Methods:** The present research analyzed the linear B-cell epitope of viscumin from European mistletoe using bioinformatics tools. We also provided references for the fast detection of biological agents. Several important tools, such as ProtParam, NCBI, PDB, T-coffee, BCPred, Bptope, Ellipro, and Ch3D were used to predict the viscumin linear epitope and its physical and chemical properties.

**Results:** The 9-mer epitope found as QQTGEEYF embedded in the A-chain of protein by the least sequence homology with other homologous rivals. Its molecular weight, theoretical isoelectric point, and the total number of negatively charged residues were 1102.1, 3.79, and 2, respectively.

**Conclusion:** Using different databases and establishing the accuracy level of >50% for linear B-cell epitope prediction, the selected epitope passed the related criteria and was introduced as a new linear epitope as a potential biological element in biosensors for cancer (viscumin) fast therapeutic detection.

## 1. Introduction

The introduction to specific and conserved short-length peptides as a biological agent in a fast detection field may result in substantial progress of many dangerous biological agents such as bacteria, viruses and toxic proteins [1, 2]. The traditional detec-

tion methods, which include conventional or laborious attitude, might be time-consuming; these steps may pass the process of target bio-analyte cultivation at a desired circumstance as well as checking the quality of final products out. Although traditional methods work, but they include unfavorable drawbacks as being laborious, time-consuming, dependent to skillful experts, lead to

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killing the time which may result in even pandemic situations or vast mortality [3-6].

Viscumin as an important cytotoxin due to its pharmacological effects extracted from mistletoe extracts are discussed in detail by the concentration of three toxic lectins: mistletoe lectin I (MLI, or viscumin), MLII, MLIII by Tonevitsky and co-workers. These proteins, as well as ricin, belong to ribosome-inactivating proteins type 2 (RIP2). The causes of the differences in activity of plant toxins as pharmacological agents, and the importance of above mentioned epitopes for neutralizing antibodies at the clinical applications of mistletoe extracts are discussed [7].

Regarding the importance of detection of the toxin in clinical and forensic medicine, it is worthy to notify that ribosome inactivating proteins (RIPs) are highly potent cytotoxins that have potential as anticancer therapeutics. Mistletoe lectin 1 (ML1) is a heterodimeric cytotoxic protein isolated from European Mistletoe and belongs to RIP class II. The aim of Beztinna and his co-workers was to systematically study ML1 cell binding, endocytosis pathway(s), subcellular processing and apoptosis activation. Additionally, co-localization studies by aforementioned researchers demonstrated the toxin transport from early endocytic vesicles to Golgi network; a retrograde road to the endoplasmic reticulum. The pro-apoptotic and anti-proliferative activity of ML1 were shown in time lapse movies and subsequently quantified. ML1 cytotoxicity was less affected in multidrug resistant tumor cell line 4T1 in contrast to commonly used chemotherapeutic drug (ML1 resistance index 6.9 vs. 13.4 for doxorubicin; IC50: ML1 1.4 ng/ml vs. doxorubicin 24000 ng/ml). This opens new opportunities for the use of ML1 as an alternative treatment in multidrug resistant cancers [8].

Bioinformatics is a scientific field where different majors such as computing, biology and information technology come to gather in order to handle and arrange derived biological information in biotechnology, genetics and molecular biology [9]. Also, bioinformatics tools for fast detection field may lead to new concepts for in silico determination of biological receptors in biosensors against dangerous bio-analysts [10].

This study is aimed at providing a route using bioinformatics and its selected software as well as databases for B-cell linear epitope prediction based on the authors personal experiments, and digging in the literature and web databases, providing an outlook on how bioinformatics may shed a helpful light especially on the epitope

prediction field. The main goal of the present study was to predict an epitope by potential application for a biosensor especially MIP-based one for mistletoe lectin 1 (ML1) or Viscumin- a plant derived therapeutic drug-fast detection.

## 2. Materials and Methods

The amino acids arrangement of ML1 was gotten from NCBI at <https://www.ncbi.nlm.nih.gov/> the physiological and biochemical characters were anticipated in view of <http://web.expasy.org/protparam/>, the optional and 3D structure was anticipated, using the National Center for Biotechnology Information (NCBI) and Protein Data Bank (PDB) information server utilizing Cn3D 4.3 from the United States National Library of Medicine that acts as a helper application for web browsers to view three-dimensional structures from The National Center for Biotechnology Information's Entrez retrieval service. The direct (constant) B-cell epitope was anticipated in light of different arrangement utilizing T-Coffee online server. Additionally, a few online web-based databases were connected for looking through the epitope areas percent of forecast with adequate precision: BCPred, Bptope, Ellipro, and so on. All sequences extracted from NCBI unless otherwise stated.

## 3. Results and Discussion

### Analysis of amino acids sequences

The accession number of amino acids of Viscumin was UniProtKB/Swiss-Prot: P81446 and its 3D structure number in PDB is 2RG9. At first, its full length amino acid sequence was derived and evaluated for extraction of B-cell epitope (Figure 1).

### Structure of Viscumin

The 3-D structure of Viscumin simulated with online Cn3D software and the result is depicted in Figure 2.

### Predicting B-cell epitope

At first, four types of protein-type toxins of type AB were extracted and examined. The sequences were obtained from the FASTA format at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov). Then, to compare their sequences and to examine their different regions; multiple sequence alignment was applied in order to find an epitope candidate. The result of the comparison of sequences in the T-coffee database is depicted in Figure 3. The result showed that 9-mer ami-

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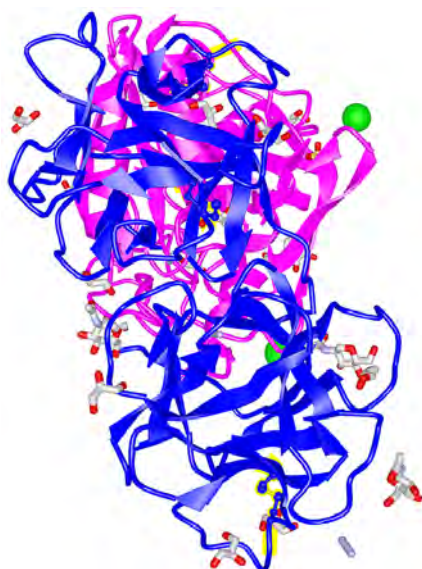
> P81446|Viscumin (Beta-galactoside-specific lectin 1)
      10      *      30      *      50      *      70
MNGHLASRRRAWVWYFLMLGQVFGATVKAETKFSYERLRLRVTHQTTGEEYFRFITLLRDYVSSGFSNEI 70
PLLRSQSTIPVSDAQRFVLVELTNEGGDSITAAIDVTNLYVVAYQAGDQSYFLRDAPRGAETHLFTGTTRS 140
SLPFNGSYPDLERYAGHRDQIPLGIDQLIQSVTALRFPGGSTRTQARSILILIQMISEAARFNPILWRAR 210
QYINSGASFLPDVYMLELETSWGQQSTQVQQSTDGVFNNPIRLAIPPGNFVTLTNVRDVIASLAIMLFVC 280
GERPSSSDVRYWPLVIRPVIADDVTCASEPTVRIVGRNGMCVDVRDDDFHDGNQIQLWPSKSNNDPNQL 350
WTIKRDGTIRNGSCLTTYGYTAGVYVMIFDCNTAVREATLWEIWNGTIINPRSNLVLAAASSGKGTTL 420
TVQTLDYTLGQWLAGNDTAPREVTIYGFRDLCMESNGGSVWVETCVISQQNRWALYGDGSIRPKQNQD 490
QCLTCGRDSVSTVINIVSCSAGSSGQRWVFTNEGAILNLKNGLAMDVAQANPKLRRIIYPATGKPNQMW 560
LPVP
    
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**Figure 1.** The full-length amino acid sequence of viscumin adapted from NCBI: available in free access

no acid segment highlighted in red (Figure 3) was the best choice to be used in follow.

After analyzing and comparing the different parts of the nine-segment C-terminal peptide sequences of the catalytic toxic chain of Viscumin with the least possible interference, QQTGEEYF was selected for further investigation, which is depicted in red with black arrow (Figure 3).



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**Figure 2.** The tertiary structure of viscumin stimulated in online Cn3D software (Yellow lines show disulfide bonds, green ball-type atoms, refer to chloride ions, three-branch structures are, glycerols (15), single capsule-like structure relies on, azide ions (3), four-branch structure depicts sulfate ions (1), and six-branch structures show 4 N-Acetyl-D-Glucosamines.

For further and more detailed studies, the isoforms sequences in the data banks of this toxin were examined and multiple aligned to determine the protection of the selected peptide using T-coffee tool (Figure 4).

#### Multiple alignments of various isomers with T-coffee

For further and more detailed studies, the isoforms sequences in the data banks of this toxin were examined and multiple aligned to determine the protection of the selected peptide using T-coffee tool (Figure 4). By profound comparison of selected isoforms, it was found that the candidate had a good conservative feature of different isoforms of toxin by multiple sequence alignment. In order to further investigate the sequences of A chain of the toxin in different databases were accomplished with different tools, using the first structure of the protein through the online network software as, Ellipro, BCPred, LPtope, bepitope, SVMTrip, Discoptope and CBTOPE (<http://www.imtech.res.in/raghava/cbtope>).

The possibility of the presence of the entire selected peptide as an epitope in the relevant sequence was investigated. The results indicated that in all cases, these peptide tools or a large portion of it are predicted to be as epitopes with precision of over 70% in most cases, and is the only option among other peptides (supplementary data). Thereafter, by referring to the Immune Epitope Database (IEDB) analysis server, epitope conservancy analysis has been accomplished at least in 80% identity level, but the identity of selected epitope determined more than 85%. Finally, it has been introduced as potential candidates for epitopes.

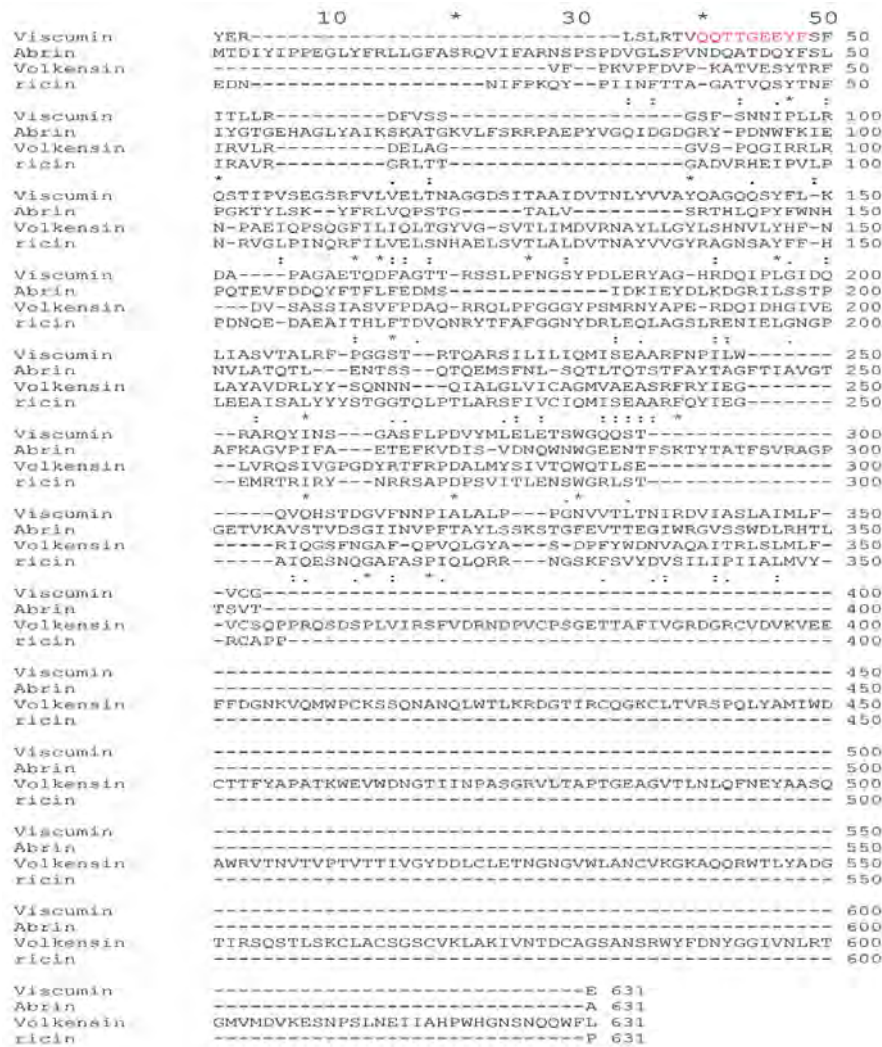


Figure 3. Multiple sequence alignment by T-coffee online server based on CLUSTAL W (1.83)

Thus, it (QTTGEEYF) was suggested as an epitope for potential use of MIP-based biosensors to solve some aspects of supramolecule challenges such as conformation stability, complexity and mass transfer phenomena. It has also submitted in EMBL-EBI with primary accession number of PRJEB24387 and secondary accession number of ERP106207, but has not yet been released publicly and therefore the full data has been submitted here completely in detail.

The evaluation of nine amino acids steric position, The steric position of selected epitope was determined by Swiss model using Cn3D online software and the result is presented (Figure 5). The full process of steric position of selected epitope is expressed and explained in figure 5. As it can be inferred from Figure 5, the selected 9-mer epitope is exposed on the surface. In addition, the chemical and physical characteristics of selected epitope were

recorded by using protparam software placed in expany complex (Figure 6).

The Instability Index (II) is computed to be 48.70. This classifies the protein as unstable but based on amino acid structure, there is no interfering amino acid for instability, thus it is stable in its matrix. Also, total number of negatively charged residues (acidic) (Asp + Glu) and positively charged residues (basic) (Arg + Lys) are 2 and 0, respectively.

#### 4. Discussion

The epitope prediction for therapeutic goals, especially in Vaccine or detection affairs showed a great potential, nowadays [11, 12]. B-cell receptors or relative antibodies are recognized by B-cell epitopes in their folded structures. Amino acid properties such as hydro-



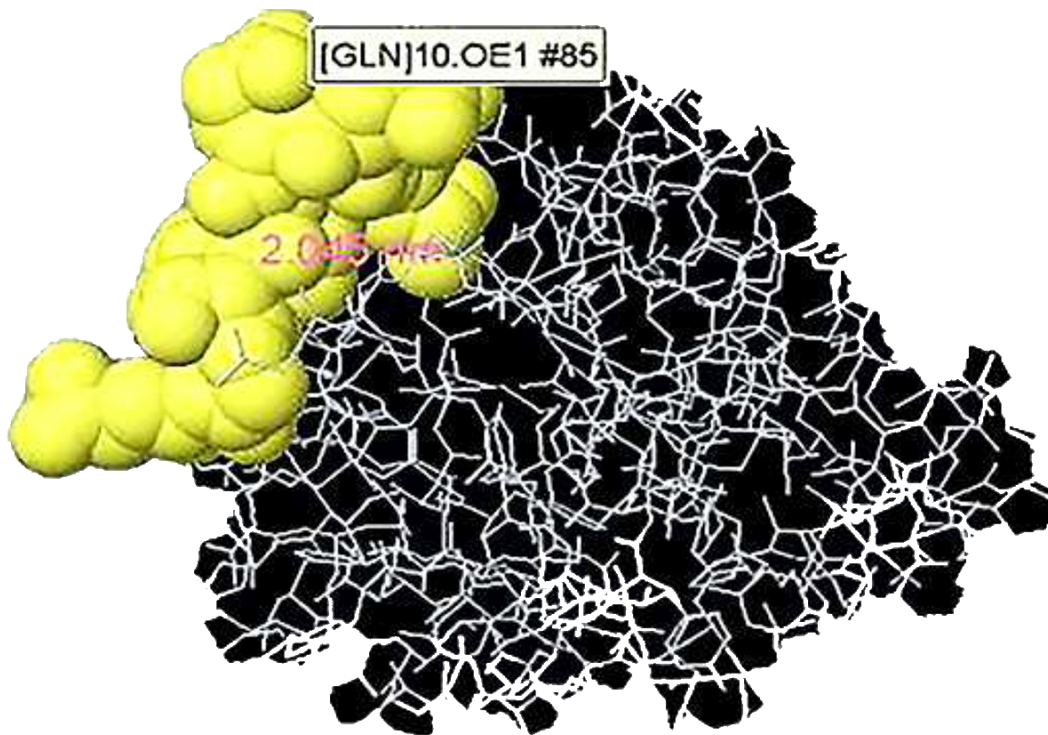
**Figure 4.** Multiple alignments of protein isoforms: gi |P81446| Viscumin; gi |2RG9| A chain; gi |G193| lectin 1; gi |G195| lectin 1 and gi |ISZ6| A chain involve; 564, 249, 564, 564 and 249 amino acids, respectively

philicity, charge, exposed surface area and secondary structure have important roles which result in continuous B-cell epitope prediction. On the other hand, 3D structure of the antigen is the basic prerequisite for B-cell epitope prediction in the batch mood [13-15]. To date, in order to predict linear B-cell epitopes, several servers and tools exist. The BCPREDS tool is a simple choice that is emerging and its accuracy is more than 50% [16]. The next one is the ABCpred server with more than 65% accuracy [11].

Bepired is another server, which infers hidden Markov model and a propensity scale method to predict the location of linear B-cell epitopes [17]. In addition, by applying protein antigen's 3-D structure, a new and more powerful tool is essential to predict linear epitopes

in contrast with other aforementioned tools defined as Ellipro. It devotes predicted epitope with a score. Based on homology modeling, a 3-D structure will be predicted for the input sequence. By the emergence of highly dangerous biological agents both with natural and genetically modified (recombinant) source imposed human community for development of more effective approaches to confront the dangerous supramolecule fast detection challenges.

The in silico approaches may be addressed by the aforementioned challenges. One of the main contributing roles of B-cell linear epitope is dedicated to molecularly imprinted polymer biosensors. The achievement in MIP-based biosensors synthesis and development return to the molecular weight of target analyte, which



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**Figure 5.** Steric position of selected epitope obtained with average horizontal width of 2 nm using online Cn3D software depicted with dense spheres at the top left corner.

is suggested being lower than 5 kDa. This caution has to be considered seriously, otherwise the chance of having a proper biosensor for practical use in complex media may be out of mind or hardly acceptable. Epitope approach is one of the new methods which proposed to encounter such a problem.

The Molecularly Imprinted Polymers (MIP) considered synthetic analogues for aptamers, antibodies, antigens, enzymes and other biological detection agents. They often offer very good levels of specificity, like their natural competitors, at a lower cost, longer durability and

resistance to inappropriate environmental conditions. As a serious rival in the evolutionary path through natural selection processes, fully synthetic MIPs have opened a new hatch to eliminate some of the constraints of natural systems with more sustainability, logical costs, and faster production to humankind.

MIPs have unique features such as high stability, easy preparation and lower logical costs than their natural rivals. In fact, a new era has begun with the advent of this type of biosensors [18-20]. Protein Bovine Hemoglobin was molded onto an array of acrylamide-based

➤ <b>QOTTGEEYF</b>	➤ <b>Total number of negatively charged residues (acidic) (Asp + Glu): 2</b>
Number of amino acids: 9	➤ <b>Total number of positively charged residues (basic) (Arg + Lys): 0</b>
Molecular weight: 1102.1 Dalt	➤ <b>Amino acid composition of epitope candidate: Carbon C 48,</b>
Theoretical pI: 3.79	Hydrogen H 67, Nitrogen N 11, Oxygen O 19, Sulfur S 0
Theoretical pI of A-chain: 5.08 249 aa	➤ <b>Aliphatic index: 0.00 and Grand average of hydropathicity (GRAVY): -1.589</b>
Theoretical pI of B-chain: 4.99 263 aa	
➤ <b>Formula: C<sub>48</sub>H<sub>67</sub>N<sub>11</sub>O<sub>19</sub>, Total number of atoms: 145, Extinction coefficients: 1490, Abs 0.1% (=1 g/l):1.352</b>	

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**Figure 6.** Biochemical characteristic of the selected epitope using protparam software located in NCBI complex

polymer hydrogels to optimize the piezoelectric sensor electrode surface [21]. In fact, this study was conducted to evaluate the complexity of protein chemistry using monomers. A cancer biomarker called quinogen- a plasma protein- for early diagnosis and cancer prediction was synthesized [22].

Another cancer biomarker, called carcinogenic embryonic carcinogenesis protein, was formed on screening electrodes of polyoxyethylene matrix, which is generally used to monitor the progression of clonal cancer [23]. The epitope-based imprinting could minimize non-specific adsorption, which is a serious problem for protein or macromolecular imprinting. Comparing the epitope with the protein molecule as a template and the formation of a protein epitope sequence, a better result was obtained by the epitope imprinting method [24]. In other research work, 15 amino acid peptides of the Japanese encephalitis virus were imprinted using a new mesenteric monomer capable of differentiating oxytocin and vasopressin [25]. Similarly, a 15 amino acid peptide for detecting yellow fever virus was imprinted as an early alternative diagnostic tool [26].

As it can be inferred, the MIP nanoparticles showed excellent capabilities for the detection of the epitope, such as the HSA protein [27]. Also, another group worked on B-cell epitope prediction and investigated its probability in detail. Although it was a valuable work but the authors did not surveyed some details as isoform alignment and region of conservancy [28]. But in present work, these deficiencies are completely carried out resulted a more powerful prediction with better accuracy. In addition, in other work, several databases were introduced for B-cell epitope prediction [29, 30]. Some of these suggestions were applied by our group in order to have more powerful prediction but it is not applied by song and his co-worker. They only used one of these databases that may not involve a powerful feedback.

#### 4. Conclusion

Bioinformatics tools present a favorable opportunity over traditional techniques such as lower costs and more speed in outputs extraction. On the other hand, it has enabled the prediction of potential B-cell epitope (linear) without being concerned to direct contact with dangerous pathogens of interest. In conclusion, suitable B-cell linear epitope involving 9-mer epitope was determined with potential application in fast detection tools especially synthetic ones (molecularly imprinted polymer based biosensors). The in silico selected peptide conservancy was approved successfully and will be tested as

a biological element candidate in future work to detect Viscumin applying a surface imprinted polymer based nanobiosensor applying epitope approach.

#### Ethical Considerations

##### Compliance with ethical guidelines

All ethical principles were considered in this article. The participants were informed about the purpose of the research and its implementation stages; they were also assured about the confidentiality of their information; Moreover, They were allowed to leave the study whenever they wish, and if desired, the results of the research would be available to them.

##### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

##### Author's contributions

All authors contributed equally in preparing all parts of the research.

##### Conflict of interest

The authors declared no conflict of interest.

##### Acknowledgements

Authors wish to thank Dr. Abbas Hajizadeh for his benevolence in the bioinformatics study.

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